EVALUATION OF BIOLOGICAL ACTIVITIES OF SELECTED MEDICINAL PLANTS

Thesis submitted for the fulfillment of the degree of

DOCTOR OF PHILOSOPHY

BY

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Department of Biological Sciences
Faculty of Basic and Applied Sciences
International Islamic University, Islamabad, Pakistan

2019

I Medicinal plants

II Traditional medicine

III Melia azedarach (MA)

IV Justicia adhatoda (JA)

V Ricinus communis (RC)

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In the Name of Allah, the Most Compassionate, the Most Merciful

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Dated: 24-10-2019

FINAL APPROVAL

It is certified that we have read the thesis submitted by Mr. Muhammad Rifaqat Ameer and it is our judgment that this project is of sufficient standard to warrant its acceptance by the International Islamic University, Islamabad for Ph.D. degree in Biotechnology.

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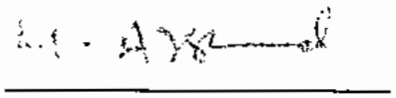


A thesis submitted to the Department of Biological Sciences, Faculty of Basic and Applied Sciences, International Islamic University, Islamabad, as a partial fulfillment of requirement for the award of Ph.D. Degree in Biotechnology

DECLARATION

I hereby declare that the work present in the following doctoral dissertation is my own effort, except where otherwise acknowledged and that the dissertation is my own composition. No part of the thesis has been previously presented for any other degree.

Dated: 24-10-2019

RIFAQAT

Muhammad Rifaqat Ameer

DEDICATED

TO

MY FAMILY

ACKNOWLEDGEMENTS

My all praise to ***Almighty Allah,*** *The omnipotent, the omnipresent, the merciful, and most compassionate, who is the entire and only source of knowledge and wisdom endowed to mankind and who blessed me with the ability to do this work. Glory and praise to our Last Prophet* ***Hazrat Muhammad (Sallallaho Alaihe Wasallam),*** *the most perfect and exalted among every borne on the surface of earth, who is forever a symbol of direction and knowledge for whole humanity and whose teachings are beam of light for us in the hours of despair and darkness that gives us new version for life.*

Completing a Ph.D is truly a marathon event, and I would not have been able to complete this journey without the aid and support of countless people over the past five years. Foremost, I would like to express my sincere gratitude to my supervisor, ***Professor Dr. Zafar Mahmood Khalid,*** *Department of Biological Sciences, International Islamic University Islamabad, who has supported me throughout my thesis with his patience, motivation and knowledge whilst allowing me the room to work in my own way. His intellectual guiding, positive and sincere criticism and continuous support throughout my research and writing of thesis, was exemplary. He helped me to grow in both my competence and in confidence as researcher. I could not have imagined having a better advisor and mentor for my Ph.D study. I doubt that I will ever be able to convey my appreciation fully, but I owe his my eternal gratitude.*

I was fortunate to have the chance to work with ***Professor Dr. Lee Hian Kee,*** *Department of Chemistry, National University of Singapore, Singapore. I am extremely grateful to him for providing research facilities to carry out my research project in his laboratory and also for his useful suggestions and guidance. I am very thankful to* ***Dr. Yuan Cheng Hui, Steven,*** *Mass Spectrometry Laboratory, and* ***Madam Frances Lim,*** *NUS Environmental Research Institute (NERI), National University of Singapore, Singapore, for his kind assistance in the GC-MS analysis to achieve the purpose of my research. I am very thankful to* ***Madam Tan Wei Ling,*** *Elemental and Thermal Lab, National University of Singapore, Singapore, for his kind assistance in the Elemental analysis. My grateful thanks to people at the Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore,*

Singapore, especially ***Dr. Gautam Sethi*** *and* ***Dr. Shanmugam Muthu*** *for their help with the cell culture and flow cytometric studies. I would like to thank all the members of* ***Professor Dr. Lee Hian Kee*** *Laboratory for their kind cooperation. Especially I would like to thank* ***Tan Sok Kiang*** *and* ***Christina Liew Shu Min,*** *with whom I had the opportunity to work. They provided a friendly and cooperative atmosphere at work and also useful feedback and insightful comments on my work.*

I would like to thank ***Dr. Ibrar Shinwari,*** *Department of Environmental Sciences, International Islamic University Islamabad for their help in the identification of plants. I would like to thank* ***Dr. Shahzad Hussain*** *and* ***Dr. Rashid Mehmood,*** *Drugs Control and Traditional Medicines Division, National Institute of Health, Islamabad for their help in the extraction, phytochemical analysis and antidiabetic activity of medicinal plants. I would like to thank* ***Mr. Hussain Ali,*** *National Institute of Health, Islamabad for their help in the antidiabetic activity of medicinal plants. I would like to thank* ***Mr. Sher Alam, Mr. Riaz Muhammad, Mr. Zafar Iqbal*** *and* ***Miss Saima*** *for their help in all the experiments conducted in the National Institute of Health, Islamabad. I would like to thank* ***Dr. Ihsan-ul-Haq,*** *Department of Pharmacy, Quaid-e-Azam University, Islamabad and* ***Dr. Muhammad Majid*** *for their help in the phytochemical analysis, antioxidant activity and cytotoxicity assay. I would like to thank* ***Dr. Syed Ali Imran Bokhari,*** *Department of Biological Sciences, International Islamic University Islamabad, for his cooperation and allow me to work in his laboratory and providing me all the facilities regarding this research work and* ***Mr. Talah Yasin*** *for their help in the antimicrobial activities of plants.*

I want to thank all the faculty members of the Department of Biological Sciences, International Islamic University Islamabad, particularly ***Dr. Muhammad Arshad Malik, Dr. Jabar Zaman Khan Khattak, Dr. Asif Mir, Dr. Bashir Ahmed, Dr. Muhammad Imran Shabbir*** *and* ***Dr. Syed Ali Imran Bokhari.*** *I want to express my special thanks to my class fellows, particularly* ***Dr. Mubin Mustafa Kiyani, Dr. Zakkarya Khan, Dr. Naqibullah Jogezai, Dr. Hasnain Alam, Dr. Ahmed Farooq, Dr. Yasir Khalil, Dr. Adil Hussain, Dr. Waqar Arshad, Dr. Ali Nadeem*** *and* ***Dr. Muhammad Daud*** *and others for their cooperation and support during my study. I wish to convey my gratitude to* ***Mr.***

Muhammad Muti-ur-Rehman, Mr. Sikandar Khan, Mr. Raza *and* ***Mr. Tariq.***

I am really thankful to ***Higher Education Commission (HEC)*** *of Pakistan for providing me financial support to accomplish this project. I would like to express my profound gratitude to HEC for giving me a golden chance to work abroad at National University of Singapore, Singapore under the* ***International Research Support Initiative Program (IRSIP).*** *I believe that HEC funding added to my strength a lot. Completing my Ph.D degree is probably the most challenging activity of my life. The best and worst moments of my doctoral journey have been shared with many people. I would like to extend my deepest appreciation to those people, who helped me in one way or another during my stay at the Department of Biological Sciences, International Islamic University, Islamabad.*

No acknowledgment could ever adequately express my feelings to my affectionate and adorable parents; my ***Father*** *and* ***Mother*** *who remembered me in their prayers and let me spare to complete my studies for a long time. I am honored to have them as my parents. Thank you for giving me a chance to prove and improve myself through all walks of my life. My father is true dense of my humblest obligations and my words will fail to give them enough credit. I am proud of my father who provided me all what I needed and remembered me in their prayers. And, of course, thank you for your constant support through the ups and downs of my academic career. It has been bumpy at times, but your confidence in me has enhanced my ability to get through it all and succeed in the end. I cannot imagine being the person I am today without such a great family and friends through the years. Thanks for everything that helped me get to this day.*

Words are lacking to express my feelings and thoughts to my ***Sisters*** *and my* ***Wife*** *for their love, trust, care and concern. Any tribute will be less for them. My special gratitude is for my Loving and Supporting* ***Wife*** *and my* ***Son,*** *who suffered a lot during my Ph.D tenure but they refused to leave my side and prayed for me. I am indebted for their loving support. From the start until the accomplishment of this manuscript, he has been my source of strength and love. It wouldn't have been this bearable if I didn't have him in my life.*

I would like to extend my deepest appreciation to those people, who helped me in one way or another to finish the task at hands, this thesis manuscript. By that time, I worked with a great number of people whose contribution in assorted ways to research and the making of the thesis deserved special mention. It is a pleasure to convey my gratitude to all of them in my humble acknowledgment. I owe a debt of gratitude to all of my school, college and university teachers, who laid seeds of enthusiasm and passion in my pursuit of knowledge. I am also thankful to all teaching, non-teaching and Research staff of the Department of Biological Sciences, International Islamic University, Islamabad for their kind behavior, encouragement and help in various ways. To my friends scattered around the world, thank you for your thoughts, well-wishes/prayers, phone calls, e-mails, texts, visits, editing advice, and being there whenever I needed a friend. Finally, I would like to thank everybody who was important to the successful completion of thesis. I would like to express my apology to those who ever had a soft corner for me but I missed to mention them personally. All the prayers and selfless love, support, guidance and affection provided by them have been extinct these days. I cannot return the favor provided by these silent supporters.

MUHAMMAD RIFAQAT AMEER

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Abbreviations	Full Name
α	Alpha
β	Beta
δ	Chemical shift
Λ	Wavelength
%	Percent
~	Similar
<	Less than
>	Greater than
°C	Degree centigrade
AA	Ascorbic acid
AAE	Ascorbic acid equivalent
AAS	Atomic absorption spectroscopy
Ab_c	Absorbance of test sample
Ab_s	Absorbance of negative control
AIDS	Acquired immune deficiency syndrome
Al	Aluminum
$AlCl_3$	Aluminum chloride
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AST	Aspartate transaminase
AST	Antimicrobial susceptibility test
AVOVA	Analysis of variance
B	Boron
Ba	Barium
$BaSO_4$	Barium sulphate
Br^-	Bromide
BSLT	Brine shrimp lethality test
BUN	Blood urea nitrogen
b.w	Body weight
$C_2H_3KO_2$	Potassium acetate
$C_2HCl_3O_2$	Trichloroacetic acid
Ca	Calcium
Cd	Cadmium
CFU	Colony forming unit
CHNS	Carbon, Hydrogen, Nitrogen, Sulphur
Cl^-	Chloride

cm	Centimeter
Co	Cobalt
Cr	Chromium
Cu	Copper
DAPI	4′, 6-Diamidino-2-Phenylindole
DC	Diabetic control
DE	Dry extract
DMEM	Dulbecco's modified eagle medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPPH	1, 1-diphenyl-2-picryl-hydrazyl
DW	Dry weight
E	Ethanol
EA	Ethyl acetate
EAC	Ehrlich ascites carcinoma
e.g.	For example
EO	Essential oil
Ev	Electron volt
F^-	Fluoride
FBS	Fetal bovine serum
Fe	Iron
$FeCl_3$	Ferric chloride
FRAP	Ferric reducing ability of plasma
GA	Gallic acid
GAE	Gallic acid equivalent
G	Gram
GC	Gas Chromatography
GC-MS	Gas Chromatography - Mass Spectrometry
H	Hour
H_2O	Water
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulphuric acid
HCL	Hydrochloric acid
HDL	High density lipoprotein
HIV	Human immunodeficiency virus
HNO_3	Nitric acid
i.p.	Intraperitoneal
i.e.	That is
IC_{50}	Fifty percent inhibition concentration

ICP-OES	Inductive Coupled Plasma - Optical Emission Spectroscopy
JA	*Justicia adhatoda*
JA-EAE	*Justicia adhatoda* ethyl acetate extract
JA-EE	*Justicia adhatoda* ethanol extract
JA-ME	*Justicia adhatoda* methanol extract
K	Potassium
Kg	Kilogram
LD_{50}	Fifty percent death
LDL	Low density lipoprotein
LFTs	Liver function tests
Li	Lithium
LPO	Lipid peroxidation
M	Methanol
M	Molar
m/z	Mass-to-charge ratio
MA	*Melia azedarach*
MA-CE	*Melia azedarach* chloroform extract
MA-EAE	*Melia azedarach* ethyl acetate extract
MA-EE	*Melia azedarach* ethanol extract
MA-HE	*Melia azedarach* hexane extract
MA-ME	*Melia azedarach* methanol extract
MF	Molecular formula
Mg	Magnesium
Mg	Milli gram
mg/dl	Milli gram per deciliter
mg/ml	Milli gram per milliliter
MIC	Minimum inhibition concentration
Ml	Milli liter
Mm	Milli meter
mM	Milli molar
Mn	Manganese
Mo	Molybdenum
MS	Mass spectrometry
MTT	3-(4, 5-Dimethylthiazol-2-yl)-2,5 diphenyltetrazoliumbromide
MW	Molecular weight
Na	Sodium
Na_2CO_3	Sodium carbonate
NaCl	Sodium chloride
NaOH	Sodium hydroxide

NC	Normal control
ND	Not detected
(NH4)2MoO4	Ammonium molybdate
Ni	Nickel
NIH	National Institute of Health
NIST	National Institute of Standards and Technology
NO	Nitric oxide
nM	Nano molar
Nm	Nano meter
NO_3^-	Nitrate
OGTT	Oral glucose tolerance test
PC	Positive control
PH	Power of hydrogen ion
PO_4^{3-}	Phosphate
Ppm	Parts per million
POD	Peroxidase
QE	Quercetin equivalent
R^2	Coefficient of determination
RBCs	Red blood cells
RC	*Ricinus communis*
RC-EAE	*Ricinus communis* ethyl acetate extract
RC-EE	*Ricinus communis* ethanol extract
RC-ME	*Ricinus communis* methanol extract
RC-RE	*Ricinus communis* root extract
Rpm	Revolution per minute
RT	Retention time
SD	Standard deviation
SDA	Sabouraud dextrose agar
SGOT	Serum glutamic oxaloacetate transaminase
SGPT	Serum glutamic pyruvates transaminase
SO_4^{2-}	Sulfate
TAC	Total antioxidant capacity
TCA	Trichloroacetic acid
TFC	Total flavonoid content
TLC	Thin layer chromatography
TNF-α	Tumor necrosis factor alpha
TPC	Total phenolic content
TRP	Total reducing power
µg	Micro gram

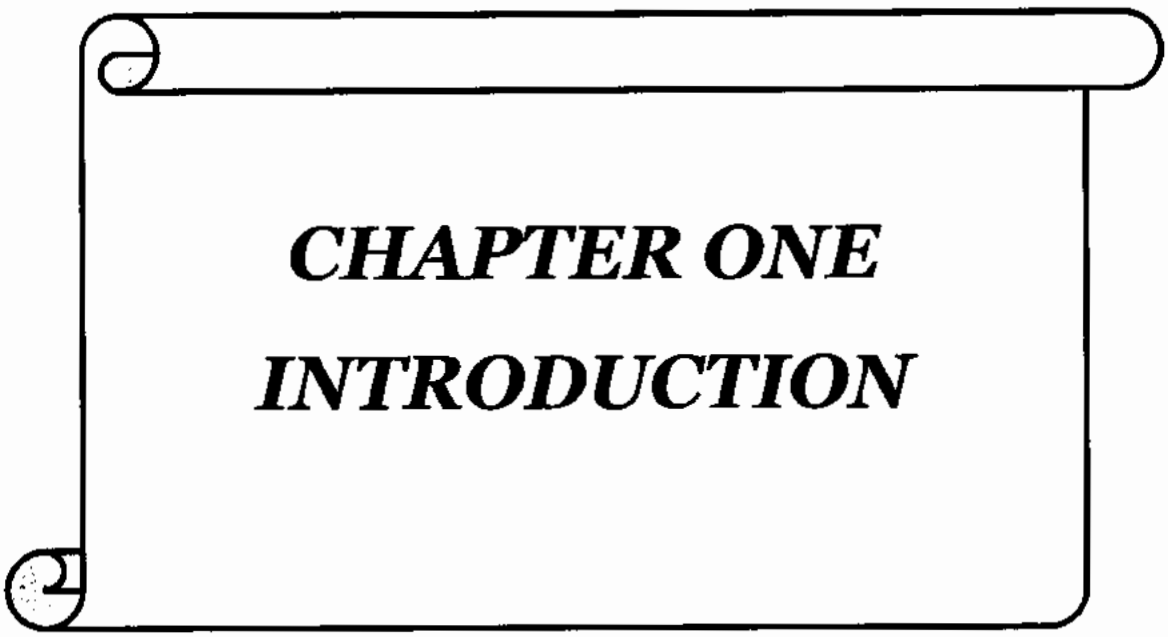
µl	Micro liter
µM	Micro molar
u/l	Units per liter
UV	Ultra violet
VLDL	Very low density lipoproteins
WBCs	White blood cells
WHO	World Health Organization
Zn	Zinc

ABSTRACT

The current study focused on evaluating the biological activities of *Melia azedarach* (MA), *Justicia adhatoda* (JA) and *Ricinus communis* (RC). The leaves of all these plants were extracted sequentially using methanol, ethanol and ethyl acetate. All the extracts were phytochemically screened. Several assays were used to assess the antioxidant potential of extracts. Alkaloids, terpenoids, tannins, glycosides, flavonoids, phenols and saponins are present in all extracts. MA-EAE showed the maximum quantity of total phenolic content (TPC). Flavonoids were found to be rich in MA-EAE. The MA-EE exhibited a maximum scavenging activity. MA-EAE has the maximum total antioxidant capacity (TAC) whereas MA-EAE showed the maximum total reducing power (TRP). JA-EE demonstrated the maximum quantity of total phenolic content (TPC). Flavonoids were found to be rich in JA-EE. The JA-ME exhibited a maximum scavenging activity. JA-EE showed the maximum total antioxidant capacity (TAC). JA-EAE showed the maximum total reducing power (TRP). RC-ME showed the maximum quantity of total phenolic content (TPC). Flavonoids were found to be rich in RC-ME. The RC-ME exhibited a maximum scavenging activity. RC-ME has the maximum total antioxidant capacity (TAC). RC-ME showed the maximum total reducing power (TRP). The cytotoxic potential in *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* leaf extracts was estimated through brine shrimp lethality test. The MA-ME showed good cytotoxicity with LD_{50} value of 15.12 µg/ml. The MA-EE showed good cytotoxicity with LD_{50} value of 51.11 µg/ml. The MA-EAE showed good cytotoxicity with LD_{50} value of 122.78 µg/ml. The JA-ME showed good cytotoxicity with LD_{50} value of 84.91 µg/ml. The JA-EE showed good cytotoxicity with LD_{50} value of 41.58 µg/ml. The JA-EAE showed good cytotoxicity with LD_{50} value of 287.50 µg/ml. The RC-ME showed good cytotoxicity with LD_{50} value of 11.14 µg/ml. The RC-EE showed good cytotoxicity with LD_{50} value of 37.67 µg/ml. The RC-EAE showed good cytotoxicity with LD_{50} value of 391.76 µg/ml. The antidiabetic activity of different plants extracts was determined. After the 28 days treatment at 400 mg/kg, both MA-ME and MA-EE decreases blood glucose levels in diabetic mice. JA-ME and JA-EE at 200 mg/kg and 400 mg/kg, respectively decrease blood glucose levels in diabetic mice. After the 28 days treatment at 400 mg/kg, both RC-ME and RC-EAE decreases blood glucose levels in diabetic mice. After the 28 days of treatment, all extracts reduced elevated cholesterol, triglycerides, LDL, VLDL, bilirubin, ALT, ALP, AST, urea, creatinine and uric acid levels and increased HDL levels. *Melia*

azedarach, *Justicia adhatoda* and *Ricinus communis* microbial activity was assessed against significant bacterial and fungal strains. The highest antibacterial activity was recorded in MA-ME against the *Staphylococcus aureus* followed by MA-EE against the *Staphylococcus aureus* and MA-EAE against the *Pseudomonas aeruginosa*. The highest antibacterial activity was recorded in JA-ME against the *Escherichia coli* followed by JA-EE against the *Escherichia coli* and JA-EAE against the *Staphylococcus aureus*. The highest antibacterial activity was recorded in RC-ME against the *Pseudomonas aeruginosa* followed by RC-EE against *Pseudomonas aeruginosa* and RC-EE against *Bacillus subtilis*. The highest antifungal activity was recorded in MA-ME against *Aspergillus flavus* followed by MA-EE against *Fusarium oxysporum* and MA-EAE against *Aspergillus flavus*. The highest antifungal activity was recorded in JA-ME against *Aspergillus niger* followed by JA-EE against *Aspergillus niger* and JA-EAE against *Aspergillus niger*. The highest antifungal activity was recorded in RC-ME against *Aspergillus flavus* followed by RC-EE against *Aspergillus flavus* and RC-EAE against *Aspergillus flavus*. The anticancer activity of all the three plant extracts was detected by MTT assay at 48 h and 72 h on HepG2 and HCCLM3 cell lines. All the extracts showed good antiproliferative activity at both time intervals. Based upon the results of MTT assay, the cell cycle arrest was performed on HepG2 cell line at 72 h, as most of the extracts showed more promising results on HepG2 cell line. MA-ME demonstrated effective results on HepG2 cell line with percent viability of 12.33%, 6.55% at 48 h and 19.72% at 72 h. The MA-ME demonstrated effective results on HCCLM3 cell line with percent viability of 22.83% at 48 h and 10.84% at 72 h. The JA-EAE demonstrated effective results on HepG2 cell line with percent viability of 18.13%, 9.84% and 6.14% at 48 h and 17.42% and 10.89% at 72 h. The JA-EAE demonstrated effective results on HCCLM3 cell line with percent viability of 15.86%, 13.30% and 11.73% at 48 h and 20.48%, 14.33% and 9.91% at 72 h. The RC-EAE demonstrated effective results on HepG2 cell line with percent viability of 12.81%, 9.17% and 8.33% at 48 h and 17.71% and 13.87% at 72 h. The RC-EAE demonstrated effective results on HCCLM3 cell line with percent viability of 9.42%, 6.15% and 5.97% at 48 h and 21.62%, 18.33% and 13.15% at 72 h. MA-ME showed the accumulation of cells in the sub-G1 phase, dose dependently with 11.24%, 64.81% and 87.21% cell cycle arrest, followed by MA-EE (26.73%, 66.14% and 81.12%) cell cycle arrest, JA-EAE (13.39%, 55.63% and 92.29%) cell cycle arrest and RC-EAE (17.38%, 70.95% and 98.86%) cell cycle arrest at the concentrations of 100 µg/ml, 500 µg/ml and 1000 µg/ml respectively. The content of elements

were determined using ICP-OES, and thus the plants contain significant levels of elements including Fe, Zn, Cu, Cr, Mn, Co, Ni, Mg, Al, Ca, Li, Na, K, Ba, B, P and Mo. Ni and Mo was absent in *Melia azedarach*. Li was absent in *Melia azedarach* and *Justicia adhatoda*. Anions of all the three plants were determined using Met Rohm Ion Chromatography system. Chloride, Fluoride, Nitrate, Phosphate and Sulfate have been found in *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis*. Bromide was absent in all three plants. The percentage of Carbon, Hydrogen, Nitrogen and Sulphur were determined using CHNS analyzer. Carbon, Hydrogen and Nitrogen were present in all the plants but Sulphur was absent in *Melia azedarach* and *Justicia adhatoda* but detected in *Ricinus communis*. All the extracts were analyzed using GC-MS. The major compounds identified in MA-ME were Chloroacetic acid allyl ester (19.92%), D-Galactose (6.82%) and 1H-Indene, 2,3-dihydro-4-methyl- (5.52%). The major compounds identified in MA-EE were 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (10.78%), Vitamin E (8.11%) and 9,12,15-Octadecatrienoic acid, (Z,Z,Z) (6.12%). The major compounds identified in MA-EAE were 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (12.66%), Dl-.alpha.-Tocopherol (10.72%) and Octacosane (5.92%). The major compounds identified in JA-ME were Phytol acetate (11.07%), 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one (6.72%) and 5-Hydroxymethylfurfural (5.85%). The major compounds identified in JA-EE were 9,12,15-Octadecatrienoic acid, (Z,Z,Z) (11.92%), Phytol, acetate (9.47%) and N-Hexadecanoic acid (6.40%). The major compounds identified in JA-EAE were 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (12.54%), 9,12-Octadecadienoic acid (Z,Z)- (9.41%) and Hexacosane (8.17%). The major compounds identified in RC-ME were Lupeol (14.90%), 9,12,15-Octadecatrienoic acid, (Z,Z,Z) (12.55%) and 1,4-Benzenediamine, N,N,N',N'-tetramethyl (12.21%). The major compounds identified in RC-EE were Lupeol (19.64%), Phytol (15.69%) and 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (15.40%). The major compounds identified in RC-EAE were 9,12-Octadecadienoic acid, methyl ester (17.19%), Lupeol (13.85%) and 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (11.92%). Pharmacological properties of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* leaves including antibacterial, antifungal, antidiabetic, anticancer, cytotoxicity and antioxidant activity may represent a new generation of potential drug candidates for the treatment of various diseases. Therefore, further clinical trials are required in the pharmaceutical industry prior to application.



CHAPTER ONE

INTRODUCTION

1.1. Medicinal plants

The bioactive molecules derived from the natural products especially plants have served as the basis of new drug discovery. Plants have fulfilled both the nutritive and curative requirements of earthlings. Plants are a source of abundant molecules possessing biological activities (Cragg *et al.*, 2012). For drugs discovery, medicinal plants have great prospective of active compounds (Lee *et al.*, 2012). Some of the plant's secondary metabolites are not essential for their growth and reproduction but when administered by humans, exert multiple pharmacological effects (Rout *et al.*, 2009). Nature is the best pharmacy and has the potential to treat various ailments of human beings. Plant based medicines for the treatment of many complex ailments like inflammatory disorders, neoplasia, diabetes and oxidative-stress induced disorders are still the mainstay. About 80% of plant derived compounds were isolated on the basis of their ethnomedicinal use (Dias *et al.*, 2012).

Pakistan has a strong herbal medicine tradition. The people of Pakistan still depend on herbal medicine (Khattak *et al.*, 1985). Pakistan has an abundance of plant resources. The plants species that have been investigated for their medicinal values had huge variety. The medicinal values of these plants are very well understood by the common people. The use of herbal medicines is very active in Pakistan. There is very abundant number of the species of the medicinal plants. In Pakistan, there are approximately 456 plants used to treat various diseases (Sheikh and Husain, 2008). For common people, allopathic medicines are expensive so they use herbal medicines which are economical for them (Bhataria *et al.*, 2008). According to some of the previous studies it is found that from the plants antibiotics, antioxidants and anti-inflammatory agents are derived (Rahman *et al.*, 2012; Bukhari *et al.*, 2012). Currently available medicines such as aspirin and quinine are isolated from the plants (Fabricant and Norman, 2011).

According to World Health Organization (WHO) about 80% population uses herbal medicines in Asia and Africa for their health. The drugs we use currently against cancer are the derivative from the plants (Komalavalli *et al.*, 2014). Plants metabolites are known as the radical scavengers and the reducing agents (Nithya and Balakrishnan, 2011). There is an increase in toxicity level because of environmental pollution, urbanization and industrialization in Pakistan. In food chains heavy metals toxicity are most common (Shinwari, 2010). Due to the lack of knowledge about the herbs for the safety, people don't use plant medicines. They think that some

of the active compounds in medicinal plants could be toxic for their health (Husain *et al.*, 2011). In Gujrat district of Pakistan, plants found there have antioxidant potential (Rafique *et al.*, 2014).

About 101 plants species of 52 families, which are used for the treatment of digestive, blood circulatory, reproductive and urinary systems are used by the people of Western Himalayan region of Pakistan (Khan *et al.*, 2012). According to reports from Ethnobotanical information, about 800 plants have antidiabetic potential. A lot of plants show antidiabetic activity when they were assessed experimentally. Metformin, the hypoglycemic drug also derived from plants (Grover *et al.*, 2002). Around 80% of people in developing countries rely on Chinese, Ayurvedic, and Unani systems of medicine for their health (Tsay and Agrawal, 2005). For the cure of different diseases since ancient time, plants have been utilized. The medicinal uses of plants growing throughout the world lie in the active constituents having direct action in the body. They are used in conventional as well as herbal medicine. Hence mankind is provided with a valuable gift from nature in the form of herbal medicines (Saxena *et al.*, 2011).

Natural products including plants, microorganisms, marine products and animals have served as an incessant reservoir of therapeutic moieties. Enhanced pathogenic resistance, advent of complex anomalies, irrational treatment approaches and emergence of adverse effects of synthetic drugs has caused a dire requirement to explore new resources to rationalize therapeutic mainstream. Natural products are potential candidates for exploring their pharmacological potential. Undoubtedly, they have paved the path to the future treatment approaches. The bioactive molecules derived from the natural products especially plants have served as the basis of new drug discovery. Plants have fulfilled both the nutritive and curative requirements of earthlings. Plants are a source of abundant molecules possessing biological activities (Cragg *et al.*, 2012). Plants have provided a foundation for the traditional systems of medicines which came into existence several years ago and are still used as remedies. Conferring to World Health Organization (WHO) survey report, about 80% of the population of world still trusts on non-conventional medicines, particularly medicinal floras for their fitness issues (Bora and Sharma, 2011).

The extended past of the herbal medications is still present in many civilizations including China, India, Arab, North Africa, Southeast Asia and America (Central, South and North) (Zhang, 2000; Tapsell *et al.*, 2006). Recently, exploration on natural products have recaptured attention due to advances in screening programs along with growing understanding of

their natural implication, identification of their sources and structural assortments (Conforti *et al.*, 2012). About 50-60% of all drugs at present available for the remedial purpose in clinical world are either natural products, natural products-derived compounds, contain active natural products-derived pharmacophores or modified natural products attached to targeting systems due to their extraordinary effectiveness and minor side effects. These natural products are desirable replacements for chemical therapeutics which have various severe adverse effects (Patwardhan *et al.*, 2004).

A large population relies on traditional medicines in many developing countries to meet the health care needs but modern medicine may exist (Vishwakarma *et al.*, 2013). Traditional medicines are the most common used medicines in the world with the herbal part are the famous and are exceedingly beneficial in the global open market (Chaudhary and Singh, 2011). Natural products have played a major role in the treatment and prevention of human diseases. Natural product medicines are derived from specific plants, terrestrial microorganisms, vertebrates and invertebrates (Newman *et al.*, 2000).

1.2. *Melia azedarach*

Melia azedarach L. is a deciduous tree belonging to the genus Meliaceae. The name *Melia azedarach* derives from the word *Melia* for the 'manna or flowering ash' indicating the similarity between the tree's leaves and that plant and *azedarach* from the name of "azadaracht" poisonous plant (Sharma and Paul, 2013). *Melia azedarach* is an original South Asia species (China, India and Iran). *Melia azedarach* is now widely distributed in Pakistan, India, Indonesia, Australia, Brazil and many countries in African and the Arab world (Rubae, 2009). Common names of *Melia azedarach* are Dhraik, Bkain, Chinaberry and White Cedar. *Melia azedarach* contains alkaloids, carbohydrates, flavonoids, saponins, sterols, esters, tannins and triterpenoids (Khan *et al.*, 2001).

Melia azedarach attains a height upto 45 m tall. Upto 30-60, maximum 120 cm in diameter, when the old bole fluted below. They've got the crown spreading. They have legs that have sparsely branched. They grow like a tree on the ornamental avenue. It's hardy and draught resistant (Seth, 2004). It grows upto 2000 m above sea level in the sub-Himalayan region. Under the natural condition during the rain, it regenerates freely. It can be propagated artificially by cutting and direct sowing. Bark is greenish-brown and smooth when young. With the age, it turns grey and fissured. Alternate leaves, 20-40 cm long. The leaflets are 3-11 pale below and the

upper surface is dark green and they produce a pungent odour when crushed. There's a long Inflorescence. The panicle of the axillary is upto 20 cm long. The flowers are violet. Sepals are five-lobed and 1 cm long. Each of the five-lobed petals. Five-lobed petals 9 cm long. The end tube is deep blue brown purple, zero point 6 cm long. Fruits are small and yellow drupe, nearly 15 mm in diameter. As a rock, they are smooth and hard and contain 4-5 black seeds. The seeds are smooth and brown and are oblongoid, 3.5 mm x 1.6 mm around the pulp (Rubae, 2009; Sultana *et al.*, 2011).

Melia azedarach barks are used to relieve thirst stomach pain, vomiting, nausea, and loss of appetite in fever (Rahmatullah *et al.*, 2010). Bark decoction is used to treat fever and pain (Kokwaro, 2009). Bark paste is used to treat piles and used as ulcer lotion (Sen *et al.*, 2010). Infusion of the stem bark is used to treat gonorrhea, malaria and to remove the parasitic worms (Dharani *et al.*, 2010). *Melia azedarach* roots are acidic, antiseptic, astringent, anthelmintic, depurative, constipating, vulnerary, expectorant, febrifuge and antiperiodic (Sen *et al.*, 2010). The leaves are highly nutritious and used as fodder. They are used in skin diseases like scabies, rheumatic pain, fever, for brushing teeth and insecticide (Rahmatullah *et al.*, 2010). *Melia azedarach* leaves are used on burns externally. They are used for cleaning the mouth, bloody piles and pyrexia (Khan *et al.*, 2011). Cure pimples, hysteria and diabetes (Sultana *et al.*, 2011).

Leaves are used for anemia, measles, jaundice, malaria treatment and the expulsion of parasite worms. Decoction is used as both astringent and as a stomach. The fruits of *Melia azedarach* are used for the preparation of tonic (Sen *et al.*, 2010). The fruits are sweet, eaten by the children and strongly vermifuge (Cropley and Haseqawa, 2007). For the treatment of diabetes pericarps fruit is used as external parasiticide (Sultana *et al.*, 2011). Flowers are effective in children against bacterial skin diseases including cellulitis and pyogenic infections (Rahmatullah *et al.*, 2010). The seeds are bitter and anthelmintic in typhoid fever, pain and helminthiasis (Sen *et al.*, 2010). Seed oil was used as antiseptic for sores and ulcers and to treat skin diseases e.g. ring worm and useful internally for malaria fever and leprosy (Khan *et al.*, 2011).

1.3. *Justicia adhatoda / Adhatoda vasica*

Justicia adhatoda L, an evergreen shrub belongs to Acanthaceae family. It is widely used in medicine preparation. It is a rare sub-herbaceous small evergreen bush distributed in Pakistan, India, Sri Lanka, Burma and Malaysia. Common names are Vasaka, Malabarnut, Adusa, Ardusi,

Bhekkar, Adhatodai and Basak (Prajapati *et al.*, 2003; Bjaj and Williams, 1995). *Justicia adhatoda* contains alkaloids, anthraquninone, flavonoids, saponins, phytosterols, triterpenoids and polyphenols (Jayapriya and Shoba, 2015).

Justicia adhatoda is a thick shrub (1.2-2.4 m), arborescent at times, 6 m. They are strong with uplifting branches opposite. The bark of the stem is yellowish. The leaves are 10-20 by 9-8 cm. When mature it's glabrous. It's dark green above, pale below. The main nerves have 10-12 pairs between them with reticulated venation. The length of petioles is 1-2.5 cm long. The flowers have 2-8 cm thick pedunculate axillary spikes. They are at the end of the branches long. The peduncles measure 3-10 cm in length. The stout of the leaves are shorter. The bracts of 0.5-1.2 cm to 1-2 cm. The subacute elliptic is glabrous. 5-7 nerved, closely reticulately veined. The bracteoles are 1.5-2 by 0.3-0.4 mm. The oblonglanceolate is acute on the margins of ciliolate. The length of the calyx is 1.3 cm and glabrous. The sepals are imbricating, oblong and sharp. Corolla is white, 2.5-3 cm in the throat with pink-coloured bars. It's outside long and pubescent. The length of the tube is 1-2 cm. The lower section is cylindrical and the upper section is filled. The upper lip length is 2/1.3 cm. The lower lip is two by1.3 cm long. The lobes are1.3 cm, rounded and lengthy. The lobe in the middle is wide. The filaments at the base are hairy and long. The ovary is pubescent. Capsules are 1.5-2 by 0.6-0.8 cm. The seeds are 5-6 mm long, orbicular-oblong and glabrous (Dhale and Kalme, 2012).

For curative effects all parts of the *Justicia adhatoda* has been used (Atal, 1980). The European medical practitioners also used this plant. The fluid extract of *Justicia adhatoda* was used in England. It is classified as a natural remedy in Sweden (Farnlof, 1998). The leaves are used for cuts, skin diseases, migraine, haemorrahge and leprosy in Southeast Asia (Adnan *et al.*, 2010). The leaves are used for cough (Lal and Yadav, 1983) and for asthma (Shah and Joshi, 1971). For haemorrahge and urinary problem, the leaves are used (Pushpangadan *et al.*, 1995). To stop the bleeding, earaches and the pus from ears, the leaves powder were used (Reddy *et al.*, 1989), to treat jaundice (Reddy *et al.*, 1988), asthma and tuberculosis (Jain and Puri, 1984).

The roots extract of *Justicia adhatoda* were used against the diabetes and disorders of the liver (Bhat *et al.*, 1978). The roots were used In the Southeast Asia to cure malaria, tuberculosis and eye diseases (Kirtikar and Basu, 1975) and gonorrhea (Siddiqui and Hussain, 1993). In South-East Asia, flowers are used for opthalmia and used for the treatment of fever, gonorrhea, cough, antispasmodic, cold, phthisis, asthma and bronchitis. The flowers of *Justicia adhatoda*

are used to improve the blood circulation (Atta-ur-Rahman *et al.*, 1986). The fruit are used for the prevention of flu, bronchitis, yellowing, fever, and diarrhea (Kirtikar and Basu, 1975).

1.4. *Ricinus communis*

Ricinus communis L. is the member of the Euphorbiaceae family. It is popularly known as castor plant. It is commonly known as 'palm of Christ', Arand, Vatari, Erando, Gida, Avanakku, Errand and Bherenda. As an ornamental plant it is widespread throughout the tropical regions (Maman and Yehezkelli, 2005). *Ricinus communis* contains carbohydrates, alkaloids, phenols, proteins and tannins (Khursheed *et al.*, 2012; Shabir *et al.*, 2011).

Ricinus communis is a shrub that grows rapidly and soft wooded. It's upto 6 m small tree. It is cultivated for leaf and flower colors. It is also cultivated for oil production. The leaves are curved, alternate and cylindrical. They have purplish petioles. The stipules are large and yellow. They are united into a cap. They are deciduous, 6-8 inches across. They have acute, coarsely serrate segments. When young they are smooth blue green and red and shining. The flowers are large and arranged on the thick rachis. Spicate panicle is beneath it. The male flowers are shortly stalked. The female flowers in the upper part are sessile. The bracts are triangular. The fruit is greenish. They are deeply-grooved and they are less than an inch long. The ovary is sharp and spreading spines. They have 3-celled and septicidally into six valves. The seeds are ovoid and flattened. Seeds are ⅝ inch long. Seeds are ¼ broad. Seeds are smooth and pinkish grey. Caruncle is large and subglobular. They are running down the centre of the ventral surface. The embryo is large in axis. It is broadly ovate and veined. The roots are light in weight. The roots are straight. Roots have few rootlets. The outer surface is yellowish brown. It is smooth (Bentley and Trimen, 2007).

There are many uses of castor oil e.g. an isolated compound Ricin has been used in bioterrorism. *Ricinus communis* L. originated in Asia and Africa and now found in the Europe and America (Olsnes *et al.*, 1974). Castor oil is produced worldwide in large quantities. A simple salting procedure will easily remove the toxin that remains in the castor meal after extracting the oil with hexane (David *et al.*, 2007). In different aspects of life, there are different uses of *Ricinus communis*. The Tons of the commercial oil were refined and used for different purposes afterwards. The plant's oil can be used as oiled fabrics, patent leather, paints, enamels and varnishes, fly-paper, typewriting and printing inks. The leaves are recommended to the women for their breasts because secretion of milk improves (Bentley and Trimen, 2007).

1.5. Extraction

The extraction requires separating medicinally active sections of plant or animal tissue from inactive components through the use of specific solvents in the standard extraction procedures. The extract was then separated to separate the individual chemicals. Specific extraction methods include (a) maceration, (b) decoction, (c) percolation, (d) infusion, (e) digestion, (f) hot continuous extraction, (g) extraction by ultrasound, (h) supercritical fluid extraction, (i) aqueous alcoholic extraction and (j) counter-current extraction (Williamson *et al.*, 1996). High solubility in phytoconstituents may be due to differences in extract yields in the plant based on their varied chemical composition (Khan *et al.*, 2015).

1.6. Phytochemical analysis

Phytochemical testing is a plant drug qualitative chemical evaluation that indicates the presence of chemical constituent's levels. The chemical analysis of the plant medicine results in the proper identification of biologically active groups (Gokhale and Kokate, 1997). Phytochemicals are produced and stored in the form of tannins, saponins, glycosides, lignin, phenols, alkaloids and flavonoids as secondary metabolites. Against the spasm and bacterial effects, purely isolated alkaloids are used (Okwu, 2005). Medicinal plants have therapeutic value possess biologically active components (Harsha *et al.*, 2002). The cardiac glycosides, saponins, volatile oils, steroids, tannins, phenols and flavonoids possess good antibacterial activity (Ahmad *et al.*, 1998; Shariff, 2001). The polyphenols and flavonoids have strong activity against bacteria (Meng *et al.*, 2001; Hideyuki *et al.*, 2002).

1.7. Antioxidant activity

It is believed that oxidative damage to cells caused by free radicals may act as a crucial factor in normal process of aging as well as in the pathogenesis of several clinical disorders (Apel and Hirt, 2004; Tang *et al.*, 2004). It has been estimated that these reactive species can cause almost 10,000 oxidative attacks per day on a single human cell (Dizdaroglu *et al.*, 2002; Li and Jackson, 2002; Marnett, 2000). Search for new free radical scavengers from natural resources is in demand owing to side effects of synthetic antioxidants like butylated hydroxytoluene (Krishnaiah *et al.*, 2007). Various assays, which determine antioxidant activities of different natural products have been developed and performed to evaluate complete spectrum of antioxidant mechanism (Yildirim *et al.*, 2000).

In total reducing power assay, Fe^{3+} ion is converted into Fe^{2+} ion resulting in development of Perl's Prussian blue colored complex. This change of color is then detected by visible spectrophotometry. It is used to determine antioxidant potential stiochiometrically relative to Fe^{2+} concentration (Bursal and Koksal, 2011). In total antioxidant capacity determination, molybdenum (VI) is reduced to molybdenum (V) which occurs at low pH as a green complex (Huang *et al.*, 2005). In DPPH free radical scavenging assay, a purple colored stable nitrogen free radical is employed. The violet chromogenic radical is reduced to a yellowish brown compound called hydrazine in the presence of antioxidant agent. This method is cost effective for the estimation of antioxidant potential (Ihtisham *et al.*, 2013).

1.8. Cytotoxicity assay

The brine shrimp lethality assay (BSLA) is in routine use for the primary screening of the isolated compounds as well as extracts for their toxicity assessment towards brine shrimp which indicates their potential cytotoxic properties. Pharmacology is simply toxicology at a lower dose, and toxicology is plainly pharmacology at a higher dose. Bioactive compounds in higher doses are nearly always toxic. (Michael *et al.*, 1956) proposed the shrimp lethality assay which was later developed by (Vanhaecke *et al.*, 1981; Sleet and Brendel, 1983). The ability to kill laboratory-cultured *Artemia nauplii* brine shrimp is the basis of this assay.

The brine shrimp cytotoxicity assay was considered as a suitable probe for preliminary evaluation of toxicity, pesticides, heavy metals, detection of fungal toxins and cytotoxicity testing of dental materials (Meyer *et al.*, 1982). It can also be extrapolated for antitumor activity and cell-line toxicity (Selvin *et al.*, 2004). Brine shrimp eggs are used as fish food and are commercially available. Several bioassay systems have utilized brine shrimp nauplii including the analyses of mycotoxins, pesticidal residues, stream pollutants, dinoflagellate toxins, anesthetics, morphine-like compounds, carcinogenicity of toxicants and phorbol esters in marine environment. Thus, screening and fractionation in discovery and monitoring of bioactive natural products is conveniently done by *in vivo* lethality in a simple organism. The synthetic compounds and natural products evaluation by using brine shrimp cytotoxicity assay not only describes cytotoxicity but also antiviral, anticancer, pesticidal and insecticidal potential (Sheikh *et al.*, 2004). This bioassay has led to the isolation of numerous novel pesticidal and antitumor natural products (Meyer *et al.*, 1982; McLaughlin *et al.*, 1991; Sam, 1993).

1.9. Antidiabetic activity

Diabetes mellitus is a multi-factor disease. It has a significant impact on the quality of life and health. 230 million people worldwide suffer from diabetes (Arumugan *et al.*, 2008). Diabetes is caused by damage to Langerhan islets cells. This mechanism makes the body produce the insulin called pancreatic hypoglycemic hormone. There are three major symptoms of Diabetes: excess urine, urinary sugar and high blood glucose (Koffi *et al.*, 2009). The metabolism of glucose and lipids is changed because of diabetes (Rajasekaran *et al.*, 2006). The changes in the lipid metabolism were demonstrated experimentally (Sochar *et al.*, 1985).

Diabetes mellitus has two main types. Type one is common in the children. Type two is common in the adults (Tripathi, 2003). Throughout the world, the number of the diabetic patients increases. In 2025, in young population, USA and China will be more prevalent for this disease. 382 million people were affected due to diabetes in 2013. The prevalence will increase to 552 million people in 2035 (Hing *et al.*, 2011). In 2014 in adults, nine percent of the diabetes was diagnosed. The deaths occurred due to diabetes were 1.5 million in 2012 (WHO, 2016). At present 7.1 million people are affected due to the diabetes in Pakistan and the ratio exceed to 11.4 million in 2030. Proper treatment is very necessary to decrease the complications in diabetic individuals (Ismail *et al.*, 2010). Medicines derived from plants provide a valuable alternative for diabetes management (Ugwuja *et al.*, 2010). Over 800 plants currently have antidiabetic potential (Patel *et al.*, 2012).

1.10. Antibacterial activity

Currently antimicrobial resistance is on top among serious health intimidations due to infections resulting from resistant bacteria. Such types of infections are mostly prevalent in susceptible patients experiencing cancer chemotherapy, dialysis due to renal failure, and surgery; principally organ transplantation for which the recipient capability to combat subsidiary infections is very essential. The causes of antibiotic resistance are complex. The need of the hour is to develop new and novel antibiotics. Medicinal plants have been in use since century for treating different ailments. The universality and usefulness of traditional medicine/medicinal herbs are obvious from their persistent use by a substantial portion of the world's inhabitants (Gilani *et al.*, 2010; Caceres *et al.*, 1995; Shinwari and Qaisar, 2011).

Number of infectious diseases has increased due to increasing incidences of opportunistic infections and emergence of antibiotics resistance. The discovery of new antimicrobial agents for pathogenic infections control has increased the demand. Existing antimicrobial remedies using folklore medicinal plants may provide strong basis for antimicrobial potential determination of plant extracts and phytochemicals (Taylor *et al.*, 2001). Antimicrobial drugs derived from plants have been included as mainstream antimicrobials owing to ineffectiveness of traditional antibiotics (Ncube *et al.*, 2008). Many natural-products chemists believe that the various potentially useful phytochemical structures can be synthesized chemically before they could be lost permanently due to species extinction. Various sensitive bioassay techniques are in continuous phase of development for the exploration of antimicrobial potential of plants. These bioassays must be simple, reproducible, specific and sensitive to detect even little quantities of antimicrobial compounds. In modern research, antimicrobial susceptibility test (AST) is considered to be an important technique to determine antimicrobial efficiency of biological extracts and isolated compounds against various pathogens (Das *et al.*, 2010).

Agar diffusion techniques most commonly assess antimicrobial capability of plant extracts; however, unable to differentiate between bactericidal and bacteriostatic effects (Nasir *et al.*, 2015). Antimicrobial potential of the plants is perhaps due to high percentage of phenolics as both are always linked together (Ravikumar *et al.*, 2009). The antimicrobial effects of flavonoids and tannins are present in the extracts (Abo *et al.*, 1999). *Staphylococcus aureus* is known to inhibit flavonoids and used as a therapy for the inflamed tissues (Ali *et al.*, 1996). The alkaloids which are derived from plants are usually found to have antimicrobial potential (Omulokoli *et al.*, 1997). Alkaloids may be helpful against AIDS associated intestinal infections (McDevitt *et al.*, 1996) as well as HIV infection (Sethi, 1979).

1.11. Antifungal activity

The synthetic antimicrobials are frequently coupled with side effects, whereas the plant based antimicrobials having good therapeutic potential with less adverse effects (Fair and Tor, 2014). The fungi are destroyers of food material. For human consumption, they are unfit (Amrouche *et al.*, 2011). Therefore, there is a requirement to constantly explore plant derived antimicrobials. Detailed investigation is desired to identify and resolve the full scale of efficiency of the antimicrobial compounds from these plants. Antimicrobial drugs derived from plants have been included as mainstream antimicrobials owing to ineffectiveness of traditional antibiotics

(Ncube *et al.*, 2008). Many natural-products chemists believe that the various potentially useful phytochemical structures can be synthesized chemically before they could be lost permanently due to species extinction (Das *et al.*, 2010).

Persistent opportunistic fungal infections have turned out to be a main factor for mortality and morbidity in immunocompromised patients (Bodey and Anaissie, 1989). *Candida* and *Aspergillus* species cause the common fungal infections. Recent trends have pointed out a shift towards infection (Groll *et al.*, 1996). Secondary metabolites possesses antifungal activity include the flavonoids and phenols of various plants (Quiroga *et al.*, 2001). Due to the presence of phenols, many investigations accredited the preventive outcome of plant extracts against microorganisms (Baydar *et al.*, 2004; Rodriguez *et al.*, 2007). There are antifungal properties of saponins (Mohanta *et al.*, 2007) and the polyphenolic compounds (Negri *et al.*, 2014).

1.12. Anticancer activity

In most countries of the world, cancer is the major cause of death. There are number of factors that influence it including diet, life style and carcinogens (Key *et al.*, 2004). Cancer is considered as one of the most prevalent ailments in the world. According to World Health Organization (WHO), more than 10 million cases of cancer new cases are reported per year worldwide (Center *et al.*, 2011). The underlying etiology of cancer, a debilitating and multipronged disorder, involves processes like epigenetic mechanisms, mutations and carcinogens etc. Prolonged inflammatory responses in the body may also initiate cancer due to production of certain chemotaxins responsible for genetic alterations. This disease has three stages: initiation, promotion, and progression (Kandouz and Batist, 2010).

Delaying or preventing the transformation of normal cells into malignant ones is an important therapeutic strategy. In this aspect, use of natural products, synthetic compounds or biological agents for the prevention and suppression of disease progression and reversal of abnormal physiological functions is in common practice. This approach is named as cancer chemoprevention (Brenner and Gescher, 2005). The MTT assay is used to determine natural product's cytotoxic capacity and synthetic compounds on various primary cells and established cancer cell lines. This method was first devised in 1983 and has gained stupendous importance in cancer research (Van-Meerloo *et al.*, 2011).

There is a continuous mitochondrial activity in metabolically active cells. This is correlated specifically with the number of live cells. To study the drug effects against different

cell lines, MTT assay had been utilized (Van-Meerloo *et al.*, 2011; Bruggisser *et al.*, 2002). To study the mammalian cell cycle, different assays have been designed. Flow cytometry has a powerful tool in the analysis of cell cycle distribution within the cell populations. Measuring fluorescence intensity using the flow cytometer enables a quantitative analysis of cell populations in different phases (Pozarowski and Darzynkiewicz, 2004).

1.13. Elemental analysis

Due to their pharmacological efficacy, which depends on their elemental concentrations, medicinal plants are effective against various diseases. Phytochemicals such as primary and secondary metabolites are produced by various combinations of major, minor and trace elements that play a curative and preventive role in most of the hazardous diseases. To assess the efficacy and functioning of drugs prepared from medicinal plants that play an important role in curing the various diseases, quantitative analysis of different elemental concentrations is necessary. Elements are known to possess profound influence in the regulation of glucose-tolerance, maintenance of the cardiac rhythms, functions of nerves and muscles, hormone regulation, blood clotting and cellular mortality (Jimoh and Oladiji, 2005).

1.14. GC-MS analysis

In the plants, the active compounds are studied using various analytical and extraction methods (Iordache *et al.*, 2009). GC-MS is an ideal method to analyze volatile and semi-volatile compounds. It is made by combining the optimal technique of separation (Gas Chromatography) with the best technique for detection (Mass Spectrometry). GC-MS is one of the best methods to assess the bioactive components of esters, acids, alcohols, long chains and branched hydrocarbons (Palawat and Lodha, 2014).

1.15. Objectives of the study

The present work was undertaken with the following objectives;

- To collect and identify the ethno botanically important medicinal plants, reported for their various biological activities from Pothohar region of Pakistan.
- To prepare the leaf extracts of *Melia azedarach, Justicia adhatoda* and *Ricinus communis* using different solvents for further studies.
- To study the qualitative and quantitative phytochemical and elemental analysis of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis.*
- To evaluate the *in vitro* antioxidant, cytotoxic, antibacterial, antifungal and anticancer activities of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis.*
- To evaluate the *in vivo* antidiabetic activity of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis.*
- Profiling of the extracts of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* through GC-MS.

CHAPTER TWO

REVIEW OF LITERATURE

The products made from the plants play an essential part in the health care sectors of the developed countries. Approximately 70-80% populations of these countries are using some form of folk medicine. Traditional medicines are the most common used medicines in the world with the herbal part are the famous and are exceedingly beneficial in the global open market (Chaudhary and Singh, 2011). Plants have provided a foundation for the traditional systems of medicines which came into existence several years ago and are still used as remedies. Conferring to World Health Organization (WHO) survey report, about 80% of the population of world still trusts on non-conventional medicines, particularly medicinal floras for their fitness issues (Bora and Sharma, 2011). For the cure of different diseases since ancient time, plants have been utilized. The medicinal uses of plants growing throughout the world lie in the active constituents having direct action in the body. They are used in conventional as well as herbal medicine. Hence mankind is provided with a valuable gift from nature in the form of herbal drugs (Saxena *et al.*, 2011).

2.1. Phytochemical analysis

Melia azedarach extract contains terpenoids, flavonoids, phenols, glycosides, alkaloids, tannins, saponins and steroids (Ahmed *et al.*, 2012a). *Melia azedarach* leaves extract contains tannins, saponins, alkaloids, Phenols, terpenoids, flavonoids glycosides and steroids (Rao *et al.*, 2012). Variety of compounds, including phytosterols, diterpene, triterpenes, terpene alcohol, flavonoid, alkane hydrocarbon, nalkanoic acid and vitamin E, found in *Melia azedarach* (Sen and Batra, 2012b). *Melia azedarach* extract contains saponins, phenols, alkaloids, tannins and flavonoids (Sultana *et al.*, 2013). Different extracts of *Melia azedarach* contains alkaloids, phenols, flavonoids, tannins, glycosides, fixed oil, carbohydrates, protein, amino acids and fats (Sumathi, 2013). *Melia azedarach* extract contains alkaloids, flavonoids, tannins, glycosides and saponins (Hossain *et al.*, 2013).

Melia azedarach extract contains alkaloids, saponins, tannins, flavonoids, terpins and steroids (Suad and Majeed, 2013). *Melia azedarach* extract contains alkaloids, flavonoids, tannins, glycosides and saponins (Asadujjaman *et al.*, 2013). Ethanolic and water extracts of *Melia azedarach* contains flavanoids, reducing sugars, alkaloids and carbohydrates (Krishnaiah and Prashanth, 2014). *Melia azedarach* extract contains tannins, phylobatannins, saponins, polyphenols, flavonoids, steroids, alkaloids, carbohydrates, glycosides, terpenoids, triterpenoids and proteins (Leela *et al.*, 2016). *Melia azedarach* extract contains phenols, tannins, saponins,

cardioactive glycosides, alkaloids, phenols, glycosides, anthraquninone glycosides, cynogenic glycosides and flavonoids (Abbas *et al.*, 2017). Phytoconstituents like saponins and tannins, carbohydrates, flavonoids, proteins etc were present in *Melia azedarach* (Babu *et al.*, 2018).

Leaves of *Adhatoda vasica* possess tannins, oils, fats, phytosterol, carbohydrate, alkaloids, flavanoids, saponins, phenolic compounds and proteins (Vankata *et al.*, 2013). Leaves of *Adhatoda vasica* possess saponins, flavanoids, amino acids, phenols, tannins, alkaloids, anthraquninone and reducing sugars (Karthikeyan *et al.*, 2014). Ethanolic leaf extract of *Adhatoda vasica* contains saponins, phenols, steroids alkaloids, flavonoids and terpenoids (Bajpaia *et al.*, 2015). *Justicia adhatoda* extract contains saponins, phytosterols, triterpenoids, alkaloids, anthraquninone, flavonoids and polyphenols (Jayapriya and Shoba, 2015). *Justicia adhatoda* extract contains anthraquninone, saponins, flavonoids, phenols, tannins, alkaloids, amino acids and reducing sugars (Desai and Patel, 2015). *Justicia adhatoda* extract contains alkaloids, glycosides, steroidal/triterpenes, resin and saponins (Abhishek *et al.*, 2014).

Ricinus communis extract contains proteins, tannins, carbohydrates, alkaloids and phenols (Khursheed *et al.*, 2012; Shabir *et al.*, 2011). *Ricinus communis* phytochemical testing contains phytate, oxalate, saponins, cynogenic glycoside, tannin, phenol, alkaloid and flavonoid (Momoh *et al.*, 2012). *Ricinus communis* contains a variety of phytochemicals like terpenoids, glycosides, reducing sugars, saponins, tannins, flavonoids and phenols (Minakshi *et al.*, 2016). *Ricinus communis* seeds contain phytochemicals like phytate, tannins, saponins, flavonoids, alkaloid, phenol and steroids (Ezenobi *et al.*, 2016).

2.2. Antioxidant activity

To assess the antioxidant activity, the different parts of *Melia azedarach* with different extracts were evaluated. In the different parts of *Melia azedarach* extracts, total phenolic and flavonoid content were calculated. The total phenolic content range was 74.43-112.10 mg GAE/g DW. The total flavonoid range was 13.32-28.11 mg CE/g DW. The total phenolic content range was 66.89-103.34 mg GAE/g DW in the ambient dried extracts and the total flavonoid range was 10.67-23.45 mg CE/g DW. The DPPH scavenging activity of sun dried extracts of *Melia azedarach* range was 55.43-63.86% and 35.57-52.11%. The range of 48.54-61.00% and 33.87-50.33% respectively in the dried form. In the sun dried extracts, higher antioxidant activity of *Melia azedarach* was found. The stem bark has stronger antioxidant activity among the different plant parts of *Melia azedarach* (Munir *et al.*, 2012).

The TPC and the antioxidant activity of MA-EE were evaluated using the DPPH assay. The result showed that the MA-EE contains the highest phenolic content. The MA-EE has the strong antioxidant activity. The high scavenging activity of extract was due to the phenolic compounds (Ahmed *et al.*, 2012b). *Melia azedarach* leaves extract have strong antioxidant activity. The leaf extracts of *Melia azedarach* using the DPPH shows the free radical scavenging properties. The leaves extract of *Melia azedarach* displayed free radical scavenging activity which is comparable to ascorbic acid (Hossain *et al.*, 2013).

The antioxidant activity of leaves of *Melia azedarach* was evaluated. The MA-EE demonstrated antioxidant activity (Asadujjaman *et al.*, 2013). The dose-dependent study of *Melia azedarach* was evaluated. The ethanol extract of *Melia azedarach* possess significant radical scavenging activity. *Melia azedarach* IC_{50} values are (a) hydroxyl radical (26.500.26 mg/ml), (b) superoxide anion (30.000.32 mg/ml) (c) nitric oxide radical (48.000.48 mg/ml) (d) DPPH radical (30.550.32 mg/ml) and (e) reducing power (22.000.22 mg/ml). This study shows that *Melia azedarach* has as an effective antioxidant (Marimuthu *et al.*, 2013). Antioxidant activity of *Melia azedarach* was performed. The result showed that green fruits have the antioxidant potential when compared with the ripe fruits against the ascorbic acid. The total phenolic content of green fruits expressed as 10.54 mg/g DW. The ripe fruits have 5.32 mg/g DW. This result shows that green fruits have the more active compounds when compared with the ripe fruits (Khan *et al.*, 2014).

The flowers of MA-EE showed the good antioxidant activity and methanol, ethyl acetate, n-butanol and dichloromethane extracts does not show the good antioxidant activity (Abbas *et al.*, 2017). The TPC and the antioxidant capacity of *Melia azedarach* leaves were determined. *Melia azedarach* young leaves have the highest TPC. They have the strongest scavenging activity against the DPPH and showed the highest oxygen radical absorbance capacity. The highest metal chelating activity was found in the immature pulp extract. We concluded from these results that *Melia azedarach* serve as an excellent source of natural antioxidants. So, *Melia azedarach* could be used for the pharmaceutical applications (Rabbet *et al.*, 2017).

Biological activities were performed by antioxidant (a) DPPH (b) H_2O_2 and (c) Nitric oxide and (d) Free radical scavenging methods. The *Melia azedarach* species of the different assay of antioxidant activity were performed. The MA-ME have higher concentration and possess more antioxidant potential than MA-CE and MA-HE when compare to ascorbic acid.

The extracts of leaves of *Melia azedarach* exhibited strong antioxidant (a) DPPH (b) H_2O_2 (c) Nitric oxide and radical scavenging activities with MA-ME, MA-CE and MA-HE respectively. The antioxidant activity of MA-ME was due to the flavonoids and phenols which are present in it. The methanol and chloroform extracts have the strong antioxidant potential (Babu *et al.*, 2018).

Vasicine was isolated from *Justicia adhatoda*. DPPH assay, FRAP assay, Acetylcholine esterase and Trypsin were evaluated. The results showed that the acetylcholine has 38.4 ± 1.2% activity and the trypsin inhibition assays have 37.4 ± 1.1% activities. Vasicine possess the good DPPH inhibition activity. In the FRAP assay, a dose dependent behavior of Vasicine was indicated (Shahwar *et al.*, 2012). Antioxidant activity of *Adhatoda vasica* methanolic extract was estimated by DPPH assay, TAC, TRP and iron chelating activity. The extracts of *Adhatoda vasica* showed the high antioxidant activities in all the antioxidant assays. The extract of *Adhatoda vasica* showed the levels of phenolics and flavonoids (Vankata *et al.*, 2013). By FRAP assay, the antioxidant activity of *Justicia adhatoda* were evaluated. The *Justicia adhatoda* antioxidant activity was found to be 0.794 mM Fe (II)/L (Giri *et al.*, 2014).

Justicia adhatoda ethanolic extract have showed good antioxidant capacity. The inhibition of the DPPH radical was 69.23%. *Justicia adhatoda* ethanolic extract had the inhibitory effects on hydroxyl radicals, superoxide and scavenging nitric oxide. This result shows the biological efficacy of leaf extract of *Justicia adhatoda* (Bajpaia *et al.*, 2015). Seed extracts of *Ricinus communis* contain antioxidant activity and it shows that it can be useful in disease treatment. The reason was that at very low concentration, it shows the high antioxidant activity. The chemical compounds (a) 12-octadecadienoic acid, methyl ester, and (b) methyl ricinoleate produces antioxidant activity. The stem and leaf extracts of *Ricinus communis* contain antioxidant activity due to the presence of the flavonoids (Gupta *et al.*, 2006).

Gallic acid, quercetin and rutin are the major phenolic compounds and they are responsible for the antioxidant activity. All these major phenolic compounds were isolated from *Ricinus communis* leaves. Indole-3-acetic acid has been extracted from the *Ricinus communis* roots (Singh *et al.*, 2009). *Ricinus communis* extract shows the maximum antioxidant properties for Quercitin and N-dimethyl ricinine. To reduce the oxidative stress in the Jaundice condition, the identified compound can serve as antioxidant compound (Jolly and Shetye, 2017). The TFC and TPC of RC-AE were determined. In *Ricinus communis* root extract, total phenolic content

obtained was 29.2 mg/g. Total flavonoid content obtained was 6 mg/gm in *Ricinus communis* root extract. This result shows that both the extracts of *Ricinus communis* contain the important bioactive compounds (Yadav and Agarwala, 2011).

2.3. Cytotoxicity assay

The study was carried out to compare the potential of *Melia azedarach* green and ripen fruits aqueous extract against brine shrimp cytotoxicity assay. The cytotoxic assay was performed at different concentrations. Green aqueous extract of the plant fruits showed significant activity when compared to the ripe fruits ($P < 0.05$).The LC_{50} value for green and ripen fruits is 18.07 and 530.2 µg/ml, respectively. For positive control, Doxorubicin was used. The LC_{50} value for Doxorubicin was 5.93 µg/ml. The degree of cytotoxicity level was observed which depend on the concentration of the drug used. The mortality rate of brine shrimp is concentration dependent. As the concentration of the plant extract increases, the percentage survival decreases. The brine shrimp activity indicates cytotoxicity as well as leads to further pharmacological activities (Apu *et al.*, 2013).

The extract of *Melia azedarach* was screened for *in vitro* cytotoxic activity by brine shrimp lethality test. *Melia azedarach* revealed a moderate toxicity with 383.58 µg/ml (Sharma and Kharel, 2019). Brine shrimp lethality test (BSLT) guided fractionation of a methanol extract of the roots of *Melia azedarach* resulted in the isolation of two new limonoids: 9α-hydroxy-12α-acetoxyfraxinellone and 7,14-epoxy-azedarachin B, together with the known compounds: 12α-hydroxyfraxinellone, 9α-hydroxyfraxinellone, azedarachin B and neoazedarachin B. The structures of two new limonoids were elucidated by analysis of spectroscopic data and comparison of their NMR data with those of the known compounds. All the compounds exhibited significant activity in the BST, in particular, azedarachin B showed remarkable BST activity with an LC_{50} value of 0.0098 µM (Fukuyama *et al.*, 2006).

The root of *Justicia adhatoda* was extracted with organic solvent and the extracts were used for the observation of cytotoxic activity. Crude extracts (n-hexane, ethyl acetate and chloroform soluble fraction) of *Justicia adhatoda*, were screened for cytotoxic activity using brine shrimp lethality bioassay. A reputed cytotoxic agent vincristine sulphate was used as a positive control. From the results of the brine shrimp lethality bioassay it can be well predicted that n-hexane, ethyl acetate and chloroform soluble fraction of methanolic crude extracts possess cytotoxic principles (with LC_{50} 1.129 µg/ml, LC_{50} 1.402 µg/ml and LC_{50} 2.130 µg/ml

respectively) comparison with positive control vincristine sulphate (with LC_{50} 0.563 µg/ml) (Meskat and Hussain, 2012).

Cytotoxicity was evaluated by brine shrimp assay. *Ricinus communis* seeds, stem, leaves, fruit and root methanolic extracts showed mild to moderate cytotoxicity against red blood cells (RBCs) of human and bovine. Brine shrimp lethality also revealed the cytotoxic nature of extracts with LC_{50} in the range of 0.22-3.70 (µg/mL) (Shaking), 1.59-60.92 (µg/mL) (Sonication) and 0.72-33.60 (µg/mL) (Soxhlet), whereas LC_{90} values were in the range of 345.42-1695.81, 660.50-14,794.40 and 641.62-15,047.80 µg/mL for Shaking, Sonication and Soxhlet extraction methods, respectively (Abbas *et al.*, 2018).

2.4. Antidiabetic activity

The sample includes *Melia azedarach's* aerial parts. Two groups of Streptozotocin induced diabetic rats were treated orally with Glibenclamide and Polyherbal extract. The blood glucose level, body weight, lipid profile, hemoglobin and liver glycogen after the 21 days of treatment were measured. Polyherbal extract and the standard drug (glibenclamide) were significant in reducing the blood glucose level, lipid profile and hemoglobin. Both the treatments increased the body weight and liver glycogen. In addition to the antidiabetic activity, polyherbal extract possess antihyperlipidemic activity (Appalaraju *et al.*, 2014). Ethanolic flower extract of *Melia azedarach* was evaluated to determine the anti-hyperglycemic effect. For fifteen days, the test drugs were administered. Blood sugar levels were determined at different days. After the administration of ethanolic flower extract of *Melia azedarach* in Streptozotocin induced diabetic animals, the blood sugar level reduces. So it was therefore concluded that MA-EE has antidiabetic activity (Asokan *et al.*, 2015).

Melia azedarach extract was intraperitoneally injected to mice. By using the tail vein sampling for four hours, plasma glucose was studied. The chronic experiment was studied for 21 days. By using the intraperitoneal glucose tolerance test for 120 mins, the glucose tolerance was studied. By the gastric retention of a radioactive marker for 20 mins, gastric emptying of a metabolically inert meal was studied. The *Melia azedarach* extracts display antidiabetic effects in mice similar to the glibenclamide. At the end of twenty-one days, administration of the *Melia azedarach* extract for long term reduced the glucose and insulin levels. The glucose tolerance test showed that glucose levels were reduced by the *Melia azedarach* extract. At the

dose of 400 mg/kg, *Melia azedarach* extract slow down the gastric emptying rate of normal and diabetic rats. The *Melia azedarach* leaf extract shows good antidiabetic activity. This reduces blood glucose levels and improves the disposal of glucose and reduces the demand for insulin (Seifu *et al.*, 2017).

The effects of leaves and roots of *Justicia adhatoda* was studied in alloxan induced diabetic rats. Oral administration of JA-EE to healthy and experimental diabetic rats reduces the levels of blood glucose from day two to sixth day of treatment. Significant results on glucose tolerance, haemoglobin, lipid profiles and body weight of experimental animals were observed (Gulfraz *et al.*, 2011). Diabetic rats were treated with the JA-EE for six weeks. Learning and memory was investigated during the fifth week of treatment. Biochemical parameters were assessed at the end of the study from the brain's cerebral cortex and hippocampus regions. In the diabetic rat brain, 70% in the cerebral cortex, AchE activity was increased. Lipid peroxidation levels have been increased by 100% in the cerebral cortex of diabetic rats. Lipid peroxidation levels have been increased by 94% in the hippocampus of diabetic rats. In the cerebral cortex and hippocampus regions of the brain, Nonprotein thiol levels, catalase and superoxide dismutase enzymatic activities were decreased. Nitrite levels in the cerebral cortex region and hippocampus regions of the brain of rats were increased to 170% and 137% respectively. In the diabetic rats, TNF-α was increased. The ethanolic extract of *adhatoda vasica* leaves attenuated the biochemical and behavioral abnormalities. The results show *Justicia adhatoda's* protective role against diabetic rats which confirms its glucose lowering and antioxidant action (Mohan *et al.*, 2014).

Ricinus communis ethanolic root extract has been tested for antidiabetic activity. This study used a dosage of 500 mg/kg body weight. This allows both healthy and type I diabetic animals to lower the fasting blood glucose. The maximum hypoglycemic effect was observed at the 8^{th} hour. The RC-RE dose to the diabetic rats showed good results in fasting blood glucose after 20 days. It showed good results on 10^{th} and 20^{th} day on the lipid profile, kidney and liver functions. Using the silica gel column chromatography, RC-RE was purified. Several different fractions of *Ricinus communis* ethanolic root extract were tested. The antidiabetic activity was shown only by the one fraction of the *Ricinus communis* ethanolic root extract. After the administration of the extract, the serum bilirubin, creatinine, SGOT, SGPT, total protein and

alkaline phosphatase did not show significant differences. To develop a powerful phytomedicine, *Ricinus communis* have a promising value (Shokeen *et al.*, 2008).

2.5. Antibacterial activity

The five extracts of *Melia azedarach* under the five different concentrations were tested. Extracts of methanol, water and ethyl acetate showed significant inhibition of the bacteria tested (Khan *et al.*, 2008). The antibacterial activity of the oil was tested against the gram positive and gram negative bacterial strains. The essential oil was sensitive to all the microorganisms (Sarkera *et al.*, 2011). To evaluate the antibacterial efficacy, disk diffusion method was followed. Against all the tested pathogens, all seed extracts have significant antibacterial activity. The ethyl acetate extract has the maximum inhibition area among all the extracts. *Melia azedarach* seed extracts could be effective antibiotics to combat both gram positive and negative human infections (Khan *et al.*, 2011). The antibacterial activity of the *Melia azedarach* leaf extracts were examined using different solvents. All extracts showed strong activity against all bacterial stains. The alcoholic extract demonstrated the highest inhibition area against all microorganisms. The zone of inhibition was minimum in petroleum ether and water extract. *Melia azedarach* alcoholic extract is a great source of new and active herbal medicines for the treatment of infections (Sen and Batra, 2012a).

All the seed, fruits, leaves and flowers of *Melia azedarach* extracts have strong antibacterial activity against all bacterial strains. In each of the factors, the size of the growth inhibition zone showed the significant difference. In forming the growth inhibitory zone, there was a significant difference between them (Neycee *et al.*, 2012). The seed oil of *Melia azedarach* has the best zone of inhibition for *Staphylococcus aureus*, *Enterobactor areogenes* and *Escherichia coli* with 30 mm, 25 mm and 23 mm respectively. The leaves EO have antibacterial effect on *Staphylococcus aureus*, *Enterobactor areogenes* and *Escherichia coli* with a diameter of 21 mm, 20 mm and 15 mm respectively. The flower EO has antibacterial effect and the inhibition area ranges from 17 mm to 19 mm (Meziane and Goumri, 2014).

Bioactive compounds of *Melia azedarach* were evaluated. For all treatments the inhibition zone ranged from 5.6 mm to 1.96 mm (Marzoqi *et al.*, 2015). The leaf extract of *Melia azedarach* solvated by ethanol and methanol extracts were subjected to test their antibacterial activity. They showed the spectrum of inhibition for the flavonoids on the *Escherichia coli* and the *Klebsiella pneaumonia* and for the alkaloids on the *Escherichia coli* and the *Staphylococcus*

aureus. For human body, leaves of *Melia azedarach*, a potential source of nutrition, minerals and useful drugs (Leela *et al.*, 2016). The antibacterial activity *Melia azedarach* against *Escherichia coli*, *Enterococcus faecalis* and *Bacillus subtilis* bacteria was evaluated. All the extracts showed significant antibacterial activity (Akacha *et al.*, 2016).

Justicia adhatoda leaf extracts have been tested for antibacterial activity. With the standard antibiotics (ciprofloxacin and ofloxacin) for bacteria, sensitivity of the bacteria was determined. The alkaloids in the extracts have been effective against the bacteria. The lowest minimum inhibitory and minimum microbicidal concentrations of *Justicia adhatoda* leaf extracts were determined against the *Pseudomonas aeruginosa*, *Escherichia coli* and *Serratia marcescens*. The highest minimum inhibitory concentration of *Justicia adhatoda* leaf extracts was determined against the *Streptococcus pyogenes*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. The leaf extracts of *Justicia adhatoda* possess good antibacterial activity. The leaf extracts of *Justicia adhatoda* is a potential source of antimicrobial agents. So this plant is useful for chemotherapy. It is also useful to control the infectious diseases (Pa and Mathew, 2012).

The effect of different extracts of *Justicia adhatoda* was tested against various bacterial strains. All the extracts showed significant antibacterial activity (Karthikeyan *et al.*, 2014). All *Justicia adhatoda* extracts display strong antibacterial activity against all bacterial strains. *Proteus vulgaris* and *Pseudomonas aeruginosa* did not show antibacterial activity (Jayapriya and Shoba, 2015). Antibacterial assay of *Justicia adhatoda* leaves was carried out using different strains of bacteria cup plate method. Ethanolic extract was effective against all the bacterial strains. Mixed effect observed in remaining two extracts (Desai and Patel, 2015). The plant's ethanol extracts display growth inhibitory effect against various bacterial strains. All extracts were more resistant to *Pseudomonas aeruginosa* (Batool *et al.*, 2017). *Ricinus communis* ethanolic leaves extract were studied against different bacterial strains. The *Ricinus communis* leaf extract showed strong activity against different bacterial strains when compared with Streptomycin sulphate (Shabir *et al.*, 2011; Khursheed *et al.*, 2012).

The antibacterial efficiency of *Ricinus communis* essential oil seeds has been assessed. The bacteria were found to be susceptible. MIC of the *Ricinus communis* extract ranged from 6.25 mg/ml to 12.50 mg/ml for bacteria. All the extracts of *Ricinus communis* were more potent against the bacterial strains (Momoh *et al.*, 2012). *Ricinus communis* oil, leaf, stems and seed extract have been screened for pathogenic bacteria. *Ricinus communis* methanol and ethanol

extracts display the highest inhibition area against the gram-positive and gram-negative bacteria. Whole parts of plant are effective in antibacterial activity assay (More *et al.*, 2014). As they are essential source of antimicrobial agents against pathogens, *Ricinus communis* was analyzed for their antibacterial activity. Against the *Escherichia coli*, the *Ricinus communis* leaves stem and roots extract showed strong antibacterial activity (Minakshi *et al.*, 2016). *Ricinus communis* show good antibacterial activity against all the bacterial strains. *Ricinus communis* seed has strong antibacterial activity (Al-Mamun *et al.*, 2016).

2.6. Antifungal activity

Melia azedarach leaf extracts have been tested for antifungal activity against the various human pathogenic fungi. All the *Melia azedarach* leaf extracts showed the significant activity. The MA-ME and MA-EE showed the maximum inhibition area. In the petroleum ether and water extract, minimum inhibition zone was determined. The MA-ME and MA-EE was a source of new and active herbal medicines for the treatment the infections (Sen and Batra, 2012a). Different concentrations of methanolic fruit extract were prepared. The MA-ME decreases the biomass of the fungus. A concentration of 3.125 mg/ml of different fractions decreases the fungal biomass. The extract of MA-ME fruit extract has a strong antifungal activity (Khan and Javaid, 2013).

The seed oil of *Melia azedarach* is the most active because it has the maximum zone of inhibition for fungal strains tested. The maximum zone of inhibition was obtained from *Candida albicans*. The 75%, 70% and 75% of zone of inhibition was obtained from EO leaves, flowers and seeds respectively. EO of leaves inhibited over 70% of the growth of *Fusarium oxysporum* and *Candida albicans*. The EO of flower shows growth inhibition of 70% for *Candida albicans* and 50% for *Fusarium oxysporum* (Meziane and Goumri, 2014). The extracts of *Melia azedarach* were found to be more effective against the fungal strains. This result suggests that *Melia azedarach* leaves are potential nutritional and mineral sources. It is useful drug for human body (Leela *et al.*, 2016).

The fungi-toxic efficacy of the extracts was tested against the yeast fungi (*Candida tropicalis*, *Cryptococcus marinus*, *Candida albicans* and *Candida krusei*) and mycelia fungi (*Aspergillus niger* and *Rhizopus oryzae*). The study revealed that n-hexane extract exhibited high inhibition against *Candida krusei*, whereas *Candida albicans* and *Candida tropicalis* showed no response and *Cryptococcus marinus* responded moderately. The methanolic extract

showed high inhibition against *Candida krusei*, whereas *Candida albicans* and *Candida tropicalis* showed no response and moderate effect against *Cryptococcus marinus*. The n-hexane and methanolic extracts of this plant showed good zone of restriction against the *Rhizopus oryzae* than the *Aspergillus niger*. Clotrimazole and Fluconazole and was taken as reference antifungal. The result clearly indicated that bark of *Melia azedarach* L. contains phytochemicals which are responsible for inhibition of fungal growth and manifestation (Khatoon *et al.*, 2016).

The extracts of *Justicia adhatoda* were tested against the fungus through the disc diffusion method. The four extracts of *Justicia adhatoda* were evaluated for their potential antifungal activity. All the extracts of *Justicia adhatoda* show the antifungal activities against all the organisms. The results provide the scientific evidence in treating the microbial diseases. The leaf extracts of *Justicia adhatoda* can be used to produce the alternative forms of antimicrobials (Jayapriya and Shoba, 2015). The study was conducted to identify the *Melia azedarach's* antifungal activity against pathogenic fungi. All the extracts of *Melia azedarach* showed the significant antifungal activity (Akacha *et al.*, 2016). The ethanolic flowers extract of *Melia azedarach* demonstrated the highest antifungal activity against the *Aspergillus flavus*. The minimum antifungal activity demonstrated against the *Aspergillus niger* by n-butanol extract. The MA-ME, MA-DME show good minimum inhibitory concentration against the *Aspergillus flavus*. *Melia azedarach* leaf extracts possess good antifungal activity. To reduce the problems of fungal pathogens, *Melia azedarach* have the potential (Abbas *et al.*, 2017).

The antifungal activity of *Justicia adhatoda* leaf extracts was evaluated. 25 µg/ml concentration was used to check the antifungal activity of *Justicia adhatoda* extracts and Vasicine. Minimum inhibitory and microbicidal concentrations were determined against all the fungal strains. The alkaloids in the *Justicia adhatoda* extracts were active against the fungal strains. The highest minimum inhibitory concentration was shown by *Cryptococcus neoformans* and *Candida albicans*. *Aspergillus flavus* showed the lowest minimum inhibitory concentration. *Justicia adhatoda*'s leaf extracts have strong antifungal activity and is a potential source of antimicrobial agents. So this plant is useful for chemotherapy. It is also useful to control the infectious diseases (Pa and Mathew, 2012).

Against the *Aspergillus niger*, *Ricinus communis* leaves extract showed significant antifungal activity. The moderate activity was observed in *Ricinus communis* leaves extract

against the *Rhizopus syphilis* and *Aspergillus flavus*. In the *Ricinus communis* ethanolic extract, the highest yield was found (Khursheed *et al.*, 2012; Shabir *et al.*, 2011). The antifungal efficiency of *Ricinus communis* seed essential oil has been evaluated. Six fungi were used in the bioassay. For fungal strains, the MIC of the *Ricinus communis* seed extract ranged from 12.50 mg/ml to 25 mg/ml (Momoh *et al.*, 2012).

2.7. Anticancer activity

The anticancer activity of MA-EE bark was evaluated. The MA-EE has shown the highest cytotoxicity in the human colon cancer cell (HCT-15). In the MTT assay, *Melia azedarach* inhibited the proliferation of HCT-15 cells. Hollow fiber assay was carried out by using the HCT-15 and SK-Hep1 via intraperitoneal and subcutaneous site and A549 human adenocarcinoma cell. The highest cytotoxicity was shown by the SK-Hep1 implanted in the intraperitoneal region. The result of the metastatic experiment using the mice was same as the hollow fiber assay. The ethanol extract of *Melia azedarach* might have a potent anticancer activity (Kim and Kang, 2009).

The twenty-eight days oral toxicity and anticancer activity of *Melia azedarach* bark extract were investigated using the HF assay. An A549 human adenocarcinoma cell was used to conduct the hollow fiber assay. The 4 mg/kg body weight of cisplatin group and 200 mg/kg body weight of hexane layer displayed the highest cytotoxicity against the A549 carcinoma cells. The 200 and150 mg/kg body weight hexane layer was given to six groups of ten male and female mice. No changes in the body weight were found in the oral toxicity study in mice hexane layer. The group treated with cisplatin decreases the body weight. The biochemical study showed an increase in bilirubin, ALT, AST, creatinine and BUN levels in groups treated with cisplatin. Cisplatin induces the reduction of white blood cells and neutrophils. The treatment with the hexane layer improved the toxicities caused by the cisplatin. This confirms that the hexane layer possess anticancer activity (Kim and Kang, 2012).

Ricinus communis chloroform leaves extract has been cytotoxic to human tumour cell lines. In the SK-MEL-28 human melanoma cells, apoptosis was induced at the concentration of 20 µg/ml. The loss of the potential of the mitochondrial membrane and the translocation of phosphatidyl serine to the surface of the cell membranes were found. It provides additional insight into the potential use of terpenoids mixtures. Terpenoids exists in the form of cancer cells as inducers of apoptosis (Darmanin *et al.*, 2009). *Foeniculum vulgare's* ethanol extract showed

significant inhibition of the proliferation of cancer cells. *Justicia adhatoda's* methanol extract also showed significant inhibition of cancer cells (Batool *et al.*, 2017).

A maximum of 24 mice containing the Ehrlich's ascites carcinoma cells were treated for six days. Mice were treated at 50 and 100 µg/ml/d/mouse with *Ricinus communis* crude protein. *Ricinus communis* seed protein growth inhibitory activity on the Ehrlich's ascites carcinoma cells was determined. Apoptotic cells are tested using DAPI staining. The castor varieties protein concentration ranged from 21 to 35 mg/ml. The molecular weight ranges from 14-200 k Da. Administration of seed protein to the mice, the growth inhibitions of EAC cells resulted in 54% growth. The DAPI staining shows the marked apoptosis characteristics including the aggregation of apoptotic bodies, condensation of cytoplasm and nuclear fragmentation. These results shows that *Ricinus communis* seed protein possess the strong anticancer activity (Al-Mamun *et al.*, 2016).

2.8. Elemental analysis

By the AAS and Titration method, the Elemental analysis of *Melia azedarach* oil shows that it contains Ca, Mg, K, Zn, Mn, Fe and P. This work might be useful for developing the applications for *Melia azedarach* seed oil (Bachheti *et al.*, 2012). Several plants of Pakistan have potential therapeutic value. These plants are used in the country for their traditional herbal medicinal system. Elemental analysis of *Justicia adhatoda* was studied by using the atomic absorption spectrophotometer to find the K, Na, Mg Cu, Ni, Zn, Mn, Cr, Cd, Co and Fe (Ghani *et al.*, 2016). Calcium was maximum in the *Justicia adhatoda*. Calcium is a rich source of important minerals. Calcium will help in the hypertension and osteoporosis. Potassium in the leaves is effective in blood pressure and constipation (Dastagir *et al.*, 2017). Using the standard analysis, *Ricinus communis* minerals and vitamin composition were determined. Most of the important minerals i.e. calcium, magnesium, sodium, phosphorus and iron were present. The vitamins are also present in *Ricinus communis* (Ezenobi *et al.*, 2016).

2.9. GC-MS analysis

The major fatty acids present in *Melia azedarach* seed oil are Linoleic acid (74.57%), Oleic acid (16.39%), Palmitic acid (5.68%) and Stearic acid (3.33%) (Bachheti *et al.*, 2012). There are 13 phytochemical bioactive compounds in the MA-ME. The compounds present in the *Melia azedarach* extract are 9,12,15-octadecatrienoate, Butanedioic acid, diethyl ester,

Dichloromethane, Propanedioic acid, diethyl ester, Octadecane, Dithiocarbamate, Hexadecanoic acid and Y-Sitosterol (Marzoqi *et al.*, 2015). There are eleven compounds were identified using the GC-MS and abundant compounds of essential oil of *Melia azedarach* were Borneol (58.60%), Bicycle (14.56%), 1,1,4a-trimethyl-5,6-dimethylenedecahydro naphthalene (5.28%), Ethanonaphthalene (2.82%), Caryophyllene oxide (2.35%), Alphacaryophyllene (1.95%), 1,2,3, trimethyl benzene (1.51%), Cycloproplejazulene (1.48%) and 2-naphthalenemethanol (1.46%) (Sarkera *et al.*, 2011).

To study the phytochemical profile using Gas Chromatography-Mass Spectrometry method. *Justicia adhatoda* leaf extracts were prepared in methanolic solvent. GC-MS analysis of JA-ME contains the major peaks: Amrinone, Phytol, N-Hexadecanoic acid and 9,12,15-Octadecatrienoic acid, (Z,Z,Z) (Jayapriya and Shoba, 2015). GC-MS analysis of *Justicia adhatoda* contains the major peaks: Phytol (57.8%), N-hentriacontane (3.92%), Nonacosane (3.65%), Pentacosane (2.65%), β-Eudesmol (1.14%) and Heneicosane (1.13%) (Shukla *et al.*, 2017). The GC-MS analysis of *Ricinus communis* essential oil contains α-thujone (31.71%), 1, 8-cineole (30.98%), α pinene (16.88%), Camphor (12.92%) and Camphene (7.48%). The coat of castor bean contains Lupeol and 30-Norlupan3β-ol-20-one (Malcolm *et al.*, 1968).

The *Ricinus communis* leaves extract contains the alkaloids: ricinine (0.55%) and N-demethylricinine (0.016%) and flavones: quercetin-3-O-β-D-xylopyranoside, quercetin3-O-β-D-glucopyranoside, kaempferol-3-O-βrutinoside and quercetin-3-O-β-rutinoside (Kang *et al.*, 1985). There are eight major compounds of *Ricinus communis* are present. The major eight compounds are 8-Octadecenal, Pregn-5-ene-3,11-dione, Aminoacetamide, Pregna-3,5-dien-9-ol-20 one, 3-(N,N dimethyllaurylammonio) propanesulfonate, 2-hydroxy-ethyl ester, 2-Methoxy-4-vinylphenol, 1,2- Cyclopentanedicarboxylic acid, 1H-Purin2-amine, Glycyl-D-asparagine, Propiolic acid, 3-(1-hydroxy-2-isopropyl-5-methylcyclohexyl) and Phytol (Hussein *et al.*, 2016).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Collection and Identification

During the month of October 2015 and again during the month of March 2017, fresh leaves of the *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* were collected from Murree Hills, Punjab province, Pakistan. The plant samples was identified and authenticated by Dr. Ibrar Shinwari, Department of Environmental Sciences, International Islamic University Islamabad and Dr. Muhammad Zafar, Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan. Voucher sample of the plants acquired (*Melia azedarach* with accession number 129781, *Justicia adhatoda* with accession number 129782 and *Ricinus communis* with accession number 129783) was submitted to the Herbarium of Pakistan, Quaid-i-Azam University Islamabad for future reference.

3.2. Preparation of extracts

Under the running water, the plants were washed thoroughly. At ambient temperature, they are shade dried for three weeks. The dried part of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* was pulverized into the powder. All the plants are stored in the airtight containers for further use. In the 2000 ml flask, 200 g of powdered plant was macerated at room temperature for 24 h using 1000 ml analytical grade solvents. The extracts were concentrated in the rotary evaporator by vacuum evaporation in order to obtain the final crude extract. After that, the extracts were dried at 40°C in the vacuum oven. All the plants dried extracts are collected in an air-tight jar and kept at 4°C until further analysis.

Figure 3.1: ***Melia azedarach*** **leaves**

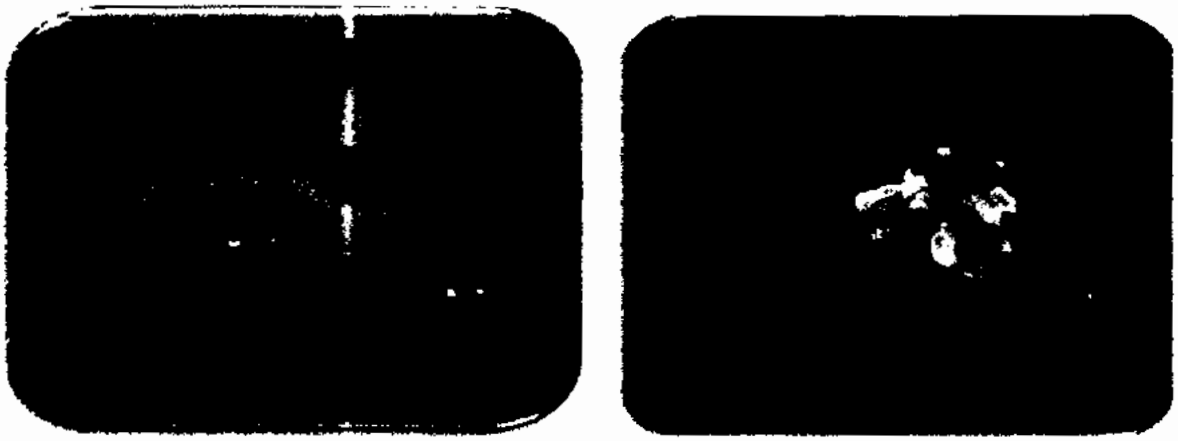


Figure 3.2: ***Justicia adhatoda*** **leaves**

Figure 3.3: ***Ricinus communis*** **leaves**

3.3. Phytochemical analysis

3.3.1. Qualitative analysis

The stock solution of all the crude extracts was prepared in dimethyl sulfoxide for the qualitative phytochemical analysis (Trease and Evans, 1989).

3.3.1.1. Test for tannins

With 10 ml of distilled water, approximately 0.5 g of each plant extract was stirred. The extract was then purified. A few drops of 1% of ferric chloride solution have been added to the filtrate's 2 ml. The presence of tannins in the extracts indicates the presence of a blue-black or blue green precipitate (Trease and Evans, 1989).

3.3.1.2. Test for steroids

In 0.2 g of each plant extract, 2 ml of acetic acid was added. The ice cooled the solution. Then the concentrated H_2SO_4 was added. The change of colour from violet to blue or bluish green demonstrates the presence of steroids in extracts (Trease and Evans, 1989).

3.3.1.3. Test for terpenoids

A small quantity of each plant extract in ethanol was dissolved. After this, was added 1 ml of the acetic anhydride and concentrated H_2SO_4. The presence of terpenoids in the extracts was shown by a colour change from pink to violet (Trease and Evans, 1989).

3.3.1.4. Test for saponins

1 g of each plant extract was boiled with 5 ml of distilled water and then filtered. Then added 3 ml of distilled water and shake vigorously for 5 minutes. Frothing indicates the presence in the extracts of saponins (Trease and Evans, 1989).

3.3.1.5. Test for flavonoids

Approximately 0.5 g of the plant extract was dissolved in ethanol. It was warmed after that, and then filtered. The filtrate was added to the three pieces of magnesium chips and added the few drops of concentrated hydrochloric acid. A pink, orange or red that has been changed to violet indicates the presence of flavonoids in the extracts (Trease and Evans, 1989).

3.3.1.6. Test for alkaloids

Using 5 ml of aqueous hydrochloric acid on the water bath, each plant extract of 0.2 g was stirred and then filtered. 1 ml was drawn separately from the filtrate into the two test tubes.

A few drops of Dragendorff's reagent have been added in the first portion. Orange-red precipitate occurrence was taken as positive. Mayer's reagent was added to the second portion of 1 ml. The precipitate in buff color shows the presence of alkaloids in the extracts (Trease and Evans, 1989).

3.3.1.7. Test for Anthraquinone

With the benzene layer, about 0.5 g of each plant extract has been shaken. Half of its own 10% ammonia solution volume was added. The presence of anthraquinone in the extracts is indicated by a pink, red or violet color in the ammoniacal phase (Trease and Evans, 1989).

3.3.1.8. Test for Phenols

Approximately 500 mg of extract in the 5 ml of distilled water was dissolved. Few drops have been added from the 5% ferric chloride solution. A dark green color shows phenols in the extracts (Trease and Evans, 1989).

3.3.1.9. Test for coumarins

With 10% of 1 ml of NaOH, 1 ml of each plant sample was reacted. The yellow color indicates that the extracts contain coumarins (Trease and Evans, 1989).

3.3.2. Quantitative analysis

The following summarized procedures quantified the total phenolic as well as flavonoid content.

3.3.2.1. Estimation of total phenolic content (TPC)

In the 96-well plate, 20 µl was transferred from the stock solution of each test sample. Then add the Folin-Ciocalteu reagent's 90 µl. For 5 mins, the plate was incubated. The reaction mixture has been added to 90 µl of Na_2CO_3. In estimating the total phenolic content, gallic acid was used as the standard. Using the microplate reader, the absorbance was taken at 630 nm of each reaction mixture. As the positive control, the gallic acid was used. The results are expressed in estimating the total phenolic content as µg gallic acid equivalent per mg dry extract (Fatima *et al.*, 2015).

3.3.2.2. Estimation of total flavonoid content (TFC)

All the plants crude extracts were transferred to each well of 96-well plate. Added the 10 µl of each 10% $AlCl_3$ and $C_2H_3KO_2$ and afterwards add 160 µl distilled water. The resulting mixture was stored at room temperature for 30 minutes. The plate absorbance was measured at

415 nm using the microplate reader. The calibration curve was drawn at the final concentrations of (6.25-100 µg/ml) using the quercetin standard. The results in the estimation of total flavonoid content are expressed as µg quercetin equivalent per mg dry extract (Sahreen *et al.*, 2013).

3.4. Antioxidant activity

Different antioxidant assays were performed in the current study to evaluate the antioxidant potential of the various plant extracts.

3.4.1. DPPH free radical scavenging activity (FRSA)

In the 96 well plates, 20 µl of various dilutions were mixed with the 180 µl of DPPH solution (9.2 mg/100 ml methanol). Using the microplate reader, the plate absorbance was measured at 517 nm. After that, the plate is incubated at 37°C for 30 mins. Percent free radical scavenging activity was calculated by using the formula: $\%FRSA = (1-Ab_s/Ab_c) \times 100$. Ab_s is the test sample absorbance. Ab_c is the absorbance of dimethyl sulfoxide (negative control). Ascorbic acid was used as the positive control. The assay was carried out in the triplicate (Haq *et al.*, 2012).

3.4.2. Total antioxidant capacity (TAC)

Phosphomolybdenum assay was used to assess the total capacity of antioxidants. It is expressed as microgram ascorbic acid equivalent per milligram dry weight of the plant (µg AAE/mg DW). There was a mixture of about 0.1 ml of each plant extract and ascorbic acid with 0.9 ml of reagent (0.6 M H_2SO_4, 4 mM $(NH_4)_2MoO_4$ solution in H_2O and 28 mM sodium phosphate). The blank contain the 0.9 ml of reagent solution and then about 0.1 ml of the dimethyl sulfoxide without the plant extract. After that put all the tubes in the water bath at 95°C for 90 mins. Afterwards, cool the tubes at room temperature. About 200 µl was transferred from each plant sample to the 96 well plates. Using the microplate reader, the plate absorbance was measured at 630 nm. The assay was carried out in the triplicate (Jafri *et al.*, 2014).

3.4.3. Total reducing power (TRP)

To estimate the reducing power of different plant extracts, Potassium ferricyanide colorimetric assay was performed. 200 µl of each extract was mixed with 400 µl of each phosphate buffer (0.2 moles/liter and PH 6.6) and 1% potassium ferricyanide in H_2O. The mixture was incubated at 50°C for 20 mins. Add the 400 µl of 10% $C_2HCl_3O_2$ in H_2O. At room temperature, centrifuged at 3000 rpm for 10 mins. The upper layer of solution was then diluted in

H_2O with the distilled water and 100 µl of 0.1% of $FeCl_3$. From this mixture, 200 µl was transferred to 96 well plates. Using the microplate reader, the plate absorbance was measured at 630 nm. The blank was prepared by adding dimethyl sulfoxide 200 µl instead of extract to the reaction mixture. The reducing power of each sample is expressed as microgram ascorbic acid equivalent per milligram dry weight (µg AAE/mg DW). The assay was carried out in the triplicate (Jafri *et al.*, 2014).

3.5. Cytotoxicity assay

The cytotoxic potential in *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* leaf extracts was estimated through brine shrimp lethality test (Apu *et al.*, 2013) with some modifications. At first, the stock solution of samples was formulated (100mg crude extracts/ml DMSO). Then 1000 µg/ml, 100 µg/ml and 10 µg/ml dilutions were formulated. A shallow rectangular tray (22x32 cm) already was filled with simulated sea water (38g sea salt/L of distilled water) in which brine shrimp eggs hatched. The tray was consisting of two portions, one of which was large and the other was small. Each portion was separated from the other through a wall consisting of many holes. In the large portion of the tray brine shrimp's eggs were spread on the surface of simulated sea water. After spreading the brine shrimp's eggs, the large portion of the tray was concealed with the aluminum foil. The surface of smaller portion was lighted up with a lamp and hatching started after 24-26 h. The newly brine shrimp larvae (nauplii) traveled towards the light portion. These nauplii were taken from the tank and shifted to the beaker by Pasteur pipette.

100 µl of sample solution with four concentrations (1000, 100, and 10 µg) was poured into respective well of 96- well plate, followed by addition of 200 µl of the simulated sea water and mixed carefully. Ten shrimps were counted under a 3x magnifying glass and then transferred to each well with the help of Pasteur pipette and 300 µl volume was maintained for each well to accomplish the required concentration of crude sample. The microplates containing shrimps were incubated at room temperature for 24 h. Then shrimps were taken out from the well with Pasteur pipette and survivor rate was counted under a 3x magnifying glass with 3x resolution against a background that is lighted. The values of LD_{50} (sample concentration required to kill 50% of shrimps) were calculated by using Table curve 2D version 4.

3.6. Antidiabetic activity

3.6.1. Experimental animals

BALB/c male mice were raised in the animal house of National Institute of Health, Islamabad. The weights were between 25-35 g, eight weeks old. The animals were kept in polypropylene cages. With a light/dark cycle of 12 h/12 h, we put 6 mice per cage at 25°C. The pellets and water were given to the animals throughout the experiment. Animal handling was conducted according to internationally accepted ethical guidelines. The research protocol was endorsed by the International Islamic University's Institutional Review Board, Islamabad (Letter No. IIU (BI&BT)/FBAS-IBBC-05).

3.6.2. Acute toxicity test

The various extracts of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* were tested for acute toxicity. To determine the acute toxicity of the extract, the guidelines 425 advocated by the Organization for Economic Cooperation and Development (OECD) were followed (OECD, 2001). For this purpose BALB/C male mice (n = 6) were orally administered at various doses of the extract (250, 500, 1000, 2000, 3000 and 5000 mg/kg) par oral, whereas saline was administered to the control mice's. The animals were examined once daily for 14 days for mortality, behavioral pattern (lethargy, sleep and salivation), changes in physical appearance, injury, pain, and signs of illness (Burger *et al.*, 2005).

3.6.3. Induction of diabetes

BALB/c male mice were fasted for 12-14 h. The fasting blood glucose levels were recorded by glucometer. After that the mice were made diabetic with alloxan monohydrate. Alloxan monohydrate was prepared prior to injection by weighing the individual weight of the animal. Thirty minutes after the administration of alloxan, food and water was given to the mice (Carvalho *et al.*, 2003). The blood glucose level of each mouse has been determined from the tail after 48 h of alloxan injection. In this study, mice above 200 mg/dl with fast blood glucose were included (Gidado *et al.*, 2005).

3.6.4. Experimental design

Fifty-four 25-35 g BALB/c male mice were housed in the aluminum cages. Water and standard chow supply ad libitum were given to the mice. Mice were acclimatized in normal

laboratory circumstances for one week (25 ± 1°C temperature, 12 h light/dark cycle and 50-60% relative humidity) prior to the start of the experiment. All the mice were randomly distributed in nine groups of six members each. Thrice a week on the alternate days, the samples were given from day one to the twenty-eighth days to the respective groups. Experimental mice were split into the groups as shown in the Table 3.1.

Table 3.1: Experimental design of antidiabetic activity of alloxan induced diabetic mice

Groups	Treatments	Description
Group 1	Normal Control	Received normal saline (0.9 % NaCl)
Group 2	Diabetic Control	Received 150 mg/kg body weight of Alloxan monohybrate
Group 3	Positive Control	Administered 10 mg/kg body weight of Glibenclamide
Group 4	MA-ME 200	Administered 200 mg/kg body weight of *Melia azedarach* (methanolic extract)
Group 5	MA-ME 400	Administered 400 mg/kg body weight of *Melia azedarach* (methanolic extract)
Group 6	MA-EE 200	Administered 200 mg/kg body weight of *Melia azedarach* (ethanolic extract)
Group 7	MA-EE 400	Administered 400 mg/kg body weight of *Melia azedarach* (ethanolic extract)
Group 8	MA-EAE 200	Administered 200 mg/kg body weight of *Melia azedarach* (ethylacetate extract)
Group 9	MA-EAE 400	Administered 400 mg/kg body weight of *Melia azedarach* (ethyl acetate extract)
Group 10	JA-ME 200	Administered 200 mg/kg body weight of *Justicia adhatoda* (methanolic extract)
Group 11	JA-ME 400	Administered 400 mg/kg body weight of *Justicia adhatoda* (methanolic extract)
Group 12	JA-EE 200	Administered 200 mg/kg body weight of *Justicia adhatoda* (ethanolic extract)
Group 13	JA-EE 400	Administered 400 mg/kg body weight of *Justicia adhatoda* (ethanolic extract)
Group 14	JA-EAE 200	Administered 200 mg/kg body weight of *Justicia adhatoda* (ethylacetate extract)
Group 15	JA-EAE 400	Administered 400 mg/kg body weight of *Justicia adhatoda* (ethylacetate extract)
Group 16	RC-ME 200	Administered 200 mg/kg body weight of *Ricinus communis* (methanolic extract)
Group 17	RC-ME 400	Administered 400 mg/kg body weight of *Ricinus communis* (methanolic extract)
Group 18	RC-EE 200	Administered 200 mg/kg body weight of *Ricinus communis* (ethanolic extract)
Group 19	RC-EE 400	Administered 400 mg/kg body weight of *Ricinus communis* (ethanolic extract)
Group 20	RC-EAE 200	Administered 200 mg/kg body weight of *Ricinus communis* (ethylacetate extract)
Group 21	RC-EAE 400	Administered 400 mg/kg body weight of *Ricinus communis* (ethylacetate extract)

3.6.5. Oral glucose tolerance test

The mice were fasted twelve hours on the test day. They were given a dose of 5 g/kg of glucose orally. The blood glucose concentrations were 0 (before glucose injection) and 2, 4, 6 and 8 h after glucose administration. Blood samples from the tail were collected (Kumar *et al.*, 2006).

3.6.6. Assessment of body weight

After 72 h, the body weights were measured after the mice were confirmed as diabetic. After the twenty-eight days, the mice's body weights were measured using the digital weighing balance.

3.6.7. Collection of blood sample

On the last day of the experiment i.e. twenty-eight days, the mice were anaesthetized by chloroform inhalation. Blood samples were collected through the abdominal aorta in the tubes under anesthesia for biochemical investigations. After centrifuge, the blood samples and serums were separated at 6000 rpm for 15 mins. The serums were analyzed for biochemical investigations.

3.6.8. Determination of biochemical parameters

The blood samples were permitted to coagulate at 25°C for 45 mins. The serum was centrifuged for 15 minutes at 6000 rpm. Serum replicates were analyzed for ALT, AST, ALP, bilirubin, urea, creatinine and uric acid, using the AMP diagnostic kits. The concentrations of TC, TG, HDL, LDL and VLDL were enzymatically measured using kits (Shah *et al.*, 2013).

3.7. Antibacterial activity

The extracts of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* were tested against the *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus auerus*. Agar well diffusion method was used for the antibacterial activity of various extracts. Strains of each bacterium were made in 0.75 ml broth culture. This comprise of 160 colony forming units (CFU) per ml. To compare the turbidity of test bacterial strains, McFarland's ($BaSO_4$) turbidity was used as a standard. Above from the surface, nutrient agar media was poured in the sterile petri plates. After that the media was left for the solidification. Then make the wells of 6 mm with the help of a sterile metallic borer. Sterile cotton swabs were used for the

swabbing of plates to ensure the growth of the particular strain on the plate. Dilutions of extracts were made in analytical methanol. The concentrations of the extracts were 1000, 500 and 100 µg/ml. Streptomycin sulphate was used as the positive control. For the single bacterium test strain, triplicate plates were prepared. Finally, the plates were incubated at 37°C for 24 h. The diameter of the inhibition zones was taken as the measure of the antibacterial activity around each well. Each experiment was carried out in triplicate. After that the mean diameter of the inhibition zone was recorded (Rios *et al.*, 1988).

3.8. Antifungal activity

Antifungal activity of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* extracts was tested against the *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum* and *Candida albicans*. Sabouraud dextrose agar (SDA) (5ml) was poured into screw caped test tube and was autoclaved for 15 minutes at 121°C. Tubes were cooled and dilutions of extracts were made in analytical methanol followed by the serial dilution method and the concentrations of extracts were 1000, 500, and 100 µg/ml, added in that non-solidified media. Tubes were solidified in a sterile environment at room temperature. Tubes were in slanting position during solidification of SDA. Tubes were inoculated after solidification with 6 mm inoculums of each fungal strain culture. Nystatin was used as the positive control. The tubes were incubated for a week at 28°C. Growth zone of inhibition was determined by measuring linear growth in mm (Rios *et al.*, 1988).

3.9. Anticancer activity

3.9.1. Cell culture

From the American Type Culture Collection (Manassas, VA), the Human hepatocellular carcinoma HepG2 and HCCLM3 cell lines were obtained. Both the HepG2 and HCCLM3 cell lines were cultured in the DMEM medium respectively with 10% of FBS in a humid atmosphere of 95% air and 5% carbon dioxide at 37°C.

3.9.2. MTT assay

Both the HCCLM3 and HepG2 cell lines were seeded in the 96-well plate at a concentration of 5000 cells/well. At the end of the treatment period, 20 µl of MTT (5 mg/ml) was added to each well. After that incubated for 4 h at 37°C. The purple formazan dye was dissolved

in the 100% dimethyl sulfoxide. By using the microplate reader (Tecan, Durham, NC, USA), the absorbance was measured at 570 nm.

3.9.3. Flow cytometric analysis

Out of the nine extracts of different plants in the MTT assay against the different cell lines we further take the four most active extracts for flow cytometric analysis against the HepG2 cell line at 72 h point. After the 72 h at the end of the treatment period, HepG2 cells were collected. After that fixed the HepG2 cells with 70% of ice cold ethanol and for 30 minutes stained with the PI (5 µg/ml). By the Cyan ADP flow cytometer (Dako Cytomation) across the cell cycle, cellular DNA content was analyzed.

3.10. Elemental analysis

3.10.1. Digestion method for samples

About 50 mg of samples is weighed accurately into glass tubes. 1 ml of concentrated HNO_3 is added. Tube is capped and place in microwave digestion system at 220°C for 15 minutes. The sample is diluted to 10 ml and filtered. The solution is analysed using the ICP.

3.10.2. Inductive Coupled Plasma - Optical Emission Spectroscopy (ICP-OES)

Multi element Standards made to 1, 5 and 10 ppm to calibrate the instrument for sample measurement. Instrument parameters are as shown in the Table 3.2.

3.11. Anionic Ion-Exchange Chromatography

Standards of 1, 5 and 10 ppm of multi anions were prepared to calibrate the instrument. Samples solutions were analysed on the instrument with parameters as follows in the Table 3.3 on the Met Rohm Ion Chromatography system.

3.12. CHNS analyzer

CHNS is measured with the Elemental Micro Vario Cube equipped with a thermal conductivity detector using the dumas method of combustion.

Table 3.2: Parameters of Inductive Coupled Plasma - Optical Emission Spectroscopy (ICP-OES)

Parameters	Setting
Purge Gas Flow	Normal
Spectral Profiling	No
Resolution	Fixed Normal
Read time	Auto
Read Delay Time	20 sec
Replicates	3
Source equilibration delay	15 sec
Plasma Flow	15.0 L/min
Auxiliary Flow	0.2 L/min
Nebulizer Flow	0.8 L/min
RF Power	1300 W
View Dist.	15.0
Plasma Viewing	Axial
Peristaltic Pump Sample Flow	1.8 mL/min
Peristaltic Pump Flush Time	25 sec
Auto sampler Wash Frequency	Between Samples
Auto sampler Pump Rate	2.5mL/min
Auto sampler Normal Time	50 sec
Processing Algorithm	Area
Points per Peak	3
Background corrections	2 points
Background correction	Two points
Calibration Equation	Linear Through 0
Spray Chamber	Scott
Nebulizer	Cross Flow

Table 3.3: Parameters of Met Rohm Ion Chromatography system

Column:	Metro Sep A Supp 5, 4.0 x 150mm x 5.0 um (Part No. 6.1006.520)
Guard Column:	Metro Sep A Supp 4/5 Guard/ 4.0 (Part No. 6.1006.500)
Eluent:	2 mM Sodium Bicarbonate, 1.3 mM Sodium carbonate
Flow Rate:	0.7 mL/min
Injection Volume:	20 UL
Pre-concentration cartridge:	Metro Sep A PCC 1 HC/4.0 (Part No. 6.1006.310)
Temperature:	30°C
Detection:	Suppressed Conductivity
Total Run Time:	20 mins

3.13. Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The chromatographic procedure was performed using the GC-MS (Agilent 7890A/5975C) with the auto sampler. 1000 ppm solutions in methanol, ethanol and ethyl acetate were prepared from methanolic, ethanolic and ethyl acetate plant extracts. Using the DB-5MS Column (30 meter × 0.25 mm and film thickness 0.25μm), 1 μl of each extract was injected for the analysis. As a carrier gas, helium gas was used at the flow rate of 1 ml/min. The analysis was carried out using the oven programming of initial temperature of 50°C for 2 mins. After that by the ramp rate of 20°C per min upto the 130°C. Then the ramp of 12°C per min to a temperature of 180°C. Finally raised the temperature upto 280°C at 3°C per min and hold for 15 mins. The ion source temperature was set at 250°C. The injection port temperature was set at 250°C. The total run time was 58.5 mins. The instrument was operated in electron impact mode with the electron energy of 70ev. Mass spectral scan range was set at 10-1050 m/z. Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST). They have more than 62,000 patterns (Abirami and Rajendran, 2012).

3.14. Statistical analysis

The data obtained in this study was presented as mean ± standard deviation. To determine the variability among the groups, one-way AVOVA was performed by Statistix 8.1. To determine the correlation of IC_{50} values of antioxidant assays by Pearson's correlation coefficient, GraphPad Prim 5 was used. The significant differences among the *in-vivo* and *in-vitro* treatment groups were calculated by the Tukey's multiple comparison and Kruskal-Wallis tests. The statistical significance was set at $P < 0.05$.

CHAPTER FOUR

RESULTS

4.1. Effect of extraction solvents on extracts yield

Extraction yield is a solvent efficiency measure for extracting particular components from the original material. Percentage extract is yield calculated as (weight of dry extract/dry material weight at the start) x 100. The extraction yield of each crude extract is shown in Figure 4.1. Among the different solvents used for extraction, methanolic extract provided the highest yield of crude extracts of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* with extraction yields of 5.72%, 7.47% and 6.95% respectively. Ethanol provided the yield of crude extracts of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* with extraction yields of 4.43%, 6.19% and 5.98% respectively. Ethyl acetate provided the yield of crude extracts of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* with extraction yields of 3.86%, 6.45% and 6.34% respectively.

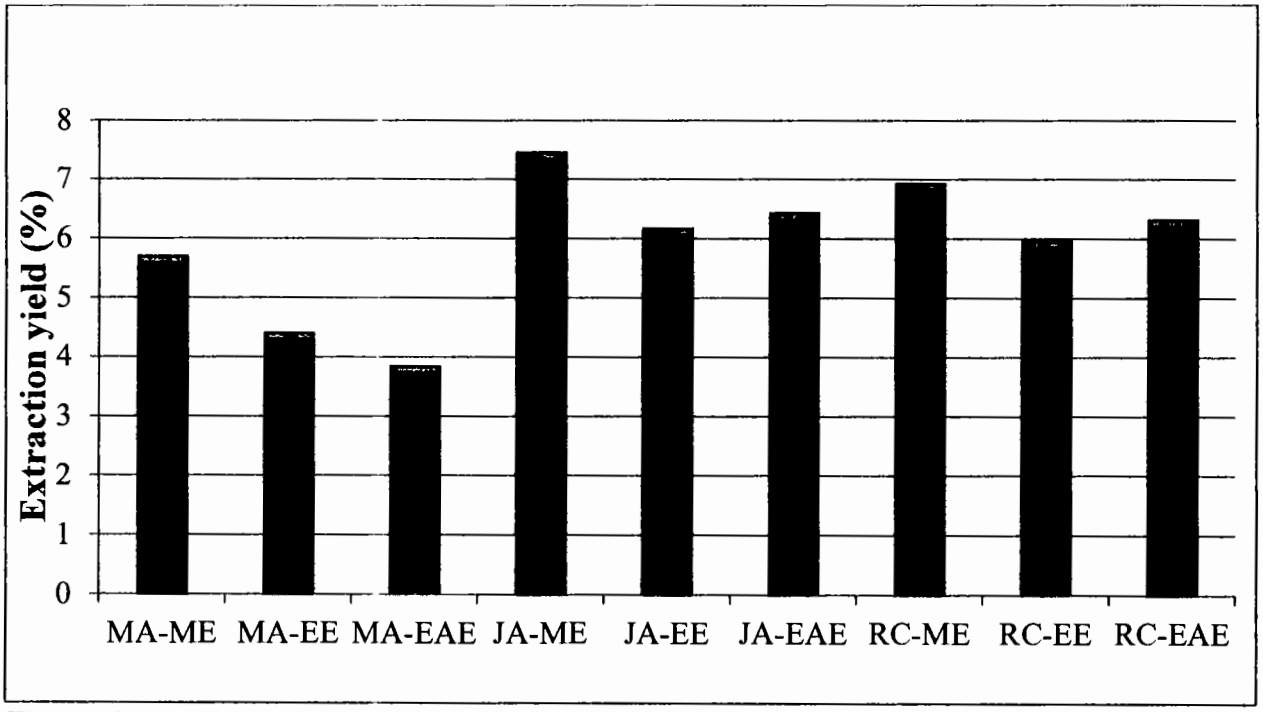


Figure 4.1: Extraction yields of crude extracts of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis*. MA-ME (*Melia azedarach* methanolic extract), MA-EE (*Melia azedarach* ethanolic extract), MA-EAE (*Melia azedarach* ethyl acetate extract). JA-ME (*Justicia adhatoda* methanolic extract), JA-EE (*Justicia adhatoda* ethanolic extract), JA-EAE (*Justicia adhatoda* ethyl acetate extract). RC-ME (*Ricinus communis* methanolic extract), RC-EE (*Ricinus communis* ethanolic extract), RC-EAE (*Ricinus communis* ethyl acetate extract).

4.2. Phytochemical analysis

To identify the bioactive and novel compounds in herbal medicine research, phytochemical screening is the first step. Phytochemical screening is a qualitative chemical evaluation of the plant drug which indicates the presence of chemical constituents. The chemical evaluation of the plant drug leads to proper identification of biologically active groups. The experimental procedures for the secondary metabolites for various phytochemical screening methods are shown in Table 4.1, 4.2 and 4.3.

The phytochemicals detected in the MA-ME, MA-EE and MA-EAE was alkaloids, saponins, tannins, terpenoids, phenols, flavonoids, steroids and anthraquinone. However, coumarins were not present in the extracts of *Melia azedarach*. The phytochemicals detected in the JA-ME, JA-EE and JA-EAE was alkaloids, saponins, tannins, terpenoids, phenols, flavonoids, steroids, anthraquinone and coumarins. The phytochemicals detected in the RC-ME, RC-EE and RC-EAE was alkaloids, saponins, tannins, terpenoids, phenols, flavonoids, steroids, anthraquinone. However, anthraquinone and coumarins were not detected in the crude extracts of *Ricinus communis*.

4.2.1. Total phenolic content

For gallic acid, considering the standard regression lines ($y = 0.0232\times + 0.0724$; $R^2 = 0.9991$), the equivalent of TPC were calculated (Figure 4.2 and 4.3). RC-ME showed the maximum quantity of TPC (153.68 ± 5.66 µg GAE/mg DE) followed by MA-EAE (107.20 ± 2.16 µg GAE/mg DE), JA-EE (101.25 ± 3.10 µg GAE/mg DE), JA-ME (94.93 ± 1.80 µg GAE/mg DE), RC-EAE (80.29 ± 4.07 µg GAE/mg DE), MA-EE (75.407 ± 2.41 µg GAE/mg DE), MA-ME (69.620 ± 3.55 µg GAE/mg DE), RC-EE (66.21 ± 3.20 µg GAE/mg DE) and JA-EAE (53.10 ± 2.77 µg GAE/mg DE).

4.2.2. Total flavonoid content

For quercetin, considering the standard regression lines ($y = 0.0254\times + 0.0483$; $R^2 = 0.9995$), the equivalent of TFC were calculated (Figure 4.4 and 4.5). Flavonoids were found to be rich in MA-EAE (94.97 ± 3.84 µg QE/mg DE) followed by JA-EE (86.44 ± 3.21 µg QE/mg DE), RC-ME (74.90 ± 4.65 µg QE/mg DE), RC-EAE (70.56 ± 4.43 µg QE/mg DE), MA-EE (55.73 ± 1.60 µg QE/mg DE), JA-ME (54.84 ± 2.66 µg QE/mg DE), RC-EE (49.50 ± 1.57 µg QE/mg DE), MA-ME (48.96 ± 2.72 µg QE/mg DE) and JA-EAE (40.23 ± 0.69 µg QE/mg DE).

Table 4.1: Phytochemical analysis of crude extracts of *Melia azedarach*

S.No	Phytochemicals	MA-ME	MA-EE	MA-EAE
1	**Alkaloids**	+	+	+
2	Saponins	+	+	+
3	**Tannins**	+	+	+
4	Terpenoids	+	+	+
5	**Phenols**	+	+	+
6	Flavonoids	+	+	+
7	**Steroids**	+	+	+
8	Coumarins	-	-	-
9	**Anthraquinone**	+	+	+

Note: (+) indicates the presence and (–) indicates the absence of phytochemical constituents.

Table 4.2: Phytochemical analysis of crude extracts of *Justicia adhatoda*

S.No	Phytochemicals	JA-ME	JA-EE	JA-EAE
1	**Alkaloids**	+	+	+
2	Saponins	+	+	+
3	**Tannins**	+	+	+
4	Terpenoids	+	+	+
5	**Phenols**	+	+	+
6	Flavonoids	+	+	+
7	**Steroids**	+	+	+
8	Coumarins	+	+	+
9	**Anthraquinone**	+	+	+

Note: (+) indicates the presence and (–) indicates the absence of phytochemical constituents.

Table 4.3: Phytochemical analysis of crude extracts of *Ricinus communis*

S.No	Phytochemicals	RC-ME	RC-EE	RC-EAE
1	Alkaloids	+	+	+
2	Saponins	+	+	+
3	Tannins	+	+	+
4	Terpenoids	+	+	+
5	Phenols	+	+	+
6	Flavonoids	+	+	+
7	Steroids	+	+	+
8	Coumarins	-	-	-
9	Anthraquinone	-	-	-

Note: (+) indicates the presence and (–) indicates the absence of phytochemical constituents.

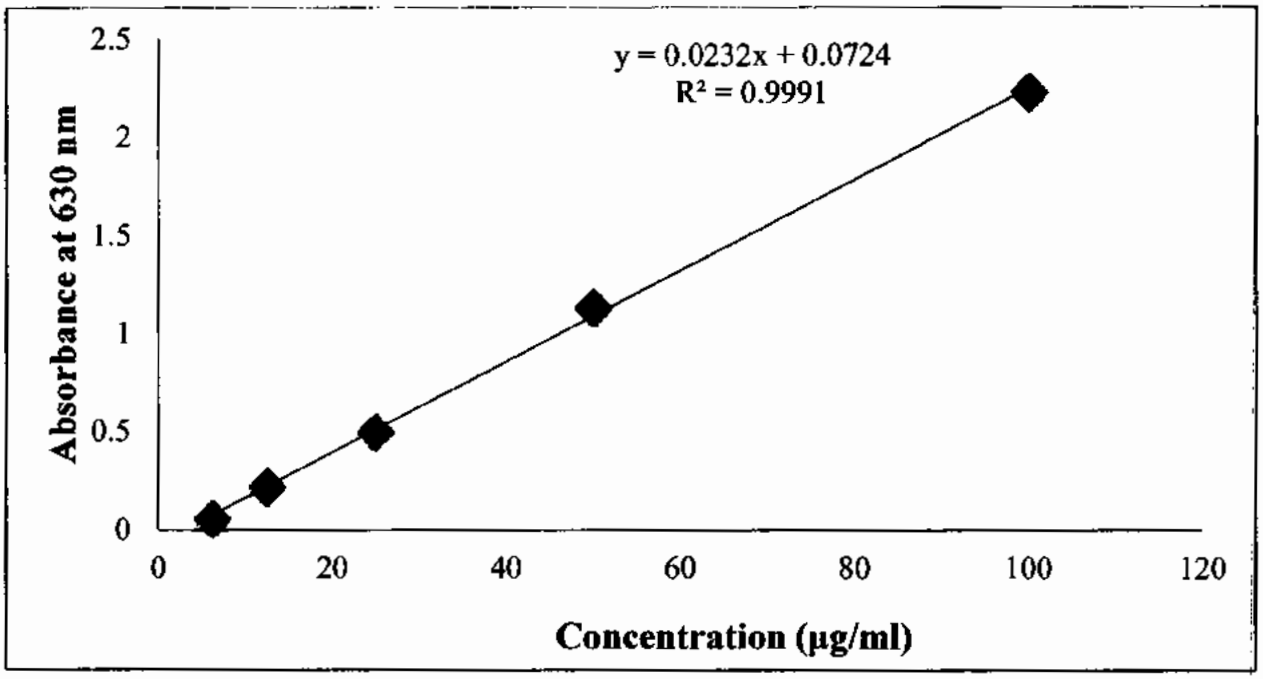


Figure 4.2: Standard curve of gallic acid for the determination of total phenolic content of extracts. Results are expressed as mean ± standard deviation (n = 3).

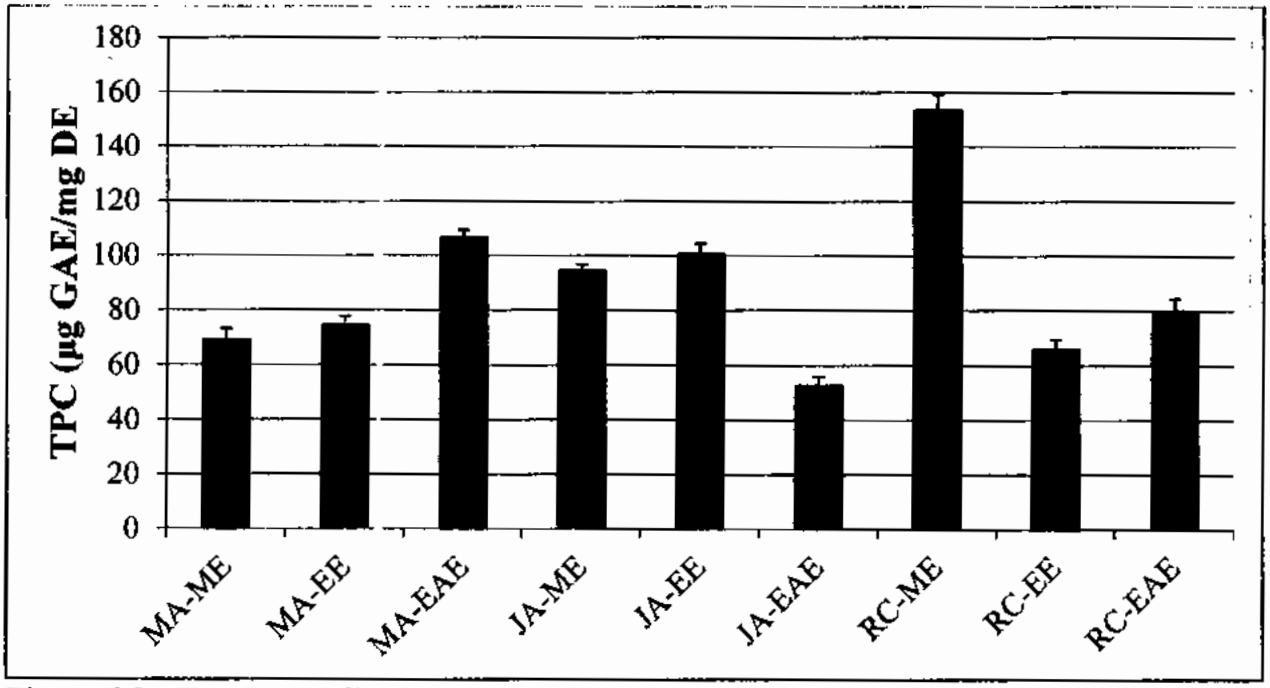


Figure 4.3: Total phenolic content of the extracts. Results are expressed as mean ± standard deviation (n = 3). TPC (total phenolic content), GAE (gallic acid equivalent) and DE (dry extract).

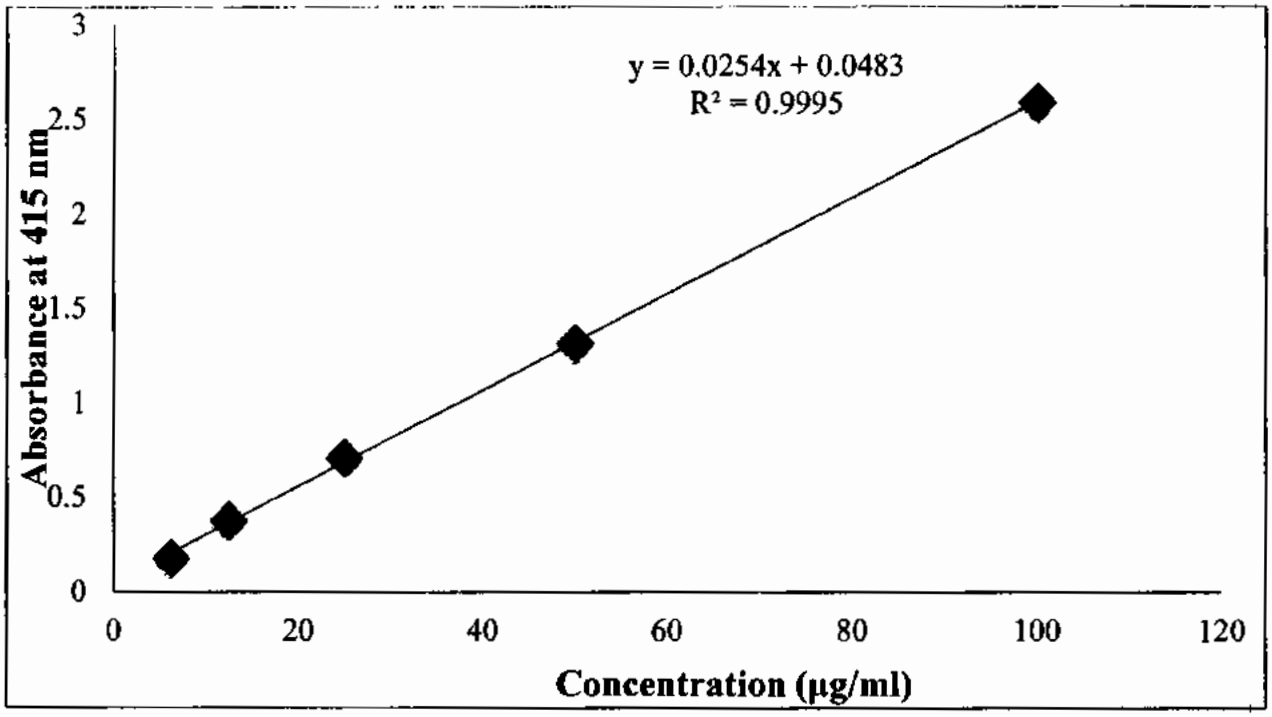


Figure 4.4: Standard curve of quercetin for the determination of total flavonoid content of extracts. Results are expressed as mean ± standard deviation (n = 3).

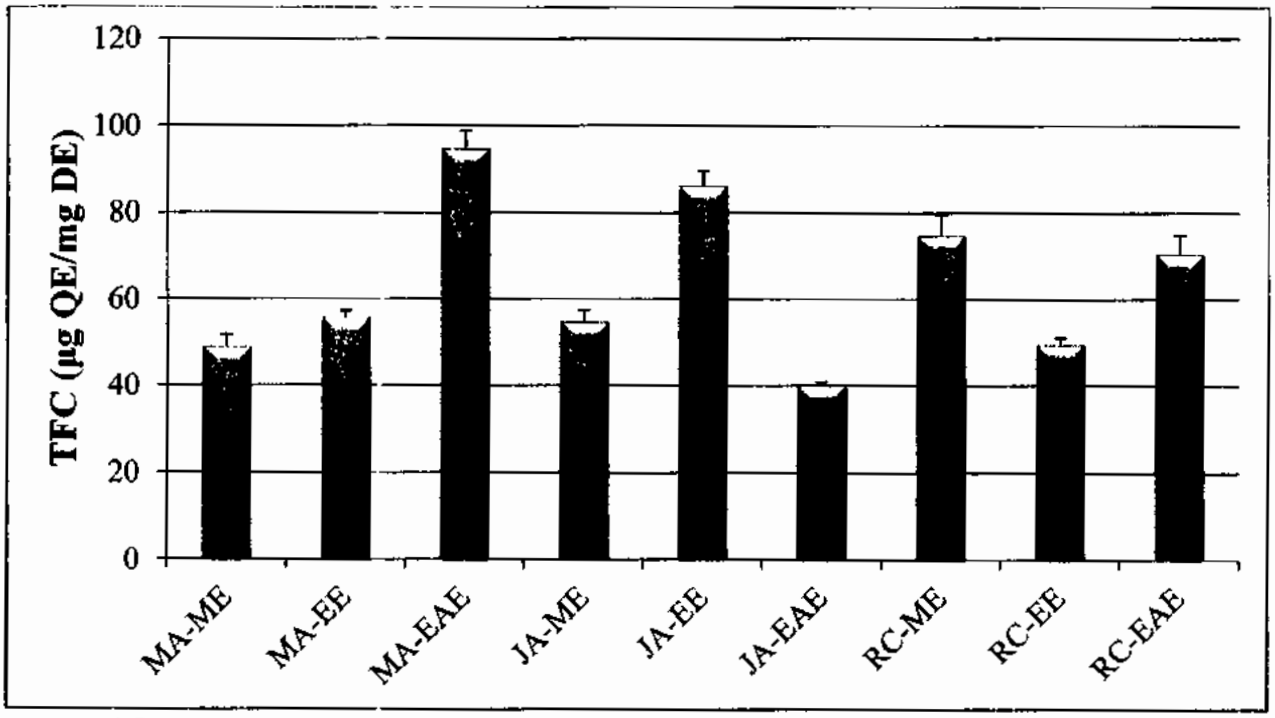


Figure 4.5: Total flavonoid content of the extracts. Results are expressed as mean ± standard deviation (n = 3). TFC (total flavonoid content), QE (quercetin equivalent) and DE (dry extract).

4.3. Antioxidant activity

4.3.1. DPPH free radical scavenging activity

The percentage inhibition of DPPH scavenging activity of crude extracts of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* are presented in Figure 4.6. The MA-ME, MA-EE and MA-EAE exhibited a maximum scavenging activity of 35.71 ± 2.71%, 44.11 ± 3.21% and 18.09 ± 1.21% at 100 µg/ ml respectively. The ascorbic acid was found to be 90.26 ± 0.91% at 100 µg/ ml. The JA-ME, JA-EE and JA-EAE exhibited a maximum scavenging activity of 68.50 ± 4.71%, 50.98 ± 3.67% and 15.63 ± 1.96% at 100 µg/ ml respectively, whereas for ascorbic acid (Standard) was found to be 90.26 ± 0.91% at 100 µg/ ml. The RC-ME, RC-EE and RC-EAE exhibited a maximum scavenging activity of 87.80 ± 5.87%, 26.59 ± 2.07% and 45.75 ± 3.67% at 100 µg/ ml respectively. The ascorbic acid was found to be 90.26 ± 0.91% at 100 µg/ ml. From all the nine extracts, RC-ME exhibited the best scavenging activity of 87.80 ± 5.87% which is approximately equal to the standard (ascorbic acid), 90.26 ± 0.91% at 100 µg/ ml.

4.3.2. Total antioxidant capacity

For ascorbic acid, considering the standard regression lines ($y = 0.0342\times + 0.0692$; $R^2 = 0.9996$), the equivalent of TAC were calculated (Figure 4.7 and 4.8). RC-ME (88.74 ± 3.03 µg AAE/mg DE) has the maximum total antioxidant capacity, followed by MA-EAE (78.99 ± 1.29 µg AAE/mg DE), RC-EAE (63.13 ± 5.25 µg AAE/mg DE), JA-EE (62.24 ± 3.92 µg AAE/mg DE), MA-EE (59.95 ± 2.11 µg AAE/mg DE), JA-ME (57.03 ± 2.38 µg AAE/mg DE), MA-ME (51.51 ± 2.86 µg AAE/mg DE), JA-EAE (43.11 ± 1.26 µg AAE/mg DE) and RC-EE (40.38 ± 1.37 µg AAE/mg DE).

4.3.3. Total reducing power

For ascorbic acid, considering the standard regression lines ($y = 0.0229\times + 0.0311$; $R^2 = 0.9955$), the equivalent of TRP were calculated (Figure 4.9 and 4.10). RC-ME (243.29 ± 8.80 µg AAE/mg DE) has the maximum total reducing power, followed by RC-EAE (148.63 ± 3.36 µg AAE/mg DE), MA-EAE (137.95 ± 3.30 µg AAE/mg DE), RC-EE (132.79 ± 2.80 µg AAE/mg DE), JA-EAE (121.74 ± 3.48 µg AAE/mg DE), MA-EE (110.48 ± 4.75 µg AAE/mg DE), JA-ME (102.60 ± 2.93 µg AAE/mg DE), MA-ME (98.74 ± 3.54 µg AAE/mg DE) and JA-EE (93.26 ± 3.54 µg AAE/mg DE).

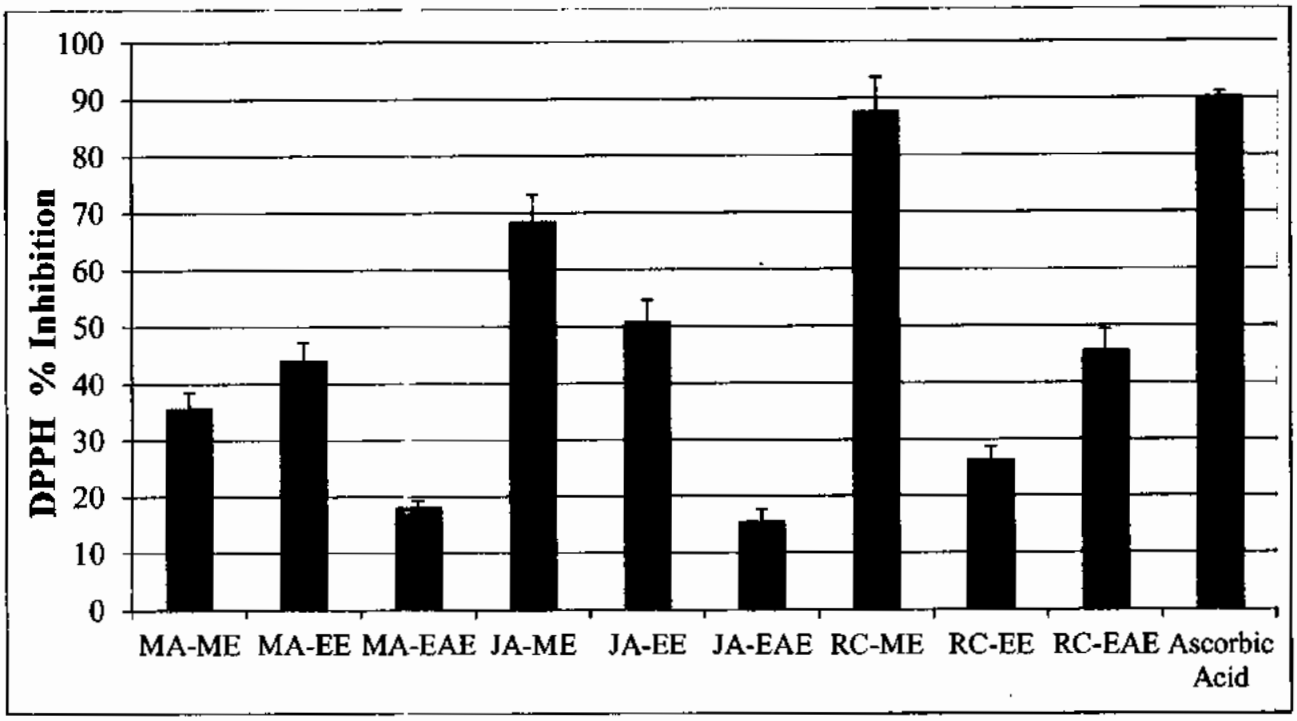


Figure 4.6: DPPH free radical scavenging activity of the extracts. Results are expressed as mean ± standard deviation (n = 3). DPPH (2, 2-Diphenyl-1-picrylhydrazyl).

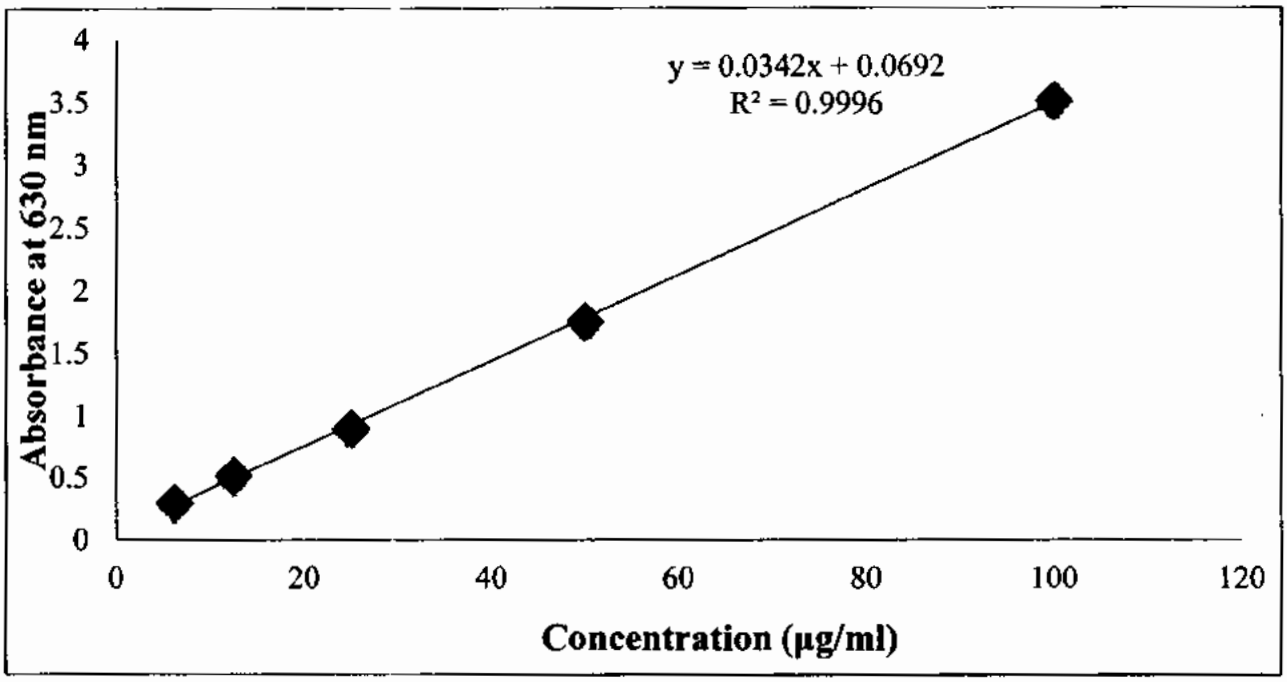


Figure 4.7: Standard curve of ascorbic acid for the determination of total antioxidant capacity of extracts. Results are expressed as mean ± standard deviation (n = 3).

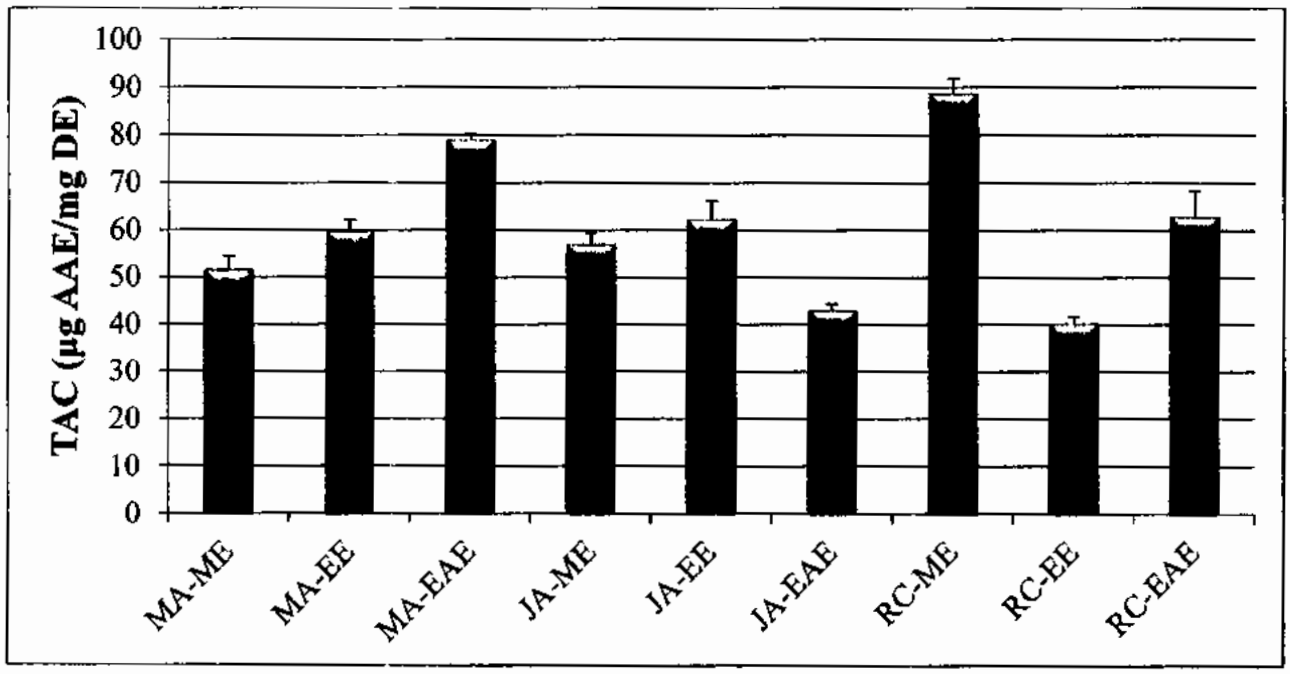


Figure 4.8: Total antioxidant capacity of the extracts. Results are expressed as mean ± standard deviation (n = 3). TAC (total antioxidant capacity), AAE (ascorbic acid equivalent) and DE (dry extract).

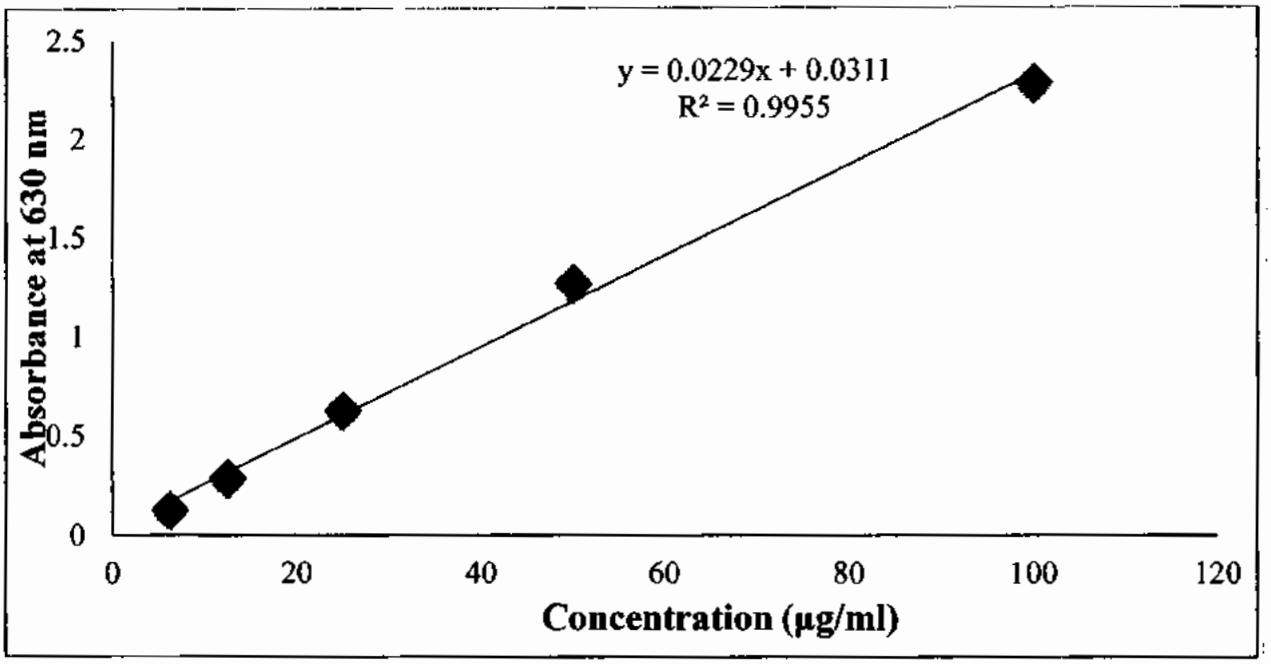


Figure 4.9: Standard curve of ascorbic acid for the determination of total reducing power of extracts. Results are expressed as mean ± standard deviation (n = 3).

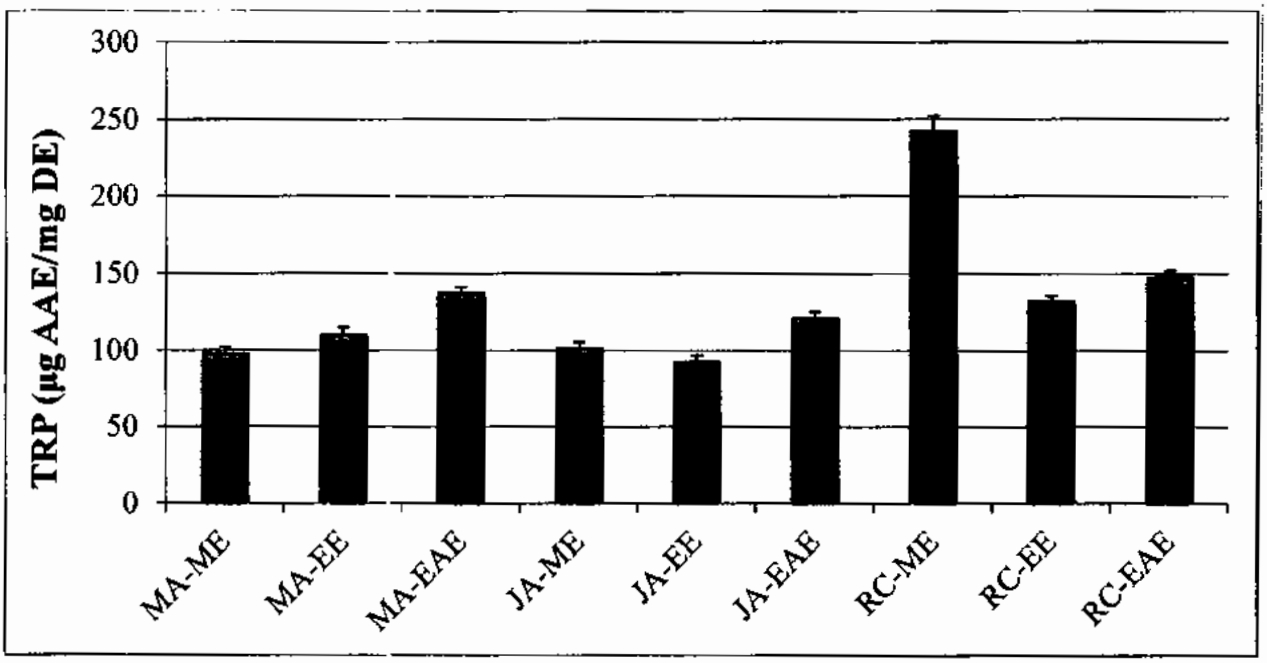


Figure 4.10: Total reducing power of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* crude extracts. Results are expressed as mean ± standard deviation (n = 3). TRP (total reducing power), AAE (ascorbic acid equivalent) and DE (dry extract).

4.4. Cytotoxicity assay

Cytotoxic effect of methanolic, ethanolic and ethyl acetate extracts of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* was evaluated using Brine shrimp lethality assay is shown in Table 4.4

4.4.1. Cytotoxicity assay of *Melia azedarach*

The crude methanol extract of *Melia azedarach* showed cytotoxicity (86.66%, 73.33% and 43.33% at 1000 µg/ml, 100 µg/ml and 10 µg/ml, respectively). The LD_{50} value calculated was 15.12 µg/ml. The crude ethanol extract of *Melia azedarach* showed cytotoxicity (83%, 60.33% and 30% at 1000 µg/ml, 100 µg/ml and 10 µg/ml, respectively). The LD_{50} value calculated was 51.11 µg/ml. The crude ethyl acetate extract of *Melia azedarach* showed cytotoxicity (73.33%, 46.66% and 23.66% at 1000 µg/ml, 100 µg/ml and 10 µg/ml, respectively). The LD_{50} value calculated was 122.78 µg/ml.

4.4.2. Cytotoxicity assay of *Justicia adhatoda*

The crude methanol extract of *Justicia adhatoda* showed cytotoxicity (63.66%, 56.33% and 33.33% at 1000 µg/ml, 100 µg/ml and 10 µg/ml, respectively). The LD_{50} value calculated was 84.91 µg/ml. The crude ethanol extract of *Justicia adhatoda* showed cytotoxicity (76%, 70.66% and 30% at 1000 µg/ml, 100 µg/ml and 10 µg/ml, respectively). The LD_{50} value calculated was 41.58 µg/ml. The crude ethyl acetate extract of *Justicia adhatoda* showed cytotoxicity (66.66%, 33.33% and 13.33% at 1000 µg/ml, 100 µg/ml and 10 µg/ml, respectively). The LD_{50} value calculated was 287.50 µg/ml.

4.4.3. Cytotoxicity assay of *Ricinus communis*

The crude methanol extract of *Ricinus communis* showed cytotoxicity (93.33%, 76.66% and 46.66% at 1000 µg/ml, 100 µg/ml and 10 µg/ml, respectively). The LD_{50} value calculated was 11.14 µg/ml. The crude ethanol extract of *Ricinus communis* showed cytotoxicity (73.33%, 63.33% and 36.66% at 1000 µg/ml, 100 µg/ml and 10 µg/ml, respectively). The LD_{50} value calculated was 37.67 µg/ml. The crude ethyl acetate extract of *Ricinus communis* showed cytotoxicity (56.66%, 40% and 26.66% at 1000 µg/ml, 100 µg/ml and 10 µg/ml, respectively). The LD_{50} value calculated was 391.76 µg/ml.

Table 4.4: Cytotoxicity effect of extracts of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis*

Extracts	Mortality (%) at various concentrations			
	1000 µg/ml	**100 µg/ml**	**10 µg/ml**	**LD_{50} µg/ml**
MA-ME	86.66	73.33	43.33	15.12
MA-EE	**83**	**60.33**	**30**	**51.11**
MA-EAE	73.33	46.66	23.66	122.78
JA-ME	**63.66**	**56.33**	**33.33**	**84.91**
JA-EE	76	70.66	30	41.58
JA-EAE	**66.66**	**33.33**	**13.33**	**287.50**
RC-ME	93.33	76.66	46.66	11.14
RC-EE	**73.33**	**63.33**	**36.66**	**37.67**
RC-EAE	56.66	40	26.66	391.76

4.5. Antidiabetic activity of *Melia azedarach*

4.5.1. Effect of *Melia azedarach* extracts on oral acute toxicity study

In the experimental animals, acute toxicity study is carried out for the determination of LD_{50} value. The LD_{50} determination was conducted in mice by OECD guideline 423. This study shows that upto the dose of 5000 mg/kg; the different extracts of *Melia azedarach* did not generate any observable signs of toxicity. This was confirmed by the absence of significant changes in the behaviours of mice such as weight loss, breathing, restlessness, diarrhea and coma etc. For two weeks, no death was observed. This result confirms that the medium lethal dose (LD_{50}) is greater than 5000 mg/kg in mice.

4.5.2. Effect of *Melia azedarach* extracts on oral glucose tolerance test

Blood glucose level of the normal control mice, Alloxan induced diabetic mice and diabetic mice treated with standard drug (glibenclamide) and different extracts at different time points after oral administration of glucose is shown in Table 4.5. No reduction in blood glucose level was observed in the normal control mice. Increase in blood glucose level was observed after 2 h and remained high over the next 8 h in the diabetic control mice. Diabetic mice treated with glibenclamide reduce the blood glucose level. Methanolic, ethanolic and ethyl acetate leaves extract of *Melia azedarach* significantly reduced the blood glucose level at 4 h and remained low over the next 4 h at 200 and 400 mg/kg dose. After the 8 h treatment, methanolic leaves extract of *Melia azedarach* at 400 mg/kg and ethanolic leaves extract of *Melia azedarach* at 400 mg/kg dose and glibenclamide decrease the blood glucose level significantly in diabetic mice.

4.5.3. Effect of *Melia azedarach* extracts on the blood glucose level in mice

Blood glucose level of the normal control mice, Alloxan induced diabetic mice and diabetic mice treated with standard drug (glibenclamide) and different extracts at different days (0 to 28^{th} day) after oral administration of glucose is shown in Table 4.6. The levels of blood glucose of diabetic mice treated with alloxan increases when compared with the normal mice. So level of blood glucose increases on 28^{th} day. After treatment with oral administration of MA-

ME, MA-EE and MA-EAE at 200 and 400 mg/kg, the levels of blood glucose was reduced when compared with the diabetic mice. The level of blood glucose of mice in MA-ME at 200 and 400 mg/kg decreases. The level of blood glucose of mice in MA-EE at 200 and 400 mg/kg decreases. The level of blood glucose of mice in MA-EAE at 200 and 400 mg/kg decreases. Glibenclamide also decreases the blood glucose level in mice after the 28 days treatment. Finally after 28 days treatment, methanolic leaves extract of *Melia azedarach* at 400 mg/kg, ethanolic leaves extract of *Melia azedarach* at the 400 mg/kg and the standard drug decreases the blood glucose level in diabetic mice ($P < 0.05$) in dose dependent manner.

4.5.4. Effect of *Melia azedarach* extracts on the body weight

The body weight increased in the normal control mice. In the diabetic mice, there was a significant decrease in the body weight. After treatment with extracts, only MA-EE at 400 mg/kg showed improvement in the body weight when compared with the diabetic and positive control treated groups. The body weight of mice in MA-ME at 200 and 400 mg/kg slightly decreases. The body weight of mice in MA-EE at 200 mg/kg slightly decreases and at 400 mg/kg slightly increases. The body weight of mice in MA-EAE at 200 and 400 mg/kg slightly decreases as described in the Table 4.7.

4.5.5. Effect of *Melia azedarach* extracts on the serum lipid profile

The Alloxan induced group showed significant elevation in the levels of cholesterol, triglycerides, LDL, VLDL and decreases the level of HDL when compared with the normal control. After 28 the days treatment with MA-ME, MA-EE and MA-EAE at 200 and 400 mg/kg, reduces the levels of cholesterol, triglycerides, LDL, VLDL and significant increase in HDL level when compared with the diabetic mice. Standard drug glibenclamide also reduces the levels of cholesterol, triglycerides, LDL, VLDL and increases the HDL level when compared with the diabetic mice as described in the Table 4.8.

4.5.6. Effect of *Melia azedarach* extracts on the liver function markers

The Alloxan induced group showed significant increase in the levels of bilirubin, ALT, ALP and AST when compared with the normal control. After the 28 days treatment with MA-ME, MA-EE and MA-EAE at 200 and 400 mg/kg, significantly reduced the elevated levels of bilirubin, ALT, ALP and AST when compared to diabetic mice. Standard drug glibenclamide

also reduces the levels bilirubin, ALT, ALP and AST when compared to diabetic mice as described in the Table 4.9.

4.5.7. Effect of *Melia azedarach* extracts on the kidney function markers

The Alloxan induced group showed significant increase in the levels of urea, creatinine and uric acid when compared with the normal group. After the 28 days treatment with MA-ME, MA-EE and MA-EAE at 200 and 400 mg/kg, reduces the levels of urea, creatinine and uric acid when compared to diabetic mice. Standard drug glibenclamide also showed significant reduction in the levels of the urea, creatinine and uric acid when compared with the diabetic mice as described in the Table 4.10.

Table 4.5: Effect of *Melia azedarach* extracts on oral glucose tolerance test in mice

Groups	Blood Glucose Levels at Varying Times (mg/dl)				
	0 Hr	2 Hr	4 Hr	6 Hr	8 Hr
NC	79.67 ± 7.25^{f}	82.33 ± 5.27^{d}	84.00 ± 4.81^{f}	81.50 ± 3.78^{g}	84.17 ± 1.47^{g}
DC	260.67 ± 10.33^{bcd}	269.67 ± 10.32^{a}	275.83 ± 8.63^{a}	277.83 ± 12.27^{a}	279.83 ± 10.17^{a}
PC	255.50 ± 10.15^{cd}	225.67 ± 2.16^{e}	198.83 ± 7.75^{e}	177.00 ± 6.81^{f}	164.50 ± 6.66^{f}
MA-ME 200	276.67 ± 10.81^{ab}	264.17 ± 10.53^{a}	253.50 ± 8.04^{b}	246.33 ± 9.41^{b}	240.83 ± 6.37^{b}
MA-ME 400	281.33 ± 8.04^{a}	265.67 ± 8.80^{a}	254.17 ± 6.37^{b}	244.67 ± 8.78^{b}	237.33 ± 5.61^{bc}
MA-EE 200	266.67 ± 9.22^{abc}	257.83 ± 7.70^{a}	248.33 ± 10.19^{b}	242.83 ± 8.11^{bc}	238.33 ± 9.83^{bc}
MA-EE 400	255.33 ± 11.12^{cd}	242.33 ± 12.19^{b}	232.67 ± 5.08^{c}	221.33 ± 7.28^{de}	216.83 ± 8.37^{de}
MA-EAE 200	$248.33 \pm 8.59d^{e}$	239.83 ± 5.45^{bc}	233.33 ± 4.58^{c}	227.83 ± 5.03^{cd}	225.33 ± 5.53^{cd}
MA-EAE 400	236.83 ± 7.67^{e}	224.50 ± 6.57^{c}	216.17 ± 8.01^{d}	209.83 ± 8.72^{e}	204.00 ± 7.09^{e}

Results are expressed as mean ± standard deviation (n=6). Means with different superscript (a-g) letters are significantly ($P < 0.05$) different from one another.

Table 4.6: Effect of *Melia azedarach* extracts on the blood glucose level in mice

Groups	Blood Glucose Level (mg/dl)				
	0 Day	7th Day	14th Day	21st Day	28th Day
NC	79.67 ± 7.25^{f}	81.83 ± 6.11^{f}	80.33 ± 9.91^{f}	84.33 ± 9.02^{f}	86.33 ± 10.94^{e}
DC	260.67 ± 10.33^{bcd}	268.00 ± 11.08^{a}	270.67 ± 14.29^{a}	275.33 ± 13.64^{a}	273.67 ± 13.00^{a}
PC	255.50 ± 10.15^{cd}	215.33 ± 8.26^{e}	179.00 ± 6.39^{e}	145.33 ± 8.33^{e}	111.67 ± 5.47^{d}
MA-ME 200	276.67 ± 10.81^{ab}	255.67 ± 6.53^{ab}	237.17 ± 6.65^{b}	222.17 ± 4.07^{b}	208.67 ± 7.23^{b}
MA-ME 400	281.33 ± 8.04^{a}	257.17 ± 8.25^{ab}	236.67 ± 3.14^{b}	218.17 ± 3.31^{b}	203.00 ± 9.35^{b}
MA-EE 200	266.67 ± 9.22^{abc}	249.17 ± 8.68^{bc}	233.33 ± 9.61^{b}	220.33 ± 8.09^{b}	206.50 ± 10.61^{b}
MA-EE 400	255.33 ± 11.12^{cd}	234.67 ± 10.37^{c}	215.83 ± 8.49^{cd}	200.67 ± 8.52^{cd}	184.50 ± 8.11^{c}
MA-EAE 200	248.33 ± 8.59^{de}	233.33 ± 6.41^{cd}	221.33 ± 5.71^{bc}	211.00 ± 6.98^{bc}	202.33 ± 10.94^{b}
MA-EAE 400	236.83 ± 7.67^{e}	218.17 ± 8.91^{de}	202.83 ± 7.57^{d}	190.50 ± 7.67^{d}	179.33 ± 6.38^{c}

Results are expressed as mean ± standard deviation (n=6). Means with different superscript (a-f) letters in the column are significantly ($P < 0.05$) different from one another.

Table 4.7: Effect of *Melia azedarach* extracts on the body weight

Groups	Changes in body weight (gm)	
	Initial day	Final day
NC	28.67 ± 2.73^{a}	32.33 ± 1.75^{a}
DC	28.33 ± 2.80^{a}	23.67 ± 1.21^{d}
PC	29.17 ± 2.71^{a}	32.17 ± 0.75^{a}
MA-ME 200	29.33 ± 1.97^{a}	28.50 ± 2.43^{bc}
MA-ME 400	31.67 ± 0.82^{a}	31.33 ± 2.33^{ab}
MA-EE 200	28.67 ± 2.59^{a}	26.17 ± 1.83^{cd}
MA-EE 400	28.33 ± 2.42^{a}	29.33 ± 1.03^{abc}
MA-EAE 200	28.83 ± 1.94^{a}	26.83 ± 1.60^{cd}
MA-EAE 400	30.67 ± 1.37^{a}	29.17 ± 2.56^{abc}

Results are expressed as mean ± standard deviation (n=6). Means with different superscript (a-d) letters in the column are significantly ($P < 0.05$) different from one another.

Table 4.8: Effect of *Melia azedarach* extracts on the serum lipid profile

Groups	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
NC	100.83 ± 5.81^{e}	91.33 ± 6.25^{e}	42.11 ± 2.87^{a}	61.83 ± 3.92^{d}	18.30 ± 1.27^{e}
DC	186.33 ± 7.14^{a}	141.17 ± 6.52^{a}	26.05 ± 2.56^{e}	136.50 ± 5.43^{a}	28.23 ± 1.30^{a}
PC	106.67 ± 6.47^{e}	97.50 ± 7.09^{e}	39.79 ± 1.45^{a}	70.33 ± 3.98^{d}	19.50 ± 1.42^{e}
MA-ME 200	139.50 ± 1.87^{bcd}	118.50 ± 3.27^{b}	31.48 ± 1.07^{cd}	98.00 ± 3.46^{c}	23.70 ± 0.65^{b}
MA-ME 400	138.50 ± 1.87^{cd}	116.17 ± 2.93^{b}	34.24 ± 0.68^{bc}	91.33 ± 2.73^{c}	23.23 ± 0.58^{b}
MA-EE 200	138.00 ± 4.69^{cd}	116.17 ± 3.71^{b}	30.87 ± 1.49^{d}	96.50 ± 5.75^{c}	23.33 ± 0.74^{b}
MA-EE 400	136.50 ± 3.27^{d}	113.83 ± 2.40^{b}	35.68 ± 1.35^{b}	93.00 ± 6.84^{c}	22.77 ± 0.48^{b}
MA-EAE 200	148.00 ± 4.38^{b}	121.83 ± 1.72^{b}	30.12 ± 0.73^{d}	111.00 ± 2.45^{b}	24.37 ± 0.34^{b}
MA-EAE 400	146.17 ± 2.79^{bc}	119.50 ± 4.37^{b}	31.27 ± 0.99^{cd}	109.00 ± 4.69^{b}	23.90 ± 0.87^{b}

Results are expressed as mean ± standard deviation (n=6). Means with different superscript (a-e) letters in the column are significantly ($P < 0.05$) different from one another.

Table 4.9: Effect of *Melia azedarach* extracts on the liver function markers

Groups	Bilirubin (mg/dl)	ALT (u/l)	ALP (u/l)	AST (u/l)
NC	0.42 ± 0.03^{e}	30.67 ± 2.87^{d}	114.67 ± 5.17^{e}	26.83 ± 4.70^{d}
DC	1.73 ± 0.13^{a}	70.83 ± 6.52^{a}	278.67 ± 20.38^{a}	91.67 ± 7.03^{a}
PC	0.52 ± 0.04^{e}	35.17 ± 2.99^{d}	128.33 ± 7.11^{e}	37.33 ± 4.67^{c}
MA-ME 200	0.89 ± 0.02^{cd}	47.50 ± 3.73^{bc}	174.83 ± 4.99^{bcd}	47.33 ± 4.17^{b}
MA-ME 400	0.87 ± 0.07^{d}	45.83 ± 1.47^{c}	169.50 ± 3.56^{cd}	46.50 ± 4.41^{b}
MA-EE 200	0.87 ± 0.04^{d}	49.83 ± 2.63^{bc}	167.00 ± 9.65^{d}	50.50 ± 3.78^{b}
MA-EE 400	0.83 ± 0.08^{d}	48.67 ± 1.63^{bc}	164.00 ± 4.33^{d}	48.50 ± 2.58^{b}
MA-EAE 200	1.11 ± 0.02^{b}	51.83 ± 3.54^{b}	188.50 ± 2.89^{b}	55.00 ± 2.83^{b}
MA-EAE 400	1.02 ± 0.06^{bc}	52.50 ± 2.59^{bc}	183.83 ± 4.11^{bc}	52.33 ± 4.97^{b}

Results are expressed as mean ± standard deviation (n=6). Means with different superscript (a-e) letters in the column are significantly ($P < 0.05$) different from one another.

Table 4.10: Effect of *Melia azedarach* extracts on the kidney function markers

Groups	Urea (mg/dl)	Creatinine (mg/dl)	Uric Acid (mg/dl)
NC	30.67 ± 2.73^{f}	0.94 ± 0.11^{c}	3.87 ± 0.37^{g}
DC	75.17 ± 2.86^{a}	2.17 ± 0.28^{a}	9.84 ± 0.27^{a}
PC	37.33 ± 1.86^{e}	1.14 ± 0.15^{c}	4.11 ± 0.23^{g}
MA-ME 200	53.00 ± 2.60^{bcd}	1.44 ± 0.02^{b}	4.78 ± 0.15^{ef}
MA-ME 400	50.17 ± 1.72^{d}	1.36 ± 0.05^{b}	4.58 ± 0.11^{f}
MA-EE 200	51.00 ± 1.41^{cd}	1.49 ± 0.03^{b}	5.39 ± 0.18^{cd}
MA-EE 400	49.67 ± 3.67^{d}	1.48 ± 0.05^{b}	5.08 ± 0.07^{de}
MA-EAE 200	56.17 ± 2.92^{b}	1.57 ± 0.04^{b}	6.00 ± 0.08^{b}
MA-EAE 400	55.33 ± 3.14^{bc}	1.55 ± 0.07^{b}	5.73 ± 0.09^{bc}

Results are expressed as mean ± standard deviation (n=6). Means with different superscript (a-g) letters in the column are significantly ($P < 0.05$) different from one another.

4.6. Antidiabetic activity of *Justicia adhatoda*

4.6.1. Effect of *Justicia adhatoda* extracts on oral acute toxicity study

In the experimental animals, acute toxicity study is carried out for the determination of LD_{50} value. The LD_{50} determination was conducted in mice by OECD guideline 423. This study shows that upto the dose of 5000 mg/kg; the different extracts of *Justicia adhatoda* did not generate any observable signs of toxicity. This was confirmed by the absence of significant changes in the behaviours of mice such as weight loss, breathing, restlessness, diarrhea and coma etc. For two weeks, no death was observed. This result confirms that the medium lethal dose (LD_{50}) is greater than 5000 mg/kg in mice.

4.6.2. Effect of *Justicia adhatoda* extracts on oral glucose tolerance test

Blood glucose level of the normal control mice, Alloxan induced diabetic mice and diabetic mice treated with standard drug (glibenclamide) and different extracts at different time points after oral administration of glucose is shown in Table 4.11. No reduction in blood glucose level was observed in the normal control mice. Increase in blood glucose level was observed after 2 h and remained high over the next 8 h in the diabetic control mice. Diabetic mice treated with glibenclamide reduce the blood glucose level. Methanolic, ethanolic and ethyl acetate leaves extract of *Justicia adhatoda* significantly reduced the blood glucose level at 4 h and remained low over the next 4 h at 200 and 400 mg/kg dose. After the 8 h treatment, methanolic leaves extract of *Justicia adhatoda* at 400 mg/kg and ethanolic leaves extract of *Justicia adhatoda* at 400 mg/kg dose and glibenclamide decrease the blood glucose level significantly in diabetic mice.

4.6.3. Effect of *Justicia adhatoda* extracts on the blood glucose level in mice

Blood glucose level of the normal control mice, Alloxan induced diabetic mice and diabetic mice treated with standard drug (glibenclamide) and different extracts at different days (0 to 28^{th} day) after oral administration of glucose is shown in Table 4.12. The levels of blood glucose of diabetic mice treated with alloxan increases when compared with the normal mice. So the level of blood glucose increases on the 28^{th} day. After treatment with oral administration of

JA-ME, JA-EE and JA-EAE at 200 and 400 mg/kg, the levels of blood glucose was reduced when compared with the diabetic mice. The level of blood glucose of mice in JA-ME at 200 and 400 mg/kg decreases. The level of blood glucose of mice in JA-EE at 200 and 400 mg/kg decreases. The level of blood glucose of mice in JA-EAE at 200 and 400 mg/kg decreases. Glibenclamide also decreases the blood glucose level in mice after 28 days treatment. Finally after 28 days treatment, methanolic leaves extract of *Justicia adhatoda* at 400 mg/kg, ethanolic leaves extract of *Justicia adhatoda* at 200 mg/kg, ethanolic leaves extract of *Justicia adhatoda* at 400 mg/kg and the standard drug decreases the blood glucose level in diabetic mice ($P < 0.05$) in dose dependent manner.

4.6.4. Effect of *Justicia adhatoda* extracts on the body weight

The body weight increased in the normal control mice. In the diabetic mice, there was a significant decrease in the body weight. After treatment with the extracts, all the extracts show improvement in the body weight when compared with the diabetic and positive treated groups except the JA-EAE at 200 mg/kg. The body weight of mice in JA-ME at 200 and 400 mg/kg slightly increases. The body weight of mice in JA-EE at 200 and 400 mg/kg slightly increases. The body weight of mice in JA-EAE at 200 mg/kg slightly decreases and at 400 mg/kg slightly increases as described in the Table 4.13.

4.6.5. Effect of *Justicia adhatoda* extracts on the serum lipid profile

The Alloxan induced group showed significant elevation in the levels of cholesterol, triglycerides, LDL, VLDL and decreases the level of HDL when compared with the normal control. After the 28 days treatment with JA-ME, JA-EE and JA-EAE at 200 and 400 mg/kg, significantly reduced the elevated levels of cholesterol, triglycerides, LDL, VLDL and significant increase in HDL level when compared to diabetic mice. Standard drug glibenclamide also reduces the levels of cholesterol, triglycerides, LDL, VLDL and significant increase in HDL level when compared with the diabetic mice as described in the Table 4.14.

4.6.6. Effect of *Justicia adhatoda* extracts on the liver function markers

The Alloxan induced group showed significant increase in the levels of bilirubin, ALT, ALP and AST when compared with the normal control. After the 28 days treatment with JA-ME, JA-EE and JA-EAE at 200 and 400 mg/kg, reduces the levels of bilirubin, ALT, ALP and AST

when compared to diabetic mice. Standard drug glibenclamide also reduces the levels of bilirubin, ALT, ALP and AST when compared to diabetic mice as described in the Table 4.15.

4.6.7. Effect of *Justicia adhatoda* extracts on the kidney function markers

The Alloxan induced group showed significant increase in the levels of urea, creatinine and uric acid when compared with the normal control. After the 28 days treatment with JA-ME, JA-EE and JA-EAE at 200 and 400 mg/kg, reduces the levels of urea, creatinine and uric acid when compared to diabetic mice. Standard drug glibenclamide also showed significant reduction in the levels urea, creatinine and uric acid when compared with the diabetic mice as described in the Table 4.16.

Table 4.11: Effect of *Justicia adhatoda* extracts on oral glucose tolerance test

Groups	Blood Glucose Levels at Varying Times (mg/dl)				
	0 Hr	2 Hr	4 Hr	6 Hr	8 Hr
NC	79.67 ± 7.25^{d}	82.33 ± 5.27^{f}	84.00 ± 4.81^{e}	81.50 ± 3.78^{f}	84.17 ± 1.47^{f}
DC	260.67 ± 10.33^{bc}	269.67 ± 10.32^{ab}	275.83 ± 8.63^{a}	277.83 ± 12.27^{a}	279.83 ± 10.17^{a}
PC	255.50 ± 10.15^{c}	225.67 ± 2.16^{e}	198.83 ± 7.75^{d}	177.00 ± 6.81^{e}	164.50 ± 6.66^{e}
JA-ME 200	277.67 ± 16.68^{ab}	261.67 ± 13.39^{bc}	248.00 ± 7.59^{b}	239.83 ± 9.45^{bc}	232.17 ± 6.59^{c}
JA-ME 400	299.67 ± 6.15^{a}	280.67 ± 3.61^{a}	264.50 ± 11.89^{a}	254.17 ± 11.49^{b}	245.50 ± 3.21^{b}
JA-EE 200	251.83 ± 11.96^{c}	238.00 ± 12.33^{de}	228.00 ± 6.26^{c}	219.50 ± 5.79^{d}	214.17 ± 6.76^{d}
JA-EE 400	297.17 ± 3.61^{a}	284.33 ± 9.01^{a}	271.50 ± 12.15^{a}	257.50 ± 6.98^{b}	249.83 ± 3.77^{b}
JA-EAE 200	240.17 ± 20.50^{c}	230.50 ± 11.52^{de}	223.50 ± 9.81^{c}	216.17 ± 15.71^{d}	213.67 ± 10.77^{d}
JA-EAE 400	257.83 ± 9.49^{bc}	246.50 ± 7.42^{cd}	236.00 ± 6.81^{bc}	229.33 ± 8.80^{cd}	223.83 ± 7.11^{cd}

Results are expressed as mean ± standard deviation (n=6). Means with different superscript (a-f) letters in the column are significantly ($P < 0.05$) different from one another.

Table 4.12: Effect of *Justicia adhatoda* extracts on the blood glucose level in mice

Groups	Blood Glucose Level (mg/dl)				
	0 Day	7^{th} Day	14^{th} Day	21^{st} Day	28^{th} Day
NC	79.67 ± 7.25^{d}	81.83 ± 6.11^{f}	80.33 ± 9.91^{f}	84.33 ± 9.02^{f}	86.33 ± 10.94^{g}
DC	260.67 ± 10.33^{bc}	268.00 ± 11.08^{ab}	270.67 ± 14.29^{a}	275.33 ± 13.64^{a}	273.67 ± 13.00^{a}
PC	255.50 ± 10.15^{c}	215.33 ± 8.26^{e}	179.00 ± 6.39^{e}	145.33 ± 8.33^{e}	111.67 ± 5.47^{f}
JA-ME 200	277.67 ± 16.68^{ab}	254.17 ± 6.52^{bc}	233.00 ± 10.33^{bc}	215.17 ± 6.61^{bc}	199.00 ± 6.07^{bc}
JA-ME 400	299.67 ± 6.15^{a}	272.83 ± 9.22^{a}	248.67 ± 7.55^{b}	226.33 ± 6.62^{b}	206.50 ± 8.69^{b}
JA-EE 200	251.83 ± 11.96^{c}	224.17 ± 7.36^{de}	203.33 ± 10.11^{d}	184.50 ± 8.81^{d}	166.33 ± 5.75^{e}
JA-EE 400	297.17 ± 3.61^{a}	269.00 ± 4.94^{ab}	244.83 ± 6.82^{b}	219.83 ± 3.13^{b}	198.00 ± 10.81^{bc}
JA-EAE 200	240.17 ± 20.50^{c}	224.50 ± 16.34^{de}	207.17 ± 14.29^{d}	192.17 ± 15.19^{d}	180.33 ± 10.19^{de}
JA-EAE 400	257.83 ± 9.49^{bc}	239.00 ± 10.02^{cd}	218.50 ± 6.83^{cd}	199.17 ± 7.93^{cd}	184.33 ± 8.06^{cd}

Results are expressed as mean ± standard deviation (n=6). Means with different superscript (a-g) letters in the column are significantly ($P < 0.05$) different from one another.

Table 4.13: Effect of *Justicia adhatoda* extracts on the body weight

Groups	Changes in body weight (gm)	
	Initial day	Final day
NC	28.67 ± 2.73^{a}	32.33 ± 1.75^{a}
DC	28.33 ± 2.80^{a}	23.67 ± 1.21^{d}
PC	29.17 ± 2.71^{a}	32.17 ± 0.75^{a}
JA-ME 200	28.67 ± 3.50^{a}	29.83 ± 2.31^{abc}
JA-ME 400	31.33 ± 2.42^{a}	32.67 ± 1.50^{a}
JA-EE 200	30.83 ± 1.83^{a}	31.33 ± 1.21^{ab}
JA-EE 400	31.67 ± 0.82^{a}	32.83 ± 1.17^{a}
JA-EAE 200	28.67 ± 1.63^{a}	27.67 ± 2.80^{a}
JA-EAE 400	27.83 ± 2.04^{a}	28.67 ± 2.42^{bc}

Results are expressed as mean ± standard deviation (n=6). Means with different superscript (a-d) letters in the column are significantly ($P < 0.05$) different from one another.

Table 4.14: Effect of *Justicia adhatoda* extracts on the serum lipid profile

Groups	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
NC	100.83 ± 5.81^{d}	91.33 ± 6.25^{c}	42.11 ± 2.87^{a}	61.83 ± 3.92^{f}	18.30 ± 1.27^{c}
DC	186.33 ± 7.14^{a}	141.17 ± 6.52^{a}	26.05 ± 2.56^{f}	136.50 ± 5.43^{a}	28.23 ± 1.30^{a}
PC	106.67 ± 6.47^{d}	97.50 ± 7.09^{c}	39.79 ± 1.45^{ab}	70.33 ± 3.98^{e}	19.50 ± 1.42^{c}
JA-ME 200	135.17 ± 2.56^{bc}	114.67 ± 3.26^{b}	33.37 ± 2.44^{de}	94.00 ± 3.58^{c}	22.93 ± 0.65^{b}
JA-ME 400	133.00 ± 3.03^{bc}	110.50 ± 3.27^{b}	36.43 ± 1.91^{bcd}	87.17 ± 3.43^{cd}	22.10 ± 0.65^{b}
JA-EE 200	133.83 ± 2.32^{bc}	111.17 ± 5.27^{b}	34.43 ± 1.63^{cde}	92.67 ± 3.73^{cd}	22.20 ± 1.05^{b}
JA-EE 400	130.67 ± 5.16^{c}	109.67 ± 3.98^{b}	37.65 ± 1.57^{bc}	85.67 ± 2.94^{d}	21.93 ± 0.79^{b}
JA-EAE 200	141.17 ± 3.54^{b}	117.33 ± 2.50^{b}	30.73 ± 1.00^{e}	107.50 ± 4.18^{b}	23.47 ± 0.51^{b}
JA-EAE 400	140.33 ± 3.67^{b}	118.67 ± 3.93^{b}	33.63 ± 2.57^{de}	104.33 ± 2.06^{b}	23.73 ± 0.79^{b}

Results are expressed as mean ± standard deviation (n=6). Means with different superscript (a-f) letters in the column are significantly ($P < 0.05$) different from one another.

Table 4.15: Effect of *Justicia adhatoda* extracts on the liver function markers

Groups	Bilirubin (mg/dl)	ALT (u/l)	ALP (u/l)	AST (u/l)
NC	0.42 ± 0.03^{d}	30.67 ± 2.87^{e}	114.67 ± 5.17^{a}	26.83 ± 4.70^{a}
DC	1.73 ± 0.13^{a}	70.83 ± 6.52^{a}	278.67 ± 20.38^{a}	91.67 ± 7.03^{a}
PC	0.52 ± 0.04^{d}	35.17 ± 2.99^{e}	128.33 ± 7.11^{e}	37.33 ± 4.67^{a}
JA-ME 200	0.84 ± 0.04^{c}	44.67 ± 3.78^{b}	168.67 ± 9.10^{bcd}	44.67 ± 4.55^{bcd}
JA-ME 400	0.83 ± 0.03^{c}	43.00 ± 2.76^{b}	163.67 ± 6.50^{cd}	43.17 ± 3.76^{cd}
JA-EE 200	0.83 ± 0.04^{c}	46.83 ± 4.71^{b}	160.17 ± 8.91^{cd}	46.67 ± 1.37^{bc}
JA-EE 400	0.79 ± 0.02^{c}	47.17 ± 2.40^{b}	157.00 ± 2.09^{d}	45.67 ± 3.45^{bc}
JA-EAE 200	1.03 ± 0.10^{b}	49.17 ± 2.32^{b}	182.00 ± 5.51^{b}	51.33 ± 3.27^{b}
JA-EAE 400	0.99 ± 0.07^{b}	48.83 ± 1.94^{b}	177.00 ± 6.63^{bc}	49.17 ± 3.54^{bc}

Results are expressed as mean ± standard deviation (n=6). Means with different superscript (a-e) letters in the column are significantly ($P < 0.05$) different from one another.

Table 4.16: Effect of *Justicia adhatoda* extracts on the kidney function markers

Groups	Urea (mg/dl)	Creatinine (mg/dl)	Uric Acid (mg/dl)
NC	30.67 ± 2.73^{f}	0.94 ± 0.11^{d}	3.87 ± 0.37^{f}
DC	75.17 ± 2.86^{a}	2.17 ± 0.28^{a}	9.84 ± 0.27^{a}
PC	37.33 ± 1.86^{e}	1.14 ± 0.15^{cd}	4.11 ± 0.23^{f}
JA-ME 200	49.00 ± 2.83^{bcd}	1.43 ± 0.06^{b}	4.73 ± 0.22^{e}
JA-ME 400	45.83 ± 1.94^{d}	1.34 ± 0.07^{bc}	4.56 ± 0.15^{e}
JA-EE 200	47.17 ± 2.56^{cd}	1.49 ± 0.04^{b}	5.39 ± 0.17^{cd}
JA-EE 400	48.17 ± 4.40^{cd}	1.44 ± 0.05^{b}	4.98 ± 0.25^{de}
JA-EAE 200	54.00 ± 4.38^{b}	1.55 ± 0.07^{b}	5.94 ± 0.06^{b}
JA-EAE 400	52.33 ± 2.51^{bc}	1.54 ± 0.03^{b}	5.64 ± 0.18^{bc}

Results are expressed as mean ± standard deviation (n=6). Means with different superscript (a-f) letters in the column are significantly ($P < 0.05$) different from one another.

4.7. Antidiabetic activity of *Ricinus communis*

4.7.1. Effect of *Ricinus communis* extracts on oral acute toxicity study

In the experimental animals, acute toxicity study is carried out for the determination of LD_{50} value. The LD_{50} determination was conducted in mice by OECD guideline 423. This study shows that upto the dose of 5000 mg/kg; the different extracts of *Ricinus communis* did not generate any observable signs of toxicity. This was confirmed by the absence of significant changes in the behaviours of mice such as weight loss, breathing, restlessness, diarrhea and coma etc. For two weeks, no death was observed. This result confirms that the medium lethal dose (LD_{50}) is greater than 5000 mg/kg in mice.

4.7.2. Effect of *Ricinus communis* extracts on oral glucose tolerance test

Blood glucose level of the normal control mice, Alloxan induced diabetic mice and diabetic mice treated with standard drug (glibenclamide) and different extracts at different time points after oral administration of glucose is shown in Table 4.17. No reduction in blood glucose level was observed in the normal control mice. Increase in blood glucose level was observed after 2 h and remained high over the next 8 h in the diabetic control mice. Diabetic mice treated with glibenclamide reduce the blood glucose level. Methanolic, ethanolic and ethyl acetate leaves extract of *Ricinus communis* significantly reduced the blood glucose level at 4 h and remained low over the next 4 h at 200 and 400 mg/kg dose. After the 8 hours treatment, methanolic leaves extract of *Ricinus communis* at 400 mg/kg, ethyl acetate leaves extract of *Ricinus communis* at 400 mg/kg and glibenclamide decrease the blood glucose level significantly in diabetic mice.

4.7.3. Effect of *Ricinus communis* extracts on the blood glucose level in mice

Blood glucose level of the normal control mice, Alloxan induced diabetic mice and diabetic mice treated with standard drug (glibenclamide) and different extracts at different days (0 to 28th day) after oral administration of glucose is shown in Table 4.18. The levels of blood glucose of diabetic mice treated with alloxan increases when compared with the normal mice. So

level of blood glucose increases on the 28th day. After treatment with oral administration of RC-ME, RC-EE and RC-EAE at 200 and 400 mg/kg, the level of blood glucose was significantly ($P < 0.05$) reduced when compared to the diabetic control mice. The level of blood glucose of mice in RC-ME at 200 and 400 mg/kg decreases. The level of blood glucose of mice in RC-EE at 200 and 400 mg/kg decreases. The level of blood glucose of mice in RC-EAE at 200 and 400 mg/kg decreases. Glibenclamide also decreases the blood glucose level in mice after 28 days treatment. Finally after 28 days treatment, methanolic leaves extract of *Ricinus communis* at 200 mg/kg and 400 mg/kg, ethyl acetate leaves extract of *Ricinus communis* at 400 mg/kg and the standard drug decreases the blood glucose level in diabetic mice ($P < 0.05$) in dose dependent manner.

4.7.4. Effect of *Ricinus communis* extracts on the body weight

The body weight increased in the normal control mice. In the diabetic mice, there was a significant decrease in the body weight. After treatment with extracts, all the extracts show improvement in the body weight when compared with the diabetic and positive control treated group except the RC-EAE at 200 mg/kg. The body weight of mice in RC-ME at 200 and 400 mg/kg slightly increases. The body weight of mice in RC-EE at 200 and 400 mg/kg slightly increases. The body weight of mice in RC-EAE at 200 mg/kg slightly decreases and at 400 mg/kg slightly increases as described in the Table 4.19.

4.7.5. Effect of *Ricinus communis* extracts on the serum lipid profile

The Alloxan induced group showed significant elevation in the levels of cholesterol, triglycerides, LDL, VLDL and decreases the level of HDL when compared with the normal control. After the 28 days treatment with RC-ME, RC-EE and RC-EAE at 200 and 400 mg/kg, reduces the levels of cholesterol, triglycerides, LDL, VLDL and significant increase in HDL level when compared to diabetic mice. Standard drug glibenclamide also reduces the levels of cholesterol, triglycerides, LDL, VLDL and significant increases the HDL level when compared with the diabetic mice as described in the Table 4.20.

4.7.6. Effect of *Ricinus communis* extracts on the liver function markers

The Alloxan induced group showed significant increase in the levels of bilirubin, ALT, ALP and AST when compared with the normal control. After the 28 days treatment with RC-

ME, RC-EE and RC-EAE at 200 and 400 mg/kg, reduces the levels of bilirubin, ALT, ALP and AST when compared to diabetic mice. Standard drug glibenclamide also reduces the levels of bilirubin, ALT, ALP and AST when compared to diabetic mice as described in the Table 4.21.

4.7.7. Effect of *Ricinus communis* extracts on the kidney function markers

The Alloxan induced group showed significant increase in the levels of urea, creatinine and uric acid when compared with the normal control. After the 28 days treatment with RC-ME, RC-EE and RC-EAE at 200 and 400 mg/kg, reduces the levels of urea, creatinine and uric acid when compared to diabetic mice. Standard drug glibenclamide also reduces the levels urea, creatinine and uric acid when compared to diabetic mice as described in the Table 4.22.

Table 4.17: Effect of *Ricinus communis* extracts on oral glucose tolerance test

Groups	Blood Glucose Levels at Varying Times (mg/dl)				
	0 Hr	2 Hr	4 Hr	6 Hr	8 Hr
NC	79.67 ± 7.25^{e}	82.33 ± 5.27^{f}	84.00 ± 4.81^{g}	81.50 ± 3.78^{f}	84.17 ± 1.47^{f}
DC	260.67 ± 10.33^{d}	269.67 ± 10.32^{ab}	275.83 ± 8.63^{a}	277.83 ± 12.27^{a}	279.83 ± 10.17^{a}
PC	255.50 ± 10.15^{d}	225.67 ± 2.16^{e}	198.83 ± 7.75^{f}	177.00 ± 6.81^{e}	164.50 ± 6.66^{e}
RC-ME 200	298.83 ± 9.68^{a}	280.83 ± 5.67^{a}	264.50 ± 8.53^{ab}	252.33 ± 8.93^{b}	245.00 ± 7.69^{b}
RC-ME 400	280.83 ± 7.44^{bc}	259.33 ± 5.00^{bc}	238.33 ± 6.65^{de}	223.00 ± 5.76^{d}	213.17 ± 9.80^{d}
RC-EE 200	261.83 ± 7.47^{d}	259.33 ± 9.69^{bc}	247.17 ± 7.98^{cd}	240.83 ± 9.64^{bc}	234.33 ± 6.12^{bc}
RC-EE 400	254.83 ± 9.23^{d}	241.33 ± 4.41^{d}	227.83 ± 5.23^{e}	217.67 ± 5.75^{d}	209.00 ± 7.24^{d}
RC-EAE 200	267.83 ± 6.58^{cd}	253.00 ± 7.48^{cd}	241.33 ± 8.89^{de}	228.50 ± 5.79^{cd}	218.33 ± 6.19^{d}
RC-EAE 400	294.83 ± 4.91^{ab}	276.17 ± 5.46^{a}	259.67 ± 10.01^{bc}	243.33 ± 7.11^{b}	231.83 ± 2.71^{c}

Results are expressed as mean ± standard deviation (n=6). Means with different superscript (a-g) letters in the column are significantly ($P < 0.05$) different from one another.

Table 4.18: Effect of *Ricinus communis* extracts on the blood glucose level in mice

Groups	Blood Glucose Level (mg/dl)				
	0 Day	7th Day	14th Day	21st Day	28th Day
NC	79.67 ± 7.25^{e}	81.83 ± 6.11^{e}	80.33 ± 9.91^{f}	84.33 ± 9.02^{f}	86.33 ± 10.94^{f}
DC	260.67 ± 10.33^{d}	268.00 ± 11.08^{a}	270.67 ± 14.29^{a}	275.33 ± 13.64^{a}	273.67 ± 13.00^{a}
PC	255.50 ± 10.15^{d}	215.33 ± 8.26^{d}	179.00 ± 6.39^{e}	145.33 ± 8.33^{e}	111.67 ± 5.47^{e}
RC-ME 200	298.83 ± 9.68^{a}	268.33 ± 6.89^{a}	243.17 ± 8.01^{b}	219.67 ± 6.56^{b}	195.67 ± 2.73^{b}
RC-ME 400	280.83 ± 7.44^{bc}	247.67 ± 8.82^{b}	218.00 ± 14.60^{cd}	191.33 ± 9.05^{d}	166.17 ± 8.95^{d}
RC-EE 200	261.83 ± 7.47^{d}	237.67 ± 8.64^{bc}	214.83 ± 8.68^{d}	195.67 ± 6.02^{cd}	178.67 ± 7.33^{cd}
RC-EE 400	254.83 ± 9.23^{d}	227.50 ± 8.31^{cd}	203.17 ± 11.91^{d}	182.17 ± 8.35^{d}	164.17 ± 6.52^{d}
RC-EAE 200	267.83 ± 6.58^{cd}	241.50 ± 6.92^{bc}	216.33 ± 8.80^{d}	195.17 ± 8.77^{cd}	177.17 ± 7.55^{cd}
RC-EAE 400	294.83 ± 4.91^{ab}	263.50 ± 5.43^{a}	235.83 ± 5.95^{bc}	210.83 ± 10.70^{bc}	188.00 ± 6.99^{bc}

Results are expressed as mean ± standard deviation (n=6). Means with different superscript (a-f) letters in the column are significantly ($P < 0.05$) different from one another.

Table 4.19: Effect of *Ricinus communis* extracts on the body weight

Groups	Changes in body weight (gm)	
	Initial day	Final day
NC	28.67 ± 2.73^{a}	32.33 ± 1.75^{ab}
DC	28.33 ± 2.80^{a}	23.67 ± 1.21^{d}
PC	29.17 ± 2.71^{a}	32.17 ± 0.75^{ab}
RC-ME 200	30.17 ± 2.23^{a}	31.67 ± 1.63^{ab}
RC-ME 400	32.33 ± 1.21^{a}	34.17 ± 0.98^{a}
RC-EE 200	29.33 ± 2.58^{a}	30.83 ± 1.60^{bc}
RC-EE 400	30.17 ± 2.13^{a}	31.83 ± 1.47^{ab}
RC-EAE 200	30.33 ± 2.87^{a}	29.67 ± 1.21^{bc}
RC-EAE 400	28.50 ± 2.67^{a}	28.67 ± 2.42^{c}

Results are expressed as mean ± standard deviation (n=6). Means with different superscript (a-d) letters in the column are significantly ($P < 0.05$) different from one another.

Table 4.20: Effect of *Ricinus communis* extracts on the serum lipid profile

Groups	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
NC	100.83 ± 5.81^{f}	91.33 ± 6.25^{e}	42.11 ± 2.87^{a}	61.83 ± 3.92^{f}	18.30 ± 1.27^{e}
DC	186.33 ± 7.14^{a}	141.17 ± 6.52^{a}	26.05 ± 2.56^{e}	136.50 ± 5.43^{a}	28.23 ± 1.30^{a}
PC	106.67 ± 6.47^{f}	97.50 ± 7.09^{de}	39.79 ± 1.45^{ab}	70.33 ± 3.98^{ef}	19.50 ± 1.42^{de}
RC-ME 200	127.17 ± 2.14^{cde}	108.67 ± 5.68^{bc}	35.96 ± 1.91^{bc}	83.17 ± 4.40^{cd}	21.73 ± 1.14^{bc}
RC-ME 400	119.83 ± 1.83^{f}	105.17 ± 3.60^{bcd}	39.27 ± 2.63^{ab}	77.83 ± 4.12^{de}	21.03 ± 0.72^{bcd}
RC-EE 200	136.83 ± 2.86^{b}	114.17 ± 5.31^{b}	33.63 ± 2.57^{cd}	103.33 ± 7.06^{b}	22.83 ± 1.06^{b}
RC-EE 400	132.67 ± 2.58^{bc}	109.83 ± 4.99^{bc}	30.74 ± 1.00^{d}	99.17 ± 5.67^{b}	21.97 ± 0.99^{bc}
RC-EAE 200	130.50 ± 1.76^{bcd}	106.17 ± 4.91^{bcd}	34.75 ± 2.81^{cd}	89.83 ± 4.67^{c}	21.23 ± 0.98^{bcd}
RC-EAE 400	123.17 ± 3.49^{de}	103.83 ± 3.82^{cd}	36.54 ± 1.25^{bc}	86.17 ± 4.26^{cd}	20.77 ± 0.77^{cd}

Results are expressed as mean ± standard deviation (n=6). Means with different superscript (a-f) letters in the column are significantly ($P < 0.05$) different from one another.

Table 4.21: Effect of *Ricinus communis* extracts on the liver function markers

Groups	Bilirubin (mg/dl)	ALT (u/l)	ALP (u/l)	AST (u/l)
NC	0.42 ± 0.03^{d}	30.67 ± 2.87^{e}	114.67 ± 5.17^{e}	26.83 ± 4.70^{d}
DC	1.73 ± 0.13^{a}	70.83 ± 6.52^{a}	278.67 ± 20.38^{a}	91.67 ± 7.03^{a}
PC	0.52 ± 0.04^{d}	35.17 ± 2.99^{de}	128.33 ± 7.11^{e}	37.33 ± 4.67^{e}
RC-ME 200	0.83 ± 0.05^{c}	41.67 ± 4.03^{bcd}	166.00 ± 9.96^{bcd}	41.50 ± 2.59^{bc}
RC-ME 400	0.81 ± 0.04^{c}	39.83 ± 3.06^{cd}	160.33 ± 10.88^{bcd}	39.17 ± 3.06^{bc}
RC-EE 200	1.005 ± 0.07^{b}	48.50 ± 0.84^{b}	179.17 ± 8.91^{b}	47.00 ± 4.56^{b}
RC-EE 400	0.98 ± 0.08^{b}	47.33 ± 2.33^{b}	170.83 ± 7.41^{bc}	44.67 ± 4.63^{bc}
RC-EAE 200	0.81 ± 0.05^{c}	43.50 ± 4.63^{bc}	157.83 ± 11.25^{cd}	43.00 ± 2.76^{bc}
RC-EAE 400	0.76 ± 0.05^{c}	44.50 ± 3.15^{bc}	149.50 ± 8.48^{d}	41.83 ± 3.06^{bc}

Results are expressed as mean ± standard deviation (n=6). Means with different superscript (a-e) letters in the column are significantly ($P < 0.05$) different from one another.

Table 4.22: Effect of *Ricinus communis* extracts on the kidney function markers

Groups	Urea (mg/dl)	Creatinine (mg/dl)	Uric Acid (mg/dl)
NC	30.67 ± 2.73^{f}	0.94 ± 0.11^{d}	3.87 ± 0.37^{g}
DC	75.17 ± 2.86^{a}	2.17 ± 0.28^{a}	9.84 ± 0.27^{a}
PC	37.33 ± 1.86^{e}	1.14 ± 0.15^{cd}	4.11 ± 0.23^{fg}
RC-ME 200	46.33 ± 3.07^{bcd}	1.40 ± 0.08^{b}	4.67 ± 0.29^{e}
RC-ME 400	43.33 ± 2.73^{de}	1.32 ± 0.09^{bc}	4.51 ± 0.23^{ef}
RC-EE 200	51.33 ± 5.09^{b}	1.54 ± 0.06^{b}	5.97 ± 0.11^{b}
RC-EE 400	49.67 ± 3.07^{bc}	1.51 ± 0.06^{b}	5.66 ± 0.21^{bc}
RC-EAE 200	44.67 ± 2.87^{cd}	1.45 ± 0.04^{b}	5.18 ± 0.19^{cd}
RC-EAE 400	45.17 ± 4.67^{bcd}	1.38 ± 0.11^{b}	4.88 ± 0.31^{de}

Results are expressed as mean ± standard deviation (n=6). Means with different superscript (a-g) letters in the column are significantly ($P < 0.05$) different from one another.

4.8. Effect of methanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on biochemical parameters

4.8.1. Effect of methanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on total cholesterol

The Alloxan induced group showed significant increase in the levels of cholesterol (100.83 ± 5.81 mg/dl to 186.33 ± 7.14 mg/dl) when compared with the normal control. Glibenclamide decreases the levels of cholesterol when compared to diabetic mice. After the 28 days treatment with MA-ME at 200 and 400 mg/kg, decreases the levels of cholesterol (139.50 ± 1.87 mg/dl and 138.50 ± 1.87 mg/dl) when compared to diabetic mice. After the 28 days treatment with JA-ME at 200 and 400 mg/kg, decreases the levels of cholesterol (135.17 ± 2.56 mg/dl and 133.00 ± 3.03 mg/dl) when compared to diabetic mice. After the 28 days treatment with RC-ME at 200 and 400 mg/kg, decreases the levels of cholesterol (127.17 ± 2.14 mg/dl and 119.83 ± 1.83 mg/dl) when compared to diabetic mice as shown in Figure 4.11.

4.8.2. Effect of methanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on triglycerides

The Alloxan induced group showed significant increase in the levels of triglycerides (91.33 ± 6.25 mg/dl to 141.17 ± 6.52 mg/dl) when compared with the normal control. Glibenclamide decreases the levels of triglycerides when compared to diabetic mice. After the 28 days treatment with MA-ME at 200 and 400 mg/kg, decreases the levels of triglycerides (118.50 ± 3.27 mg/dl and 116.17 ± 2.93 mg/dl) when compared to diabetic mice. After the 28 days treatment with JA-ME at 200 and 400 mg/kg, decreases the levels of triglycerides (114.67 ± 3.26 mg/dl and 110.50 ± 3.27 mg/dl) when compared to diabetic mice. After the 28 days treatment with RC-ME at 200 mg/kg and 400 mg/kg, decreases the levels of triglycerides (108.67 ± 5.68 mg/dl and 105.17 ± 3.60 mg/dl) when compared to diabetic mice as shown in Figure 4.12.

4.8.3. Effect of methanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on high-density lipoprotein

The Alloxan induced group showed significant decrease in the levels of HDL (42.11 ± 2.87 mg/dl to 26.05 ± 2.56 mg/dl) when compared with the normal control. Glibenclamide

increases the levels of HDL when compared to diabetic mice. After the 28 days treatment with MA-ME at 200 and 400 mg/kg, increases the levels of HDL (31.48 ± 1.07 mg/dl and 34.24 ± 0.68 mg/dl) when compared to diabetic mice. After the 28 days treatment with JA-ME at 200 and 400 mg/kg, increases the levels of HDL (33.37 ± 2.44 mg/dl and 36.43 ± 1.91 mg/dl) when compared to diabetic mice. After the 28 days treatment with RC-ME at 200 and 400 mg/kg, increases the levels of HDL (35.96 ± 1.91 mg/dl and 39.27 ± 2.63 mg/dl) when compared to diabetic mice as shown in Figure 4.13.

4.8.4. Effect of methanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on low-density lipoprotein

The Alloxan induced group showed significant increase in the levels of LDL (61.83 ± 3.92 mg/dl to 136.50 ± 5.43 mg/dl) when compared with the normal control. Glibenclamide decreases the levels of LDL when compared to diabetic mice. After the 28 days treatment with MA-ME at 200 and 400 mg/kg, decreases the levels of LDL (98.00 ± 3.46 mg/dl and 91.33 ± 2.73 mg/dl) when compared to diabetic mice. After the 28 days treatment with JA-ME at 200 and 400 mg/kg, decreases the levels of LDL (94.00 ± 3.58 mg/dl and 87.17 ± 3.43 mg/dl) when compared to diabetic mice. After the 28 days treatment with RC-ME at 200 and 400 mg/kg, decreases the levels of LDL (83.17 ± 4.40 mg/dl and 77.83 ± 4.12 mg/dl) when compared to diabetic mice as shown in Figure 4.14.

4.8.5. Effect of methanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on very-low-density lipoprotein

The Alloxan induced group showed significant increase in the levels of VLDL (18.30 ± 1.27 mg/dl to 28.23 ± 1.30 mg/dl) as compared to normal control. Glibenclamide decreases the levels of VLDL when compared to diabetic mice. After the 28 days treatment with MA-ME at 200 and 400 mg/kg, decreases the levels of VLDL (23.70 ± 0.65 mg/dl and 23.23 ± 0.58 mg/dl) when compared to diabetic mice. After the 28 days treatment with JA-ME at 200 and 400 mg/kg, decreases the levels of VLDL (22.93 ± 0.65 mg/dl and 22.10 ± 0.65 mg/dl) when compared to diabetic mice. After the 28 days treatment with RC-ME at 200 and 400 mg/kg, decreases the levels of VLDL (21.73 ± 1.14 mg/dl and 21.03 ± 0.72 mg/dl) when compared to diabetic mice as shown in Figure 4.15.

4.8.6. Effect of methanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on bilirubin

The Alloxan induced group showed significant increase in the levels of bilirubin (0.42 ± 0.03 mg/dl to 1.73 ± 0.13 mg/dl) as compared to normal control. Glibenclamide decreases the levels of bilirubin when compared to diabetic mice. After the 28 days treatment with MA-ME at 200 and 400 mg/kg, decreases the levels of bilirubin (0.89 ± 0.02 mg/dl and 0.87 ± 0.07 mg/dl) when compared to diabetic mice. After the 28 days treatment with JA-ME at 200 and 400 mg/kg, decreases the levels of bilirubin (0.84 ± 0.04 mg/dl and 0.83 ± 0.03 mg/dl) when compared to diabetic mice. After the 28 days treatment with RC-ME at 200 and 400 mg/kg, decreases the levels of bilirubin (0.83 ± 0.05 mg/dl and 0.81 ± 0.04 mg/dl) when compared to diabetic mice as shown in Figure 4.16.

4.8.7. Effect of methanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on alanine aminotransferase

The Alloxan induced group showed significant increase in the levels of ALT (30.67 ± 2.87 u/l to 70.83 ± 6.52 u/l) as compared to normal control. Glibenclamide decreases the levels of ALT when compared to diabetic mice. After the 28 days treatment with MA-ME at 200 and 400 mg/kg, decreases the levels of ALT (47.50 ± 3.73 u/l and 45.83 ± 1.47 u/l) when compared to diabetic mice. After the 28 days treatment with JA-ME at 200 and 400 mg/kg, decreases the levels of ALT (44.67 ± 3.78 u/l and 43.00 ± 2.76 u/l) when compared to diabetic mice. After the 28 days treatment with RC-ME at 200 and 400 mg/kg, decreases the levels of ALT (41.67 ± 4.03 u/l and 39.83 ± 3.06 u/l) when compared to diabetic mice as shown in Figure 4.17.

4.8.8. Effect of methanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on alkaline phosphatase

The Alloxan induced group showed significant increase in the levels of ALP (114.67 ± 5.17 u/l to 278.67 ± 20.38 u/l) as compared to normal control. Glibenclamide decreases the levels of ALP when compared to diabetic mice. After the 28 days treatment with MA-ME at 200 and 400 mg/kg, decreases the levels of ALP (174.83 ± 4.99 u/l and 169.50 ± 3.56 u/l) when compared to diabetic mice. After the 28 days treatment with JA-ME at 200 and 400 mg/kg, decreases the levels of ALP (168.67 ± 9.10 u/l and 163.67 ± 6.50 u/l) when compared to diabetic

mice. After the 28 days treatment with RC-ME at 200 and 400 mg/kg, decreases the levels of ALP (166.00 ± 9.96 u/l and 160.33 ± 10.88 u/l) when compared to diabetic mice as shown in Figure 4.18.

4.8.9. Effect of methanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on aspartate aminotransferase

The Alloxan induced group showed significant increase in the levels of AST (26.83 ± 4.70 u/l to 91.67 ± 7.03 u/l) as compared to normal control. Glibenclamide decreases the levels of AST when compared to diabetic mice. After the 28 days treatment with MA-ME at 200 and 400 mg/kg, decreases the levels of AST (47.33 ± 4.17 u/l and 46.50 ± 4.41 u/l) when compared to diabetic mice. After the 28 days treatment with JA-ME at 200 and 400 mg/kg, decreases the levels of AST (44.67 ± 4.55 u/l and 43.17 ± 3.76 u/l) when compared to diabetic mice. After the 28 days treatment with RC-ME at 200 and 400 mg/kg, decreases the elevated levels of AST (41.50 ± 2.59 u/l and 39.17 ± 3.06 u/l) when compared to diabetic mice as shown in Figure 4.19.

4.8.10. Effect of methanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on urea

The Alloxan induced group showed significant increase in the levels of urea (30.67 ± 2.73 mg/dl to 75.17 ± 2.86 mg/dl) as compared to normal control. Glibenclamide decreases the levels of urea when compared to diabetic mice. After the 28 days treatment with MA-ME at 200 and 400 mg/kg, decreases the levels of urea (53.00 ± 2.60 mg/dl and 50.17 ± 1.72 mg/dl) when compared to diabetic mice. After the 28 days treatment with JA-ME at 200 and 400 mg/kg, decreases the levels of urea (49.00 ± 2.83 mg/dl and 45.83 ± 1.94 mg/dl) when compared to diabetic mice. After the 28 days treatment with RC-ME at 200 and 400 mg/kg, decreases the levels of urea (46.33 ± 3.07 mg/dl and 43.33 ± 2.73 mg/dl) when compared to diabetic mice as shown in Figure 4.20.

4.8.11. Effect of methanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on creatinine

The Alloxan induced group showed significant increase in the levels of creatinine (0.94 ± 0.11 mg/dl to 2.17 ± 0.28 mg/dl) as compared to normal control. Glibenclamide decreases the levels of creatinine when compared to diabetic mice. After the 28 days treatment with MA-ME at

200 and 400 mg/kg, decreases the levels of creatinine (1.44 ± 0.02 mg/dl and 1.36 ± 0.05 mg/dl) when compared to diabetic mice. After the 28 days treatment with JA-ME at 200 and 400 mg/kg, decreases the levels of creatinine (1.43 ± 0.06 mg/dl and 1.34 ± 0.07 mg/dl) when compared to diabetic mice. After the 28 days treatment with RC-ME at 200 and 400 mg/kg, decreases the levels of creatinine (1.40 ± 0.08 and 1.32 ± 0.09) when compared to diabetic mice as shown in Figure 4.21.

4.8.12. Effect of methanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on uric acid

The Alloxan induced group showed significant increase in the levels of uric acid (3.87 ± 0.37 mg/dl to 9.84 ± 0.27 mg/dl) as compared to normal control. Glibenclamide decreases the levels of uric acid when compared to diabetic mice. After the 28 days treatment with MA-ME at 200 and 400 mg/kg, decreases the levels of uric acid (4.78 ± 0.15 mg/dl and 4.58 ± 0.11 mg/dl) when compared to diabetic mice. After the 28 days treatment with JA-ME at 200 and 400 mg/kg, decreases the levels of uric acid (4.73 ± 0.22 mg/dl and 4.56 ± 0.15 mg/dl) when compared to diabetic mice. After the 28 days treatment with RC-ME at 200 and 400 mg/kg, decreases the levels of uric acid (4.67 ± 0.29 mg/dl and 4.51 ± 0.23 mg/dl) when compared to diabetic mice as shown in Figure 4.22.

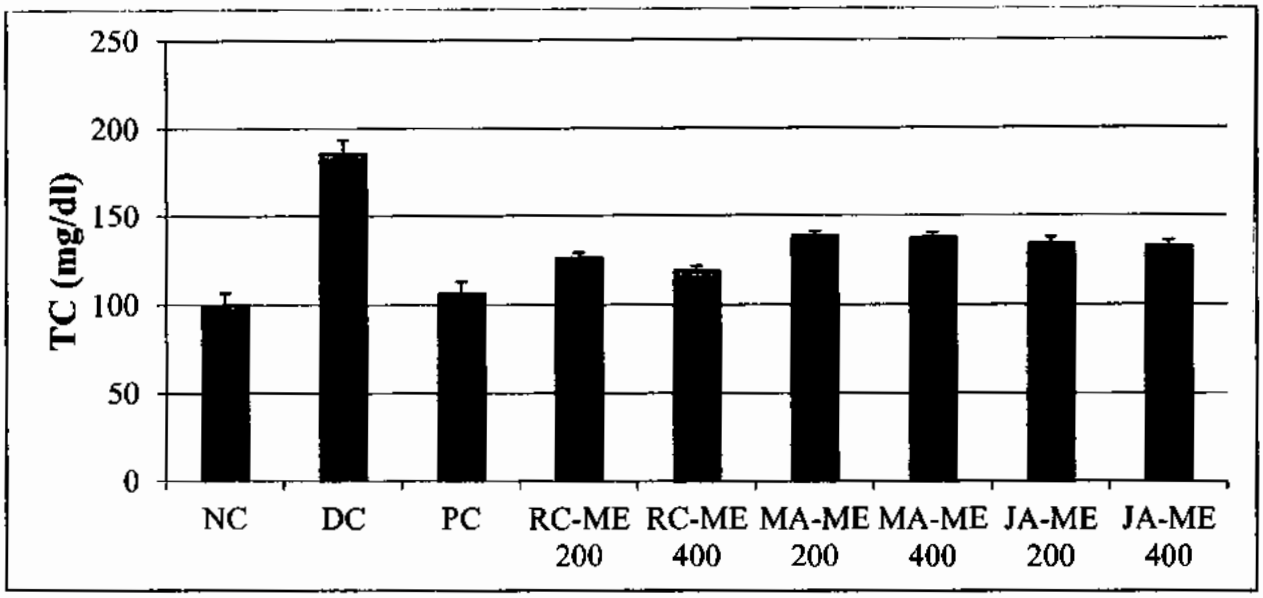


Figure 4.11: Effect of methanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on total cholesterol. Results are expressed as mean ± standard deviation (n=6).

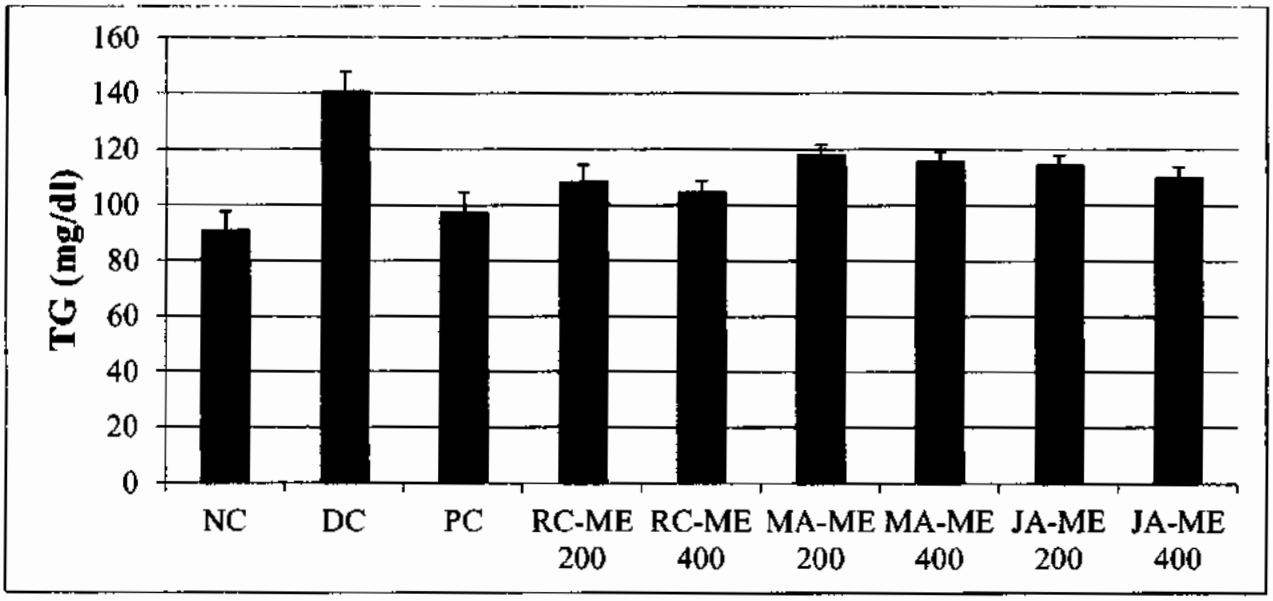


Figure 4.12: Effect of methanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on triglycerides. Results are expressed as mean ± standard deviation (n=6).

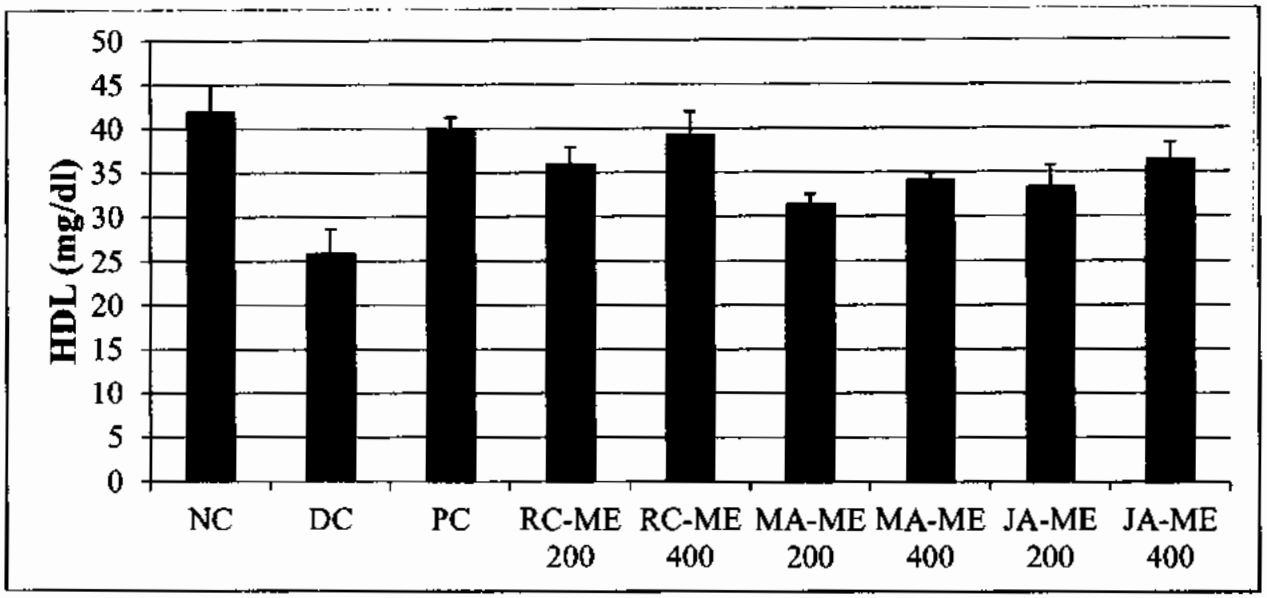


Figure 4.13: Effect of methanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on high-density lipoprotein. Results are expressed as mean ± standard deviation (n=6).

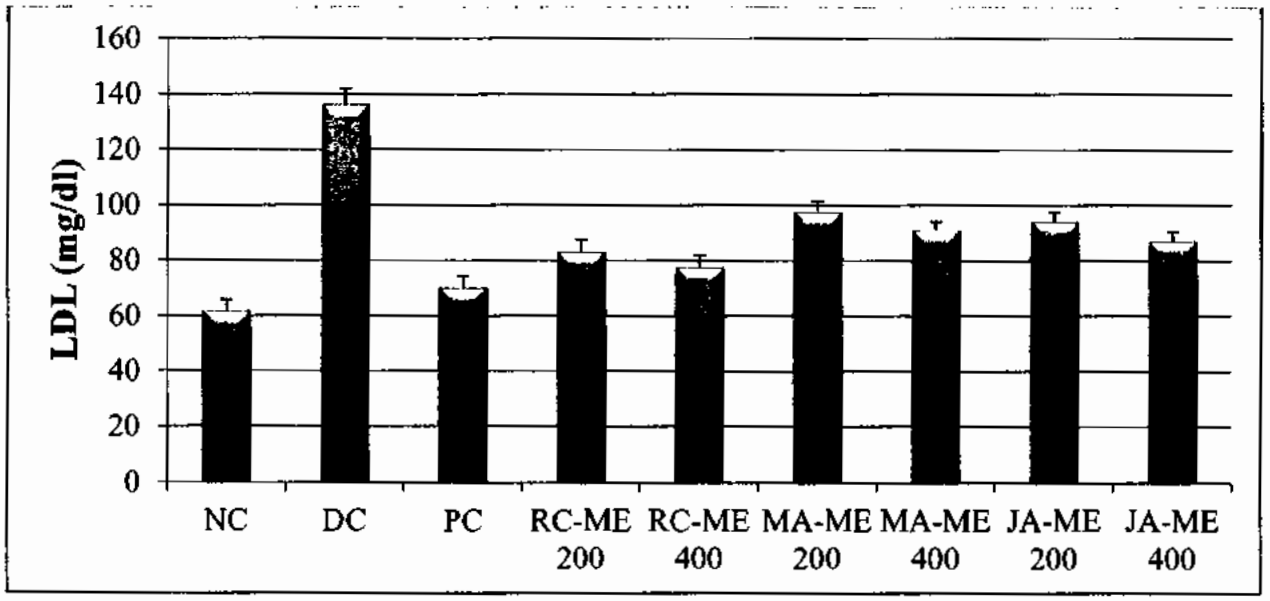


Figure 4.14: Effect of methanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on low-density lipoprotein. Results are expressed as mean ± standard deviation (n=6).

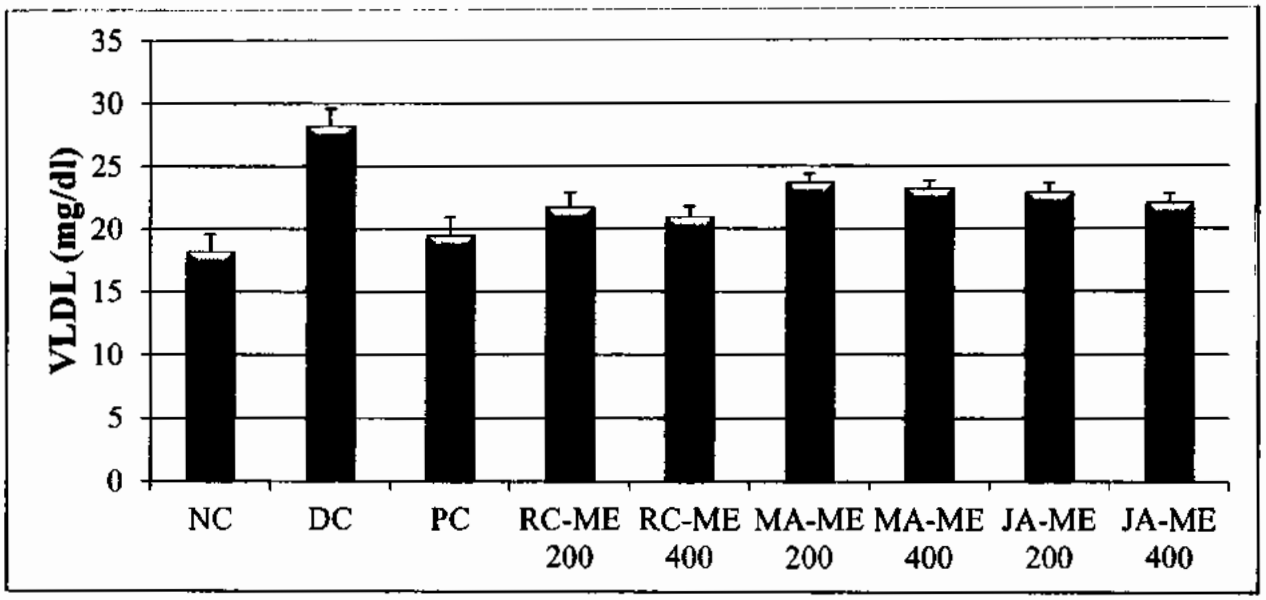


Figure 4.15: Effect of methanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on very-low-density lipoprotein. Results are expressed as mean ± standard deviation (n=6).

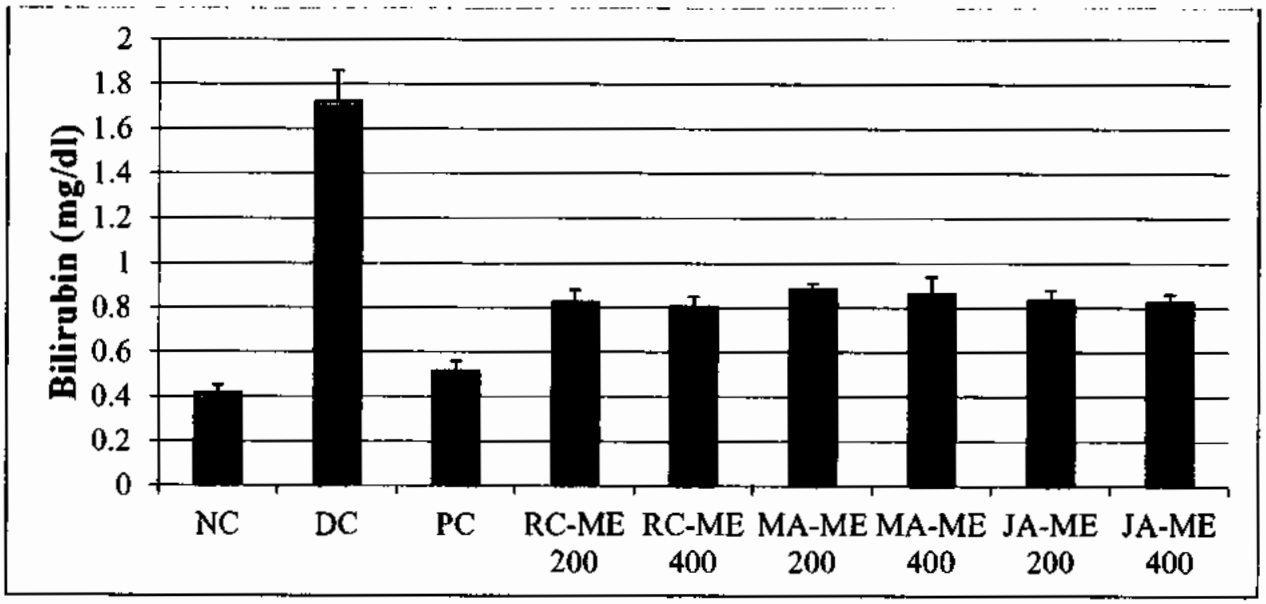


Figure 4.16: Effect of methanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on bilirubin. Results are expressed as mean ± standard deviation (n=6).

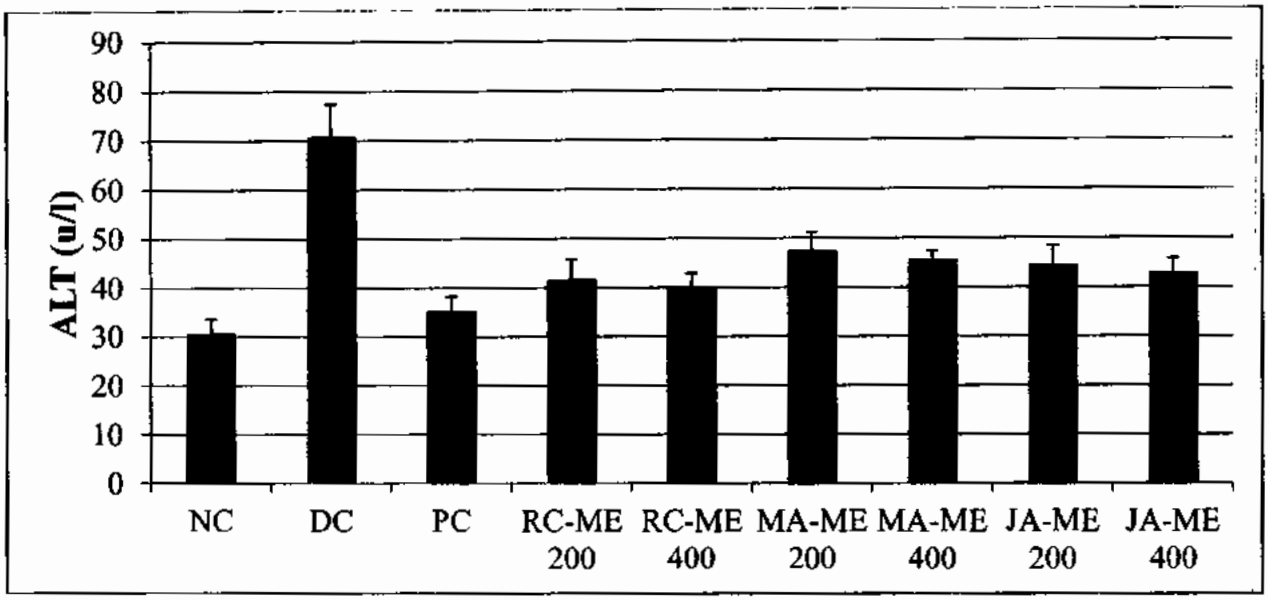


Figure 4.17: Effect of methanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on alanine aminotransferase. Results are expressed as mean ± standard deviation (n=6).

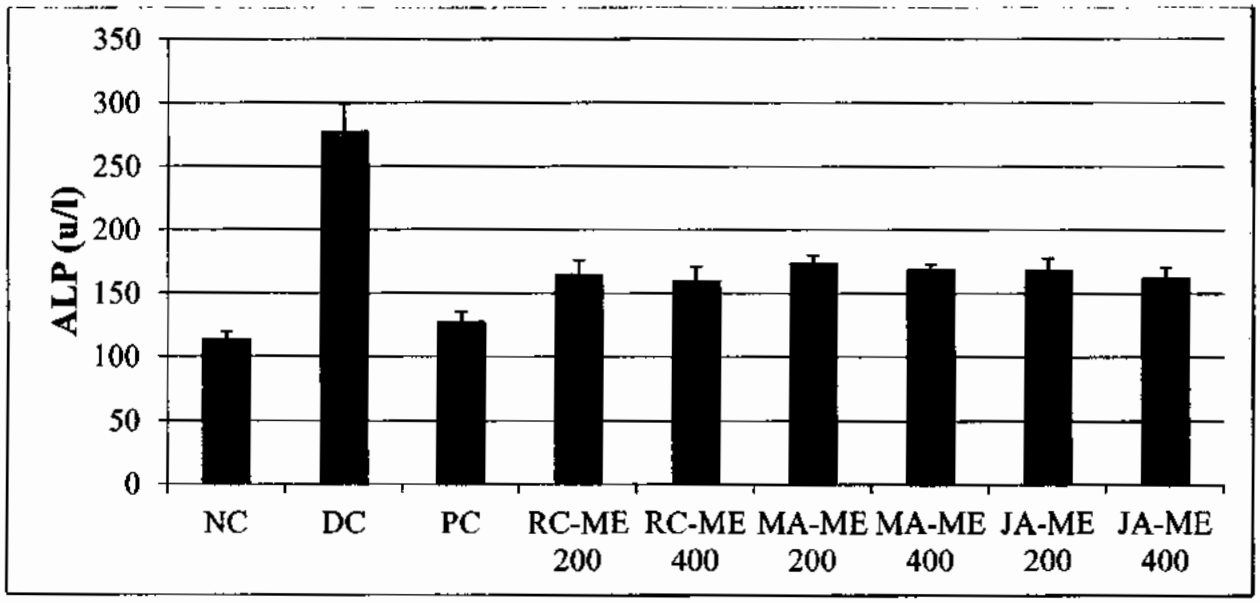


Figure 4.18: Effect of methanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on alkaline phosphatase. Results are expressed as mean ± standard deviation (n=6).

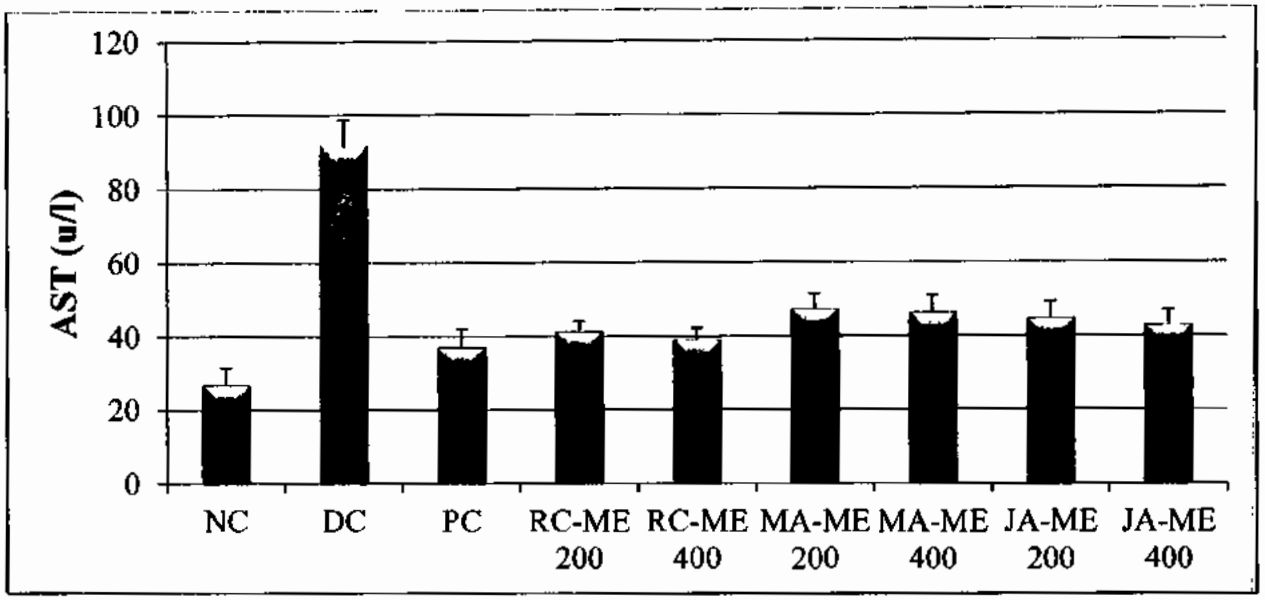


Figure 4.19: Effect of methanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on aspartate aminotransferase. Results are expressed as mean ± standard deviation (n=6).

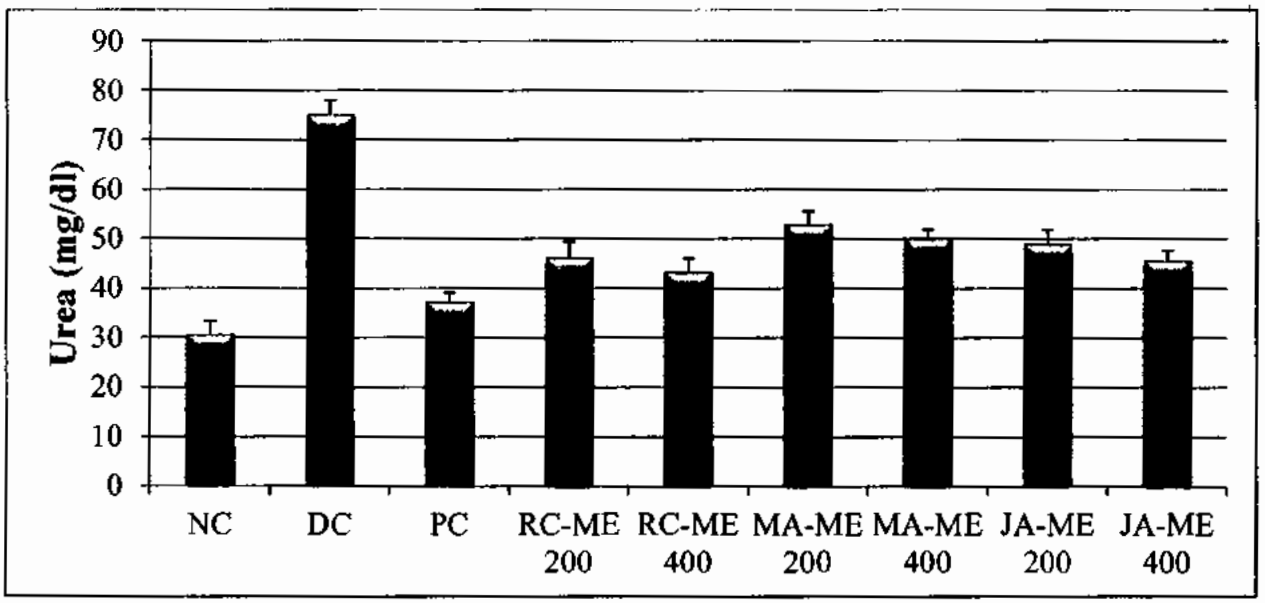


Figure 4.20: Effect of methanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on urea. Results are expressed as mean ± standard deviation (n=6).

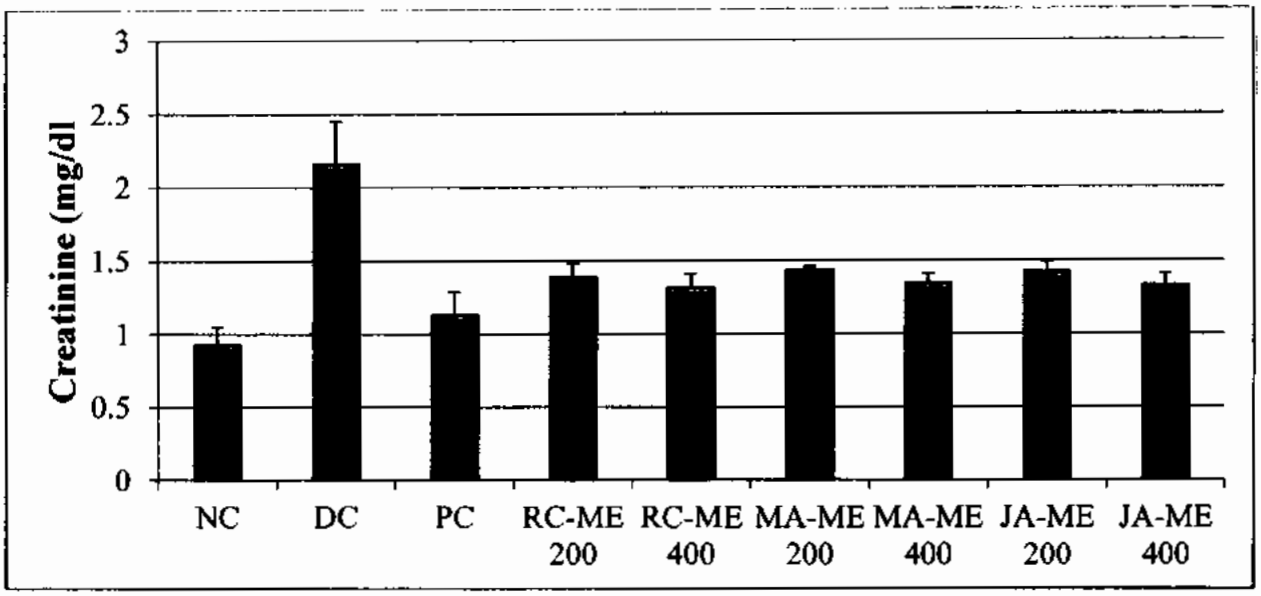


Figure 4.21: Effect of methanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on creatinine. Results are expressed as mean ± standard deviation (n=6).

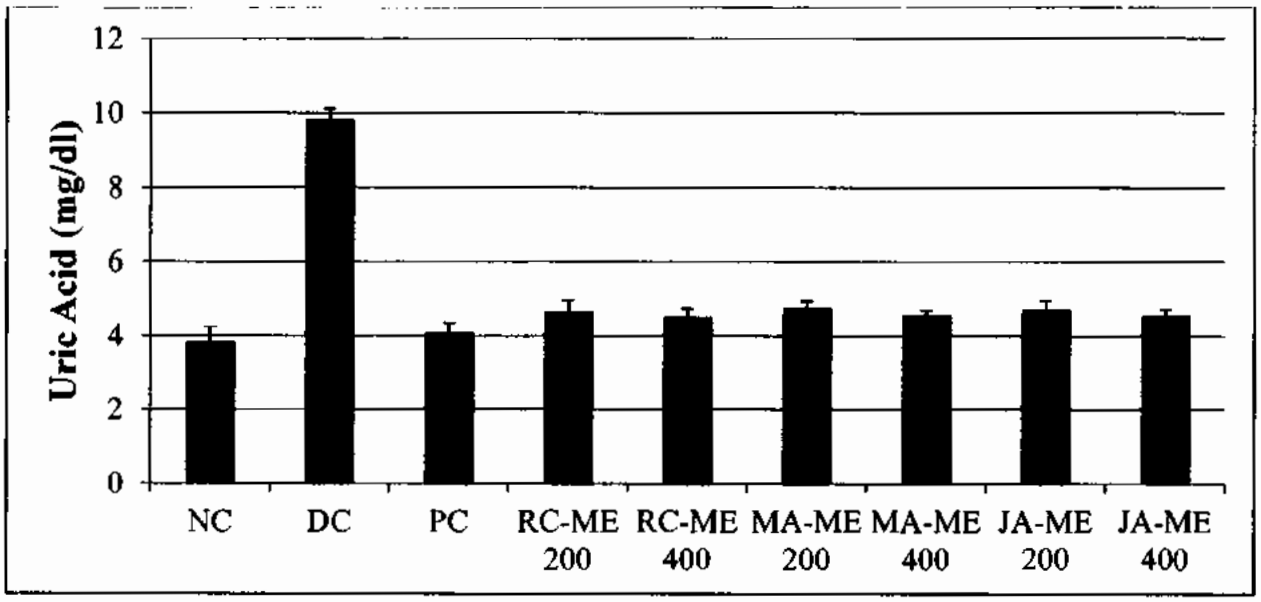


Figure 4.22: Effect of methanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on uric acid. Results are expressed as mean ± standard deviation (n=6).

4.9. Effect of ethanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on biochemical parameters

4.9.1. Effect of ethanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on total cholesterol

The Alloxan induced group showed significant increase in the levels of cholesterol (100.83 ± 5.81 mg/dl to 186.33 ± 7.14 mg/dl) as compared to normal control. Glibenclamide decreases the levels of cholesterol when compared to diabetic mice. After the 28 days treatment with MA-EE at 200 and 400 mg/kg, decreases the levels of cholesterol (138.00 ± 4.69 mg/dl and 136.50 ± 3.27 mg/dl) when compared to diabetic mice. After the 28 days treatment with JA-EE at 200 and 400 mg/kg, decreases the levels of cholesterol (133.83 ± 2.32 mg/dl and 130.67 ± 5.16 mg/dl) when compared to diabetic mice. After the 28 days treatment with RC-EE at 200 and 400 mg/kg, decreases the levels of cholesterol (136.83 ± 2.86 mg/dl and 132.67 ± 2.58 mg/dl) when compared to diabetic mice as shown in Figure 4.23.

4.9.2. Effect of ethanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on triglycerides

The Alloxan induced group showed significant increase in the levels of triglycerides (91.33 ± 6.25 mg/dl to 141.17 ± 6.52 mg/dl) as compared to normal control. Glibenclamide decreases the levels of triglycerides when compared to diabetic mice. After the 28 days treatment with MA-EE at 200 and 400 mg/kg, decreases the levels of triglycerides (116.17 ± 3.71 mg/dl and 113.83 ± 2.40 mg/dl) when compared to diabetic mice. After the 28 days treatment with JA-EE at 200 and 400 mg/kg, decreases the levels of triglycerides (111.17 ± 5.27 mg/dl and 109.67 ± 3.98 mg/dl) when compared to diabetic mice. After the 28 days treatment with RC-EE at 200 and 400 mg/kg, decreases the levels of triglycerides (114.17 ± 5.31 mg/dl and 109.83 ± 4.99 mg/dl) when compared to diabetic mice as shown in Figure 4.24.

4.9.3. Effect of ethanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on high-density lipoprotein

The Alloxan induced group showed significant decrease in the levels of HDL (42.11 ± 2.87 mg/dl to 26.05 ± 2.56 mg/dl) as compared to normal control. Glibenclamide increases the

levels of HDL when compared to diabetic mice. After the 28 days treatment with MA-EE at 200 and 400 mg/kg, increases the levels of HDL (30.87 ± 1.49 mg/dl and 35.68 ± 1.35 mg/dl) when compared to diabetic mice. After the 28 days treatment with JA-EE at 200 and 400 mg/kg, increases the levels of HDL (34.43 ± 1.63 mg/dl and 37.65 ± 1.57 mg/dl) when compared to diabetic mice. After the 28 days treatment with RC-EE at 200 and 400 mg/kg, increases the levels of HDL (33.63 ± 2.57 mg/dl and 30.74 ± 1.00 mg/dl) when compared to diabetic mice as shown in Figure 4.25.

4.9.4. Effect of ethanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on low-density lipoprotein

The Alloxan induced group showed significant increase in the levels of LDL (61.83 ± 3.92 mg/dl to 136.50 ± 5.43 mg/dl) as compared to normal control. Glibenclamide decreases the levels of LDL when compared to diabetic mice. After the 28 days treatment with MA-EE at 200 and 400 mg/kg, decreases the levels of LDL (96.50 ± 5.75 mg/dl and 93.00 ± 6.84 mg/dl) when compared to diabetic mice. After the 28 days treatment with JA-EE at 200 and 400 mg/kg, decreases the levels of LDL (92.67 ± 3.73 mg/dl and 85.67 ± 2.94 mg/dl) when compared to diabetic mice. After the 28 days treatment with RC-EE at 200 and 400 mg/kg, decreases the levels of LDL (103.33 ± 7.06 mg/dl and 99.17 ± 5.67 mg/dl) when compared to diabetic mice as shown in Figure 4.26.

4.9.5. Effect of ethanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on very-low-density lipoprotein

The Alloxan induced group showed significant increase in the levels of VLDL (18.30 ± 1.27 mg/dl to 28.23 ± 1.30 mg/dl) as compared to normal control. Glibenclamide decreases the levels of VLDL when compared to diabetic mice. After the 28 days treatment with MA-EE at 200 and 400 mg/kg, decreases the levels of VLDL (23.33 ± 0.74 mg/dl and 22.77 ± 0.48 mg/dl) when compared to diabetic mice. After the 28 days treatment with JA-EE at 200 and 400 mg/kg, decreases the levels of VLDL (22.20 ± 1.05 mg/dl and 21.93 ± 0.79 mg/dl) when compared to diabetic mice. After the 28 days treatment with RC-EE at 200 and 400 mg/kg, decreases the levels of VLDL (22.83 ± 1.06 mg/dl and 21.97 ± 0.99 mg/dl) when compared to diabetic mice as shown in Figure 4.27.

4.9.6. Effect of ethanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on bilirubin

The Alloxan induced group showed significant increase in the levels of bilirubin (0.42 ± 0.03 mg/dl to 1.73 ± 0.13 mg/dl) as compared to normal control. Glibenclamide decreases the levels of bilirubin when compared to diabetic mice. After the 28 days treatment with MA-EE at 200 and 400 mg/kg, decreases the levels of bilirubin (0.87 ± 0.04 mg/dl and 0.83 ± 0.08 mg/dl) when compared to diabetic mice. After the 28 days treatment with JA-EE at 200 and 400 mg/kg, decreases the levels of bilirubin (0.83 ± 0.04 mg/dl and 0.79 ± 0.02 mg/dl) when compared to diabetic mice. After the 28 days treatment with RC-EE at 200 and 400 mg/kg, decreases the levels of bilirubin (1.005 ± 0.07 mg/dl and 0.98 ± 0.08 mg/dl) when compared to diabetic mice as shown in Figure 4.28.

4.9.7. Effect of ethanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on alanine aminotransferase

The Alloxan induced group showed significant increase in the levels of ALT (30.67 ± 2.87 u/l to 70.83 ± 6.52 u/l) as compared to normal control. Glibenclamide decreases the levels of ALT when compared to diabetic mice. After the 28 days treatment with MA-EE at 200 and 400 mg/kg, decreases the levels of ALT (49.83 ± 2.63 u/l and 48.67 ± 1.63 u/l) when compared to diabetic mice. After the 28 days treatment with JA-EE at 200 and 400 mg/kg, decreases the levels of ALT (46.83 ± 4.71 u/l and 47.17 ± 2.40 u/l) when compared to diabetic mice. After the 28 days treatment with RC-EE at 200 and 400 mg/kg, decreases the levels of ALT (48.50 ± 0.84 u/l and 47.33 ± 2.33 u/l) when compared to diabetic mice as shown in Figure 4.29.

4.9.8. Effect of ethanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on alkaline phosphatase

The Alloxan induced group showed significant increase in the levels of ALP (114.67 ± 5.17 u/l to 278.67 ± 20.38 u/l) as compared to normal control. Glibenclamide decreases the levels of ALP when compared to diabetic mice. After the 28 days treatment with MA-EE at 200 and 400 mg/kg, decreases the levels of ALP (167.00 ± 9.65 u/l and 164.00 ± 4.33 u/l) when compared to diabetic mice. After the 28 days treatment with JA-EE at 200 and 400 mg/kg, decreases the levels of ALP (160.17 ± 8.91 u/l and 157.00 ± 2.09 u/l) when compared to diabetic

mice. After the 28 days treatment with RC-EE at 200 and 400 mg/kg, decreases the levels of ALP (179.17 ± 8.91 u/l and 170.83 ± 7.41 u/l) when compared to diabetic mice as shown in Figure 4.30.

4.9.9. Effect of ethanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on aspartate aminotransferase

The Alloxan induced group showed significant increase in the levels of AST (26.83 ± 4.70 u/l to 91.67 ± 7.03 u/l) as compared to normal control. Glibenclamide decreases the levels of AST when compared to diabetic mice. After the 28 days treatment with MA-EE at 200 and 400 mg/kg, decreases the levels of AST (50.50 ± 3.78 u/l and 48.50 ± 2.58 u/l) when compared to diabetic mice. After the 28 days treatment with JA-EE at 200 and 400 mg/kg, decreases the levels of AST (46.67 ± 1.37 u/l and 45.67 ± 3.45 u/l) when compared to diabetic mice. After the 28 days treatment with RC-EE at 200 and 400 mg/kg, decreases the levels of AST (47.00 ± 4.56 u/l and 44.67 ± 4.63 u/l) when compared to diabetic mice as shown in Figure 4.31.

4.9.10. Effect of ethanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on urea

The Alloxan induced group showed significant increase in the levels of urea (30.67 ± 2.73 mg/dl to 75.17 ± 2.86 mg/dl) as compared to normal control. Glibenclamide decreases the levels of urea when compared to diabetic mice. After the 28 days treatment with MA-EE at 200 and 400 mg/kg, decreases the levels urea (51.00 ± 1.41 mg/dl and 49.67± 3.67 mg/dl) when compared to diabetic mice. After the 28 days treatment with JA-EE at 200 and 400 mg/kg, decreases the levels urea (47.17 ± 2.56 mg/dl and 48.17 ± 4.40 mg/dl) when compared to diabetic mice. After the 28 days treatment with RC-EE at the dose of 200 and 400 mg/kg, decreases the levels urea (51.33 ± 5.09 mg/dl and 49.67 ± 3.07 mg/dl) when compared to diabetic mice as shown in Figure 4.32.

4.9.11. Effect of ethanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on creatinine

The Alloxan induced group showed significant increase in the levels of creatinine (0.94 ± 0.11 mg/dl to 2.17 ± 0.28 mg/dl) as compared to normal control. Glibenclamide decreases the levels of creatinine when compared to diabetic mice. After the 28 days treatment with MA-EE at

200 and 400 mg/kg, decreases the levels of creatinine (1.49 ± 0.03 mg/dl and 1.48 ± 0.05 mg/dl) when compared to diabetic mice. After the 28 days treatment with JA-EE at 200 and 400 mg/kg, decreases the levels of creatinine (1.49 ± 0.04 mg/dl and 1.44 ± 0.05 mg/dl) when compared to diabetic mice. After the 28 days treatment with RC-EE at 200 and 400 mg/kg, decreases the levels of creatinine (1.54 ± 0.06 mg/dl and 1.51 ± 0.06 mg/dl) when compared to diabetic mice as shown in Figure 4.33.

4.9.12. Effect of ethanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on uric acid

The Alloxan induced group showed significant increase in the levels of uric acid (3.87 ± 0.37 mg/dl to 9.84 ± 0.27 mg/dl) as compared to normal control. Glibenclamide decreases the levels of uric acid when compared to diabetic mice. After the 28 days treatment with MA-EE at 200 and 400 mg/kg, decreases the levels of uric acid (5.39 ± 0.18 mg/dl and 5.08 ± 0.07 mg/dl) when compared to diabetic mice. After the 28 days treatment with JA-EE at 200 and 400 mg/kg, decreases the levels of uric acid (5.39 ± 0.17 mg/dl and 4.98 ± 0.25 mg/dl) when compared to diabetic mice. After the 28 days treatment with RC-EE at 200 and 400 mg/kg, decreases the levels of uric acid (5.97 ± 0.11 mg/dl and 5.66 ± 0.21 mg/dl) when compared to diabetic mice as shown in Figure 4.34.

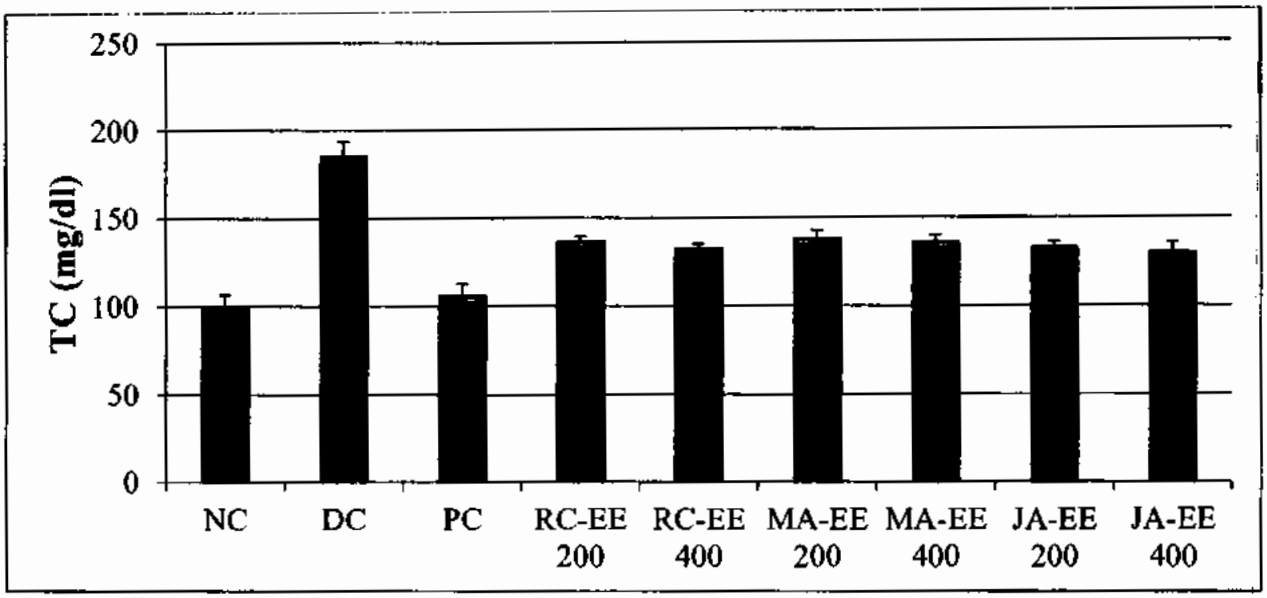


Figure 4.23: Effect of ethanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on total cholesterol. Results are expressed as mean ± standard deviation (n=6).

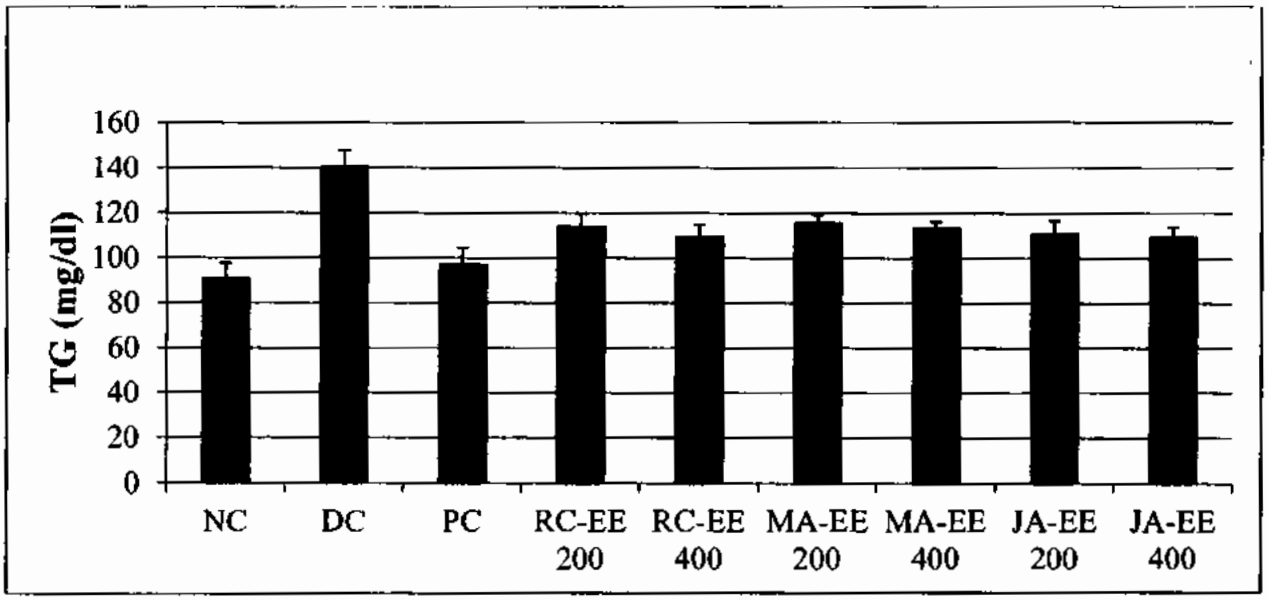


Figure 4.24: Effect of ethanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on triglycerides. Results are expressed as mean ± standard deviation (n=6).

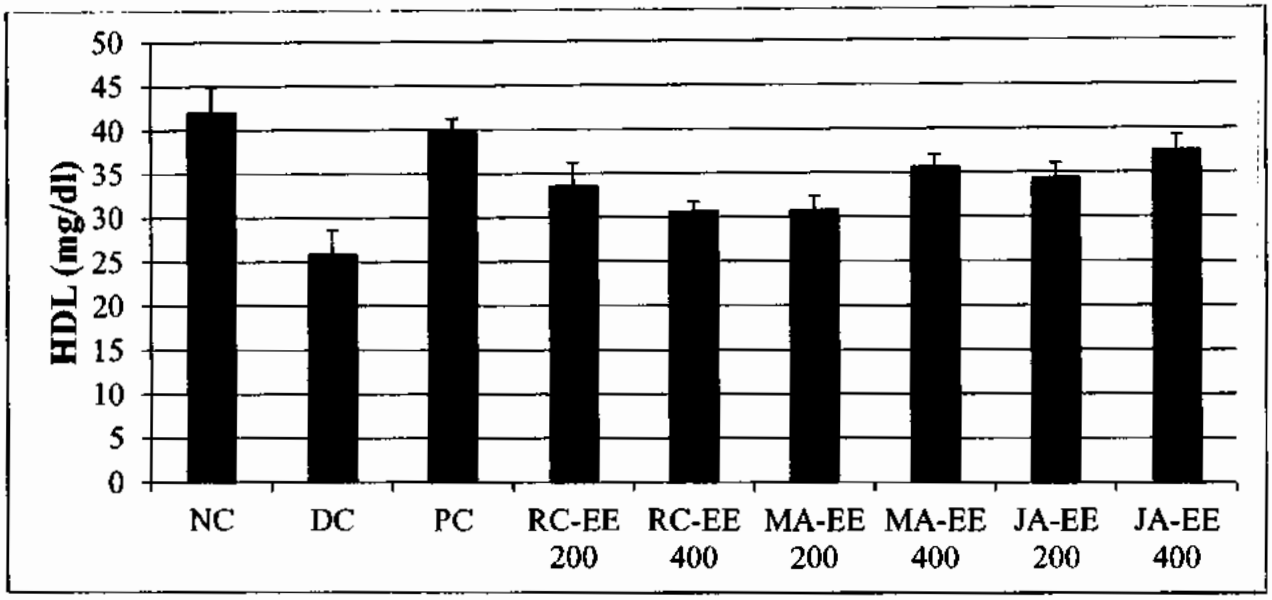


Figure 4.25: Effect of ethanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on high-density lipoprotein. Results are expressed as mean ± standard deviation (n=6).

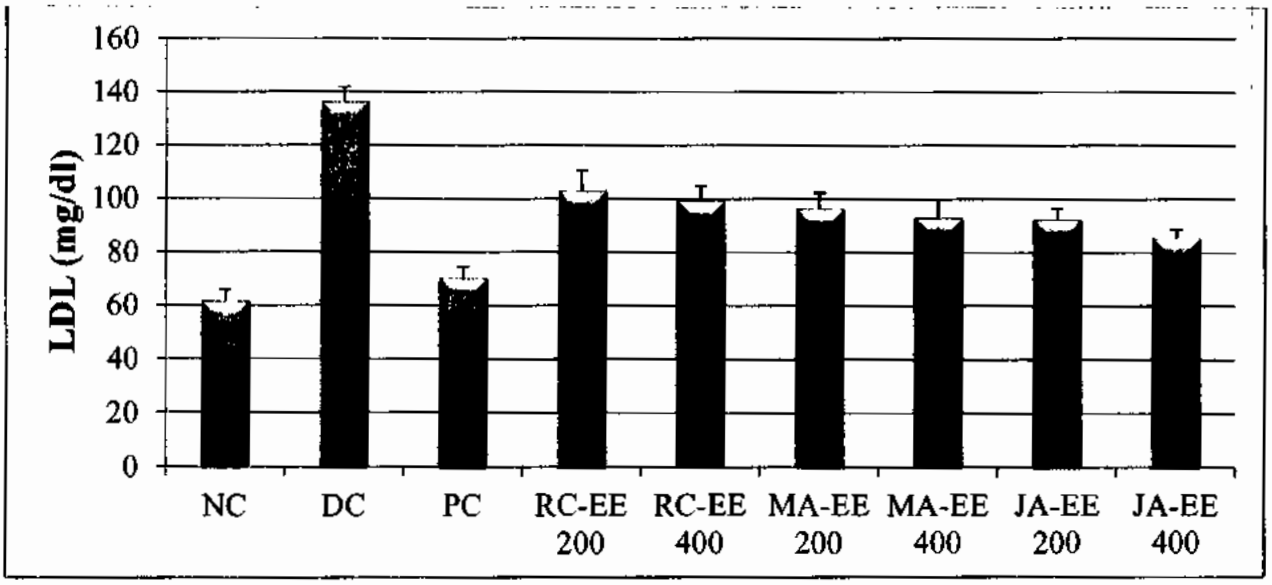


Figure 4.26: Effect of ethanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on low-density lipoprotein. Results are expressed as mean ± standard deviation (n=6).

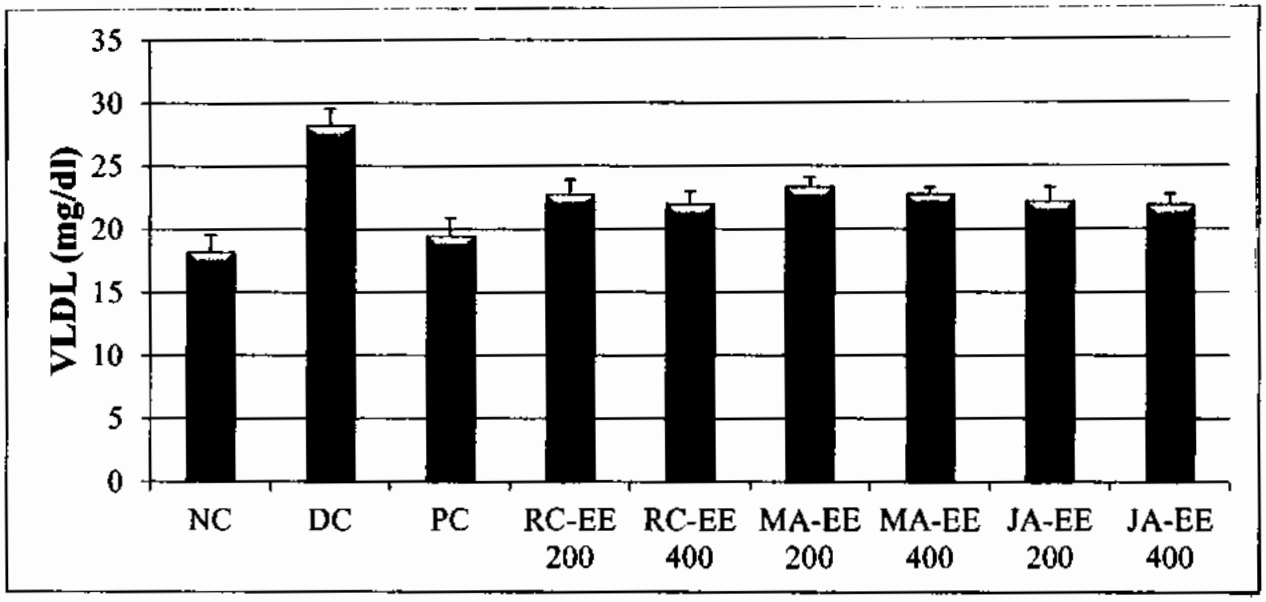


Figure 4.27: Effect of ethanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on very-low-density lipoprotein. Results are expressed as mean ± standard deviation (n=6).

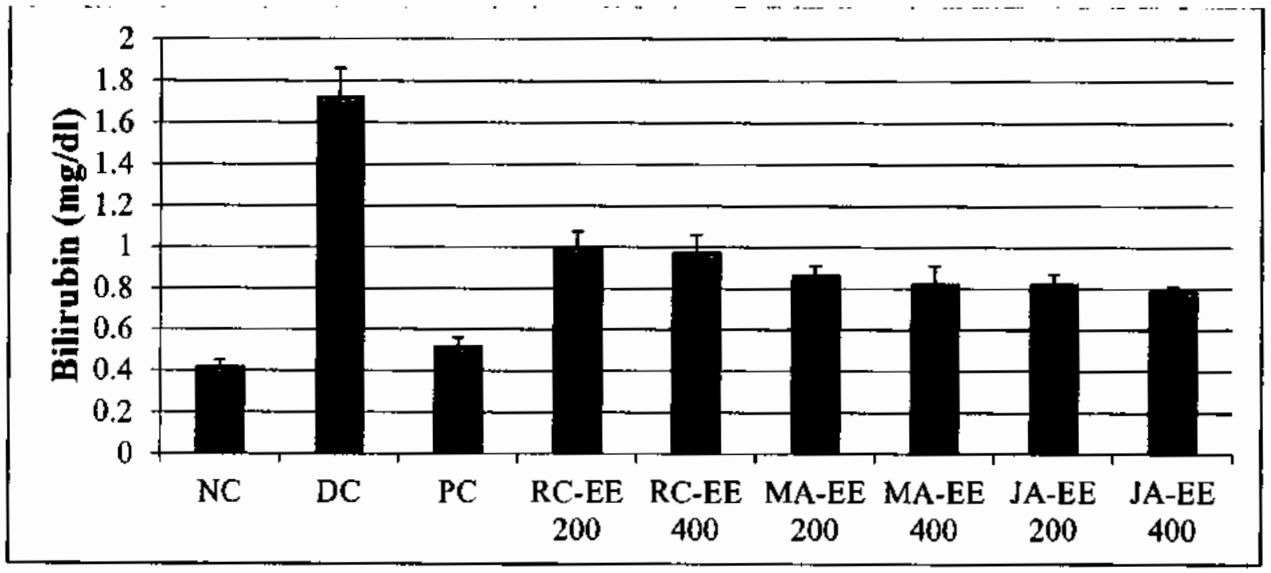


Figure 4.28: Effect of ethanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on bilirubin. Results are expressed as mean ± standard deviation (n=6).

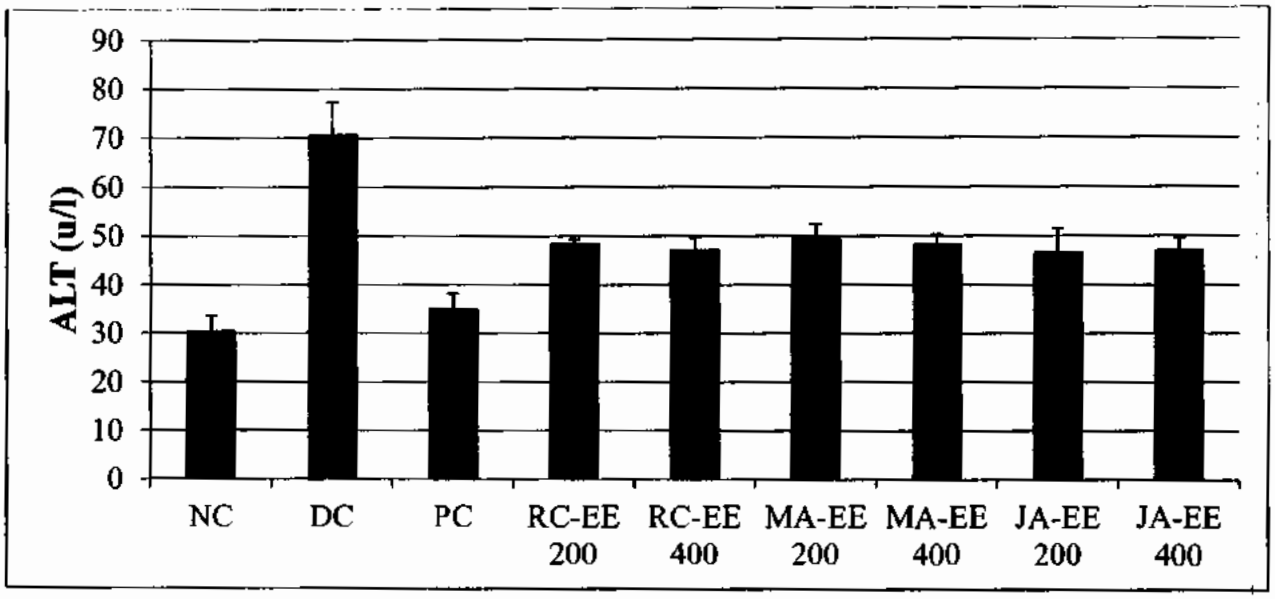


Figure 4.29: Effect of ethanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on alanine aminotransferase. Results are expressed as mean ± standard deviation (n=6).

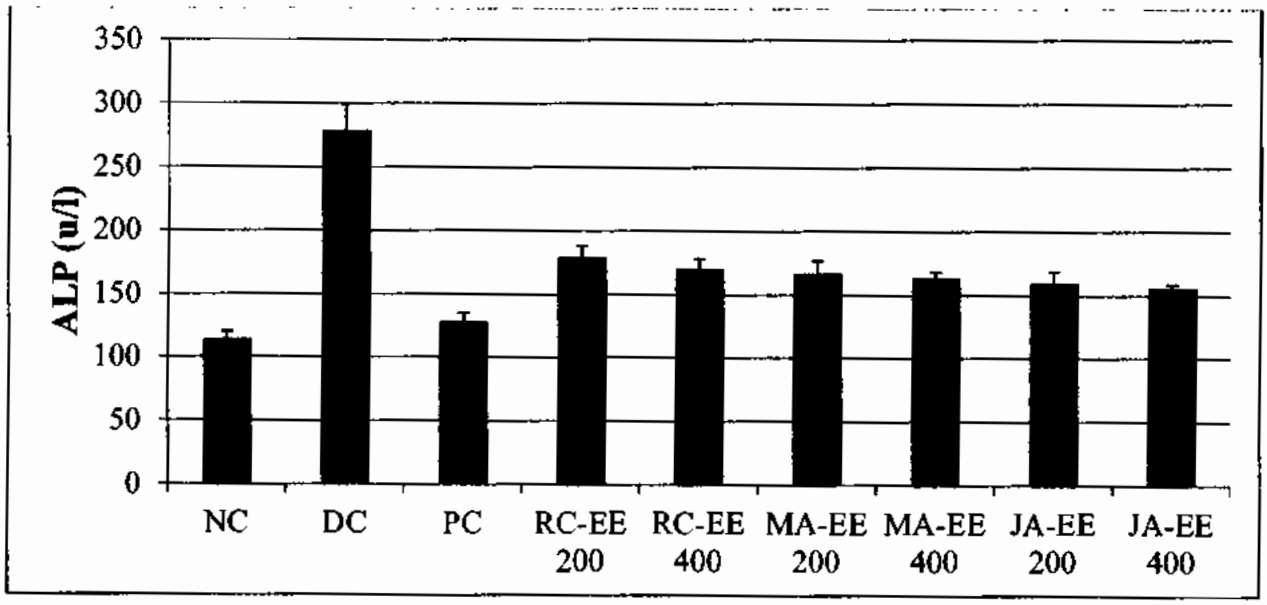


Figure 4.30: Effect of ethanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on alkaline phosphatase. Results are expressed as mean ± standard deviation (n=6).

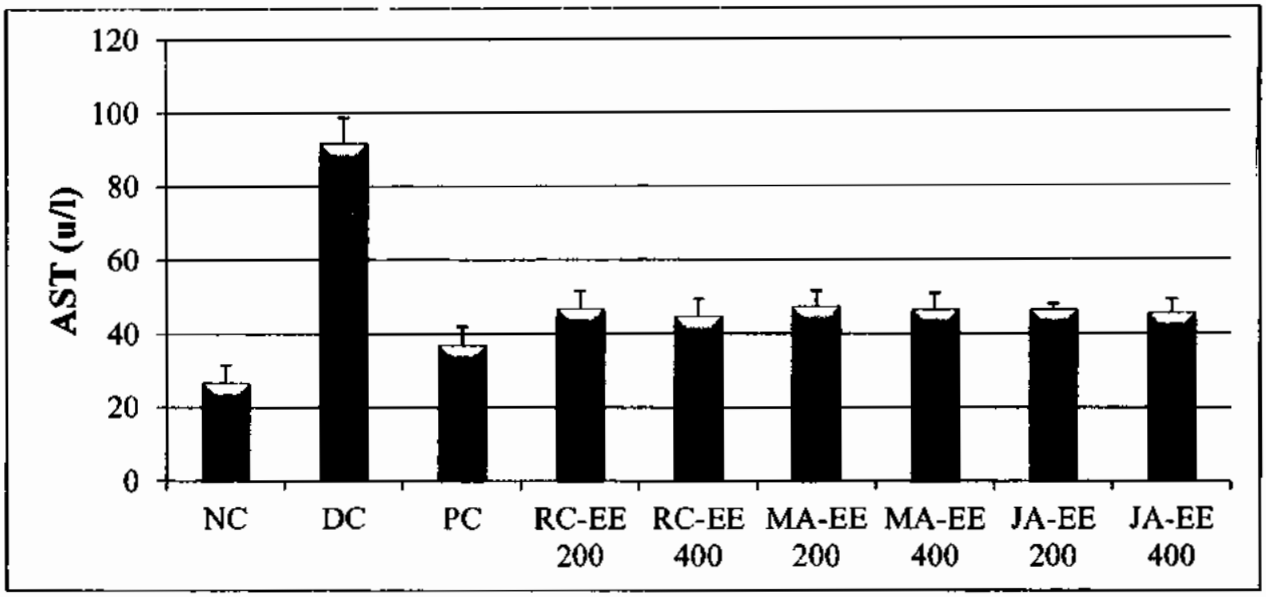


Figure 4.31: Effect of ethanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on aspartate aminotransferase. Results are expressed as mean ± standard deviation (n=6).

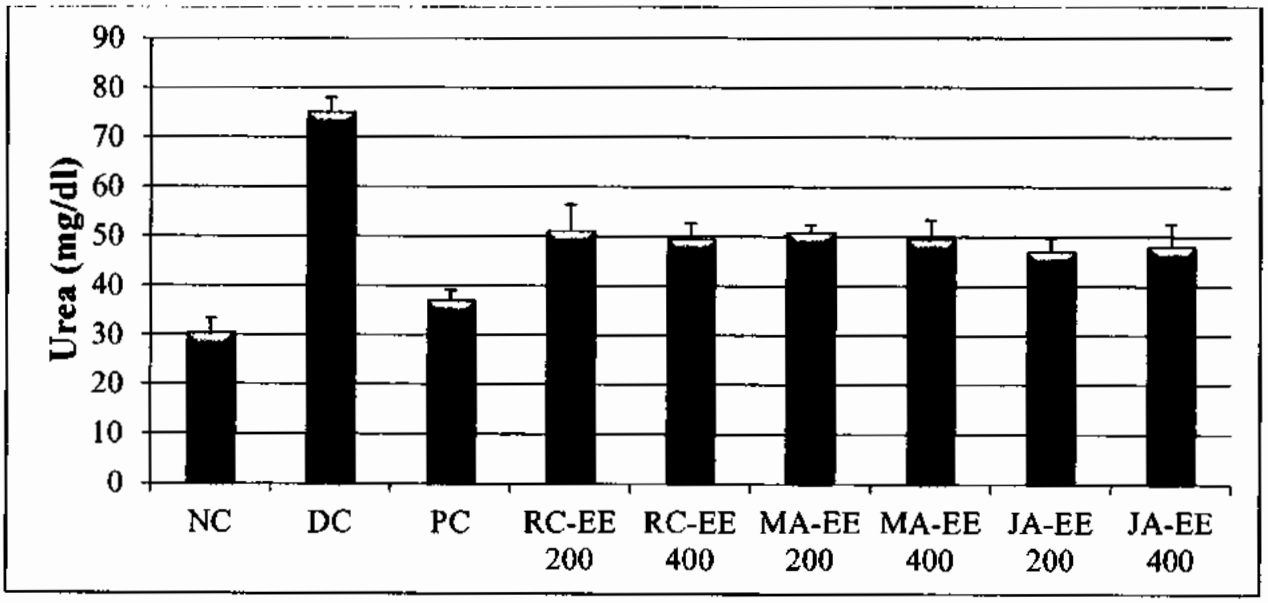


Figure 4.32: Effect of ethanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on urea. Results are expressed as mean ± standard deviation (n=6).

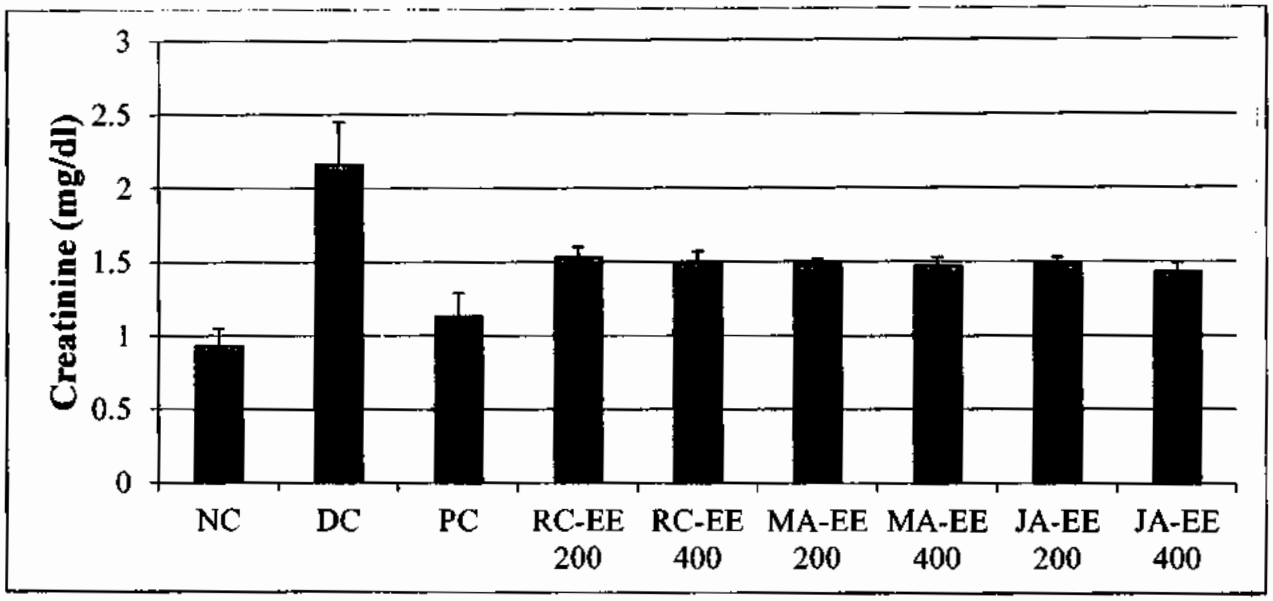


Figure 4.33: Effect of ethanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on creatinine. Results are expressed as mean ± standard deviation (n=6).

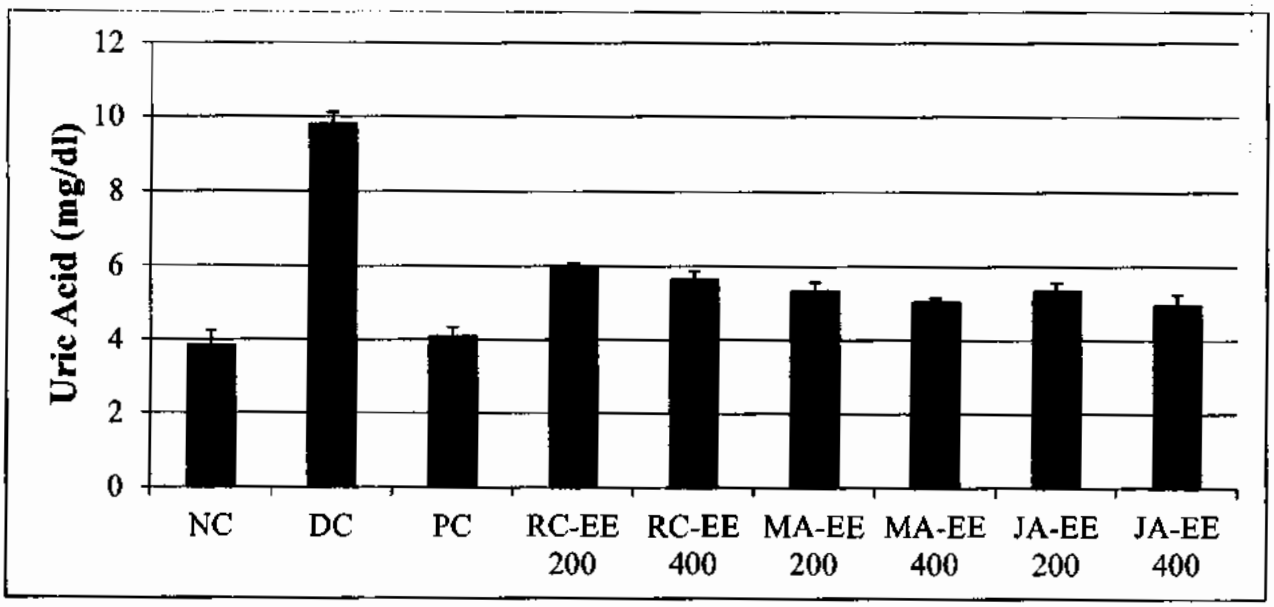


Figure 4.34: Effect of ethanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on uric acid. Results are expressed as mean ± standard deviation (n=6).

4.10. Effect of ethyl acetate extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on biochemical parameters

4.10.1. Effect of ethyl acetate extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on total cholesterol

The Alloxan induced group showed significant increase in the levels of cholesterol (100.83 ± 5.81 mg/dl to 186.33 ± 7.14 mg/dl) as compared to normal control. Glibenclamide decreases the levels of cholesterol when compared to diabetic mice. After the 28 days treatment with MA-EAE at 200 and 400 mg/kg, decreases the levels of cholesterol (148.00 ± 4.38 mg/dl and 146.17 ± 2.79 mg/dl) when compared to diabetic mice. After the 28 days treatment with JA-EAE at 200 and 400 mg/kg, decreases the levels of cholesterol (141.17 ± 3.54 mg/dl and 140.33 ± 3.67 mg/dl) when compared to diabetic mice. After the 28 days treatment with RC-EAE at 200 and 400 mg/kg, decreases the levels of cholesterol (130.50 ± 1.76 mg/dl and 123.17 ± 3.49 mg/dl) when compared to diabetic mice as shown in Figure 4.35.

4.10.2. Effect of ethyl acetate extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on triglycerides

The Alloxan induced group showed significant increase in the levels of triglycerides (91.33 ± 6.25 mg/dl to 141.17 ± 6.52 mg/dl) as compared to normal control. Glibenclamide decreases the levels of triglycerides when compared to diabetic mice. After the 28 days treatment with MA-EAE at 200 and 400 mg/kg, decreases the levels of triglycerides (121.83 ± 1.72 mg/dl and 119.50 ± 4.37 mg/dl) when compared to diabetic mice. After the 28 days treatment with JA-EAE at 200 and 400 mg/kg, decreases the levels of triglycerides (117.33 ± 2.50 mg/dl and 118.67 ± 3.93 mg/dl) when compared to diabetic mice. After the 28 days treatment with RC-EAE at 200 and 400 mg/kg, decreases the levels of triglycerides (106.17 ± 4.91 mg/dl and 103.83 ± 3.82 mg/dl) when compared to diabetic mice as shown in Figure 4.36.

4.10.3. Effect of ethyl acetate extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on high-density lipoprotein

The Alloxan induced group showed significant decrease in the levels of HDL (42.11 ± 2.87 mg/dl to 26.05 ± 2.56 mg/dl) as compared to normal control. Glibenclamide increases the levels of HDL when compared to diabetic mice. After the 28 days treatment with MA-EAE at 200 and 400 mg/kg, increases the levels of HDL (30.12 ± 0.73 mg/dl and 31.27 ± 0.99 mg/dl) when compared to diabetic mice. After the 28 days treatment with JA-EAE at 200 and 400 mg/kg, increases the levels of HDL (30.73 ± 1.00 mg/dl and 33.63 ± 2.57 mg/dl) when compared to diabetic mice. After the 28 days treatment with RC-EAE at 200 and 400 mg/kg, increases the levels of HDL (34.75 ± 2.81 mg/dl and 36.54 ± 1.25 mg/dl) when compared to diabetic mice as shown in Figure 4.37.

4.10.4. Effect of ethyl acetate extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on low-density lipoprotein

The Alloxan induced group showed significant increase in the levels of LDL (61.83 ± 3.92 mg/dl to 136.50 ± 5.43 mg/dl) as compared to normal control. Glibenclamide decreases the levels of LDL when compared to diabetic mice. After the 28 days treatment with MA-EAE at 200 and 400 mg/kg, decreases the levels of LDL (111.00 ± 2.45 mg/dl and 109.00 ± 4.69 mg/dl) when compared to diabetic mice. After the 28 days treatment with JA-EAE at 200 and 400 mg/kg, decreases the levels of LDL (107.50 ± 4.18 mg/dl and 104.33 ± 2.06 mg/dl) when compared to diabetic mice. After the 28 days treatment with RC-EAE at 200 and 400 mg/kg, decreases the levels of LDL (89.83 ± 4.67 mg/dl and 86.17 ± 4.26 mg/dl) when compared to diabetic mice as shown in Figure 4.38.

4.10.5. Effect of ethyl acetate extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on very-low-density lipoprotein

The Alloxan induced group showed significant increase in the levels of VLDL (18.30 ± 1.27 mg/dl to 28.23 ± 1.30 mg/dl) as compared to normal control. Glibenclamide decreases the levels of VLDL when compared to diabetic mice. After the 28 days treatment with MA-EAE at 200 and 400 mg/kg, decreases the levels of VLDL (24.37 ± 0.34 mg/dl and 23.90 ± 0.87 mg/dl) when compared to diabetic mice. After the 28 days treatment with JA-EAE at 200 and 400

mg/kg, decreases the levels of VLDL (23.47 ± 0.51 mg/dl and 23.73 ± 0.79 mg/dl) when compared to diabetic mice. After the 28 days treatment with RC-EAE at 200 and 400 mg/kg, decreases the levels of VLDL (21.23 ± 0.98 mg/dl and 20.77 ± 0.77 mg/dl) when compared to diabetic mice as shown in Figure 4.39.

4.10.6. Effect of ethyl acetate extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on bilirubin

The Alloxan induced group showed significant increase in the levels of bilirubin (0.42 ± 0.03 mg/dl to 1.73 ± 0.13 mg/dl) as compared to normal control. Glibenclamide decreases the levels of bilirubin when compared to diabetic mice. After the 28 days treatment with MA-EAE at 200 and 400 mg/kg, decreases the levels of bilirubin (1.11 ± 0.02 mg/dl and 1.02 ± 0.06 mg/dl) when compared to diabetic mice. After the 28 days treatment with JA-EAE at 200 and 400 mg/kg, decreases the levels of bilirubin (1.03 ± 0.10 mg/dl and 0.99 ± 0.07 mg/dl) when compared to diabetic mice. After the 28 days treatment with RC-EAE at 200 and 400 mg/kg, decreases the levels of bilirubin (0.81 ± 0.05 mg/dl and 0.76 ± 0.05 mg/dl) when compared to diabetic mice as shown in Figure 4.40.

4.10.7. Effect of ethyl acetate extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on alanine aminotransferase

The Alloxan induced group showed significant increase in the levels of ALT (30.67 ± 2.87 u/l to 70.83 ± 6.52 u/l) as compared to normal control. Glibenclamide decreases the levels of ALT when compared to diabetic mice. After the 28 days treatment with MA-EAE at 200 and 400 mg/kg, decreases the levels of ALT (51.83 ± 3.54 u/l and 52.50 ± 2.59 u/l) when compared to diabetic mice. After the 28 days treatment with JA-EAE at 200 and 400 mg/kg, decreases the levels of ALT (49.17 ± 2.32 u/l and 48.83 ± 1.94 u/l) when compared to diabetic mice. After the 28 days treatment with RC-EAE at 200 and 400 mg/kg, decreases the levels of ALT (43.50 ± 4.63 u/l and 44.50 ± 3.15 u/l) when compared to diabetic mice as shown in Figure 4.41.

4.10.8. Effect of ethyl acetate extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on alkaline phosphatase

The Alloxan induced group showed significant increase in the levels of ALP (114.67 ± 5.17 u/l to 278.67 ± 20.38 u/l) as compared to normal control. Glibenclamide decreases the

levels of ALP when compared to diabetic mice. After the 28 days treatment with MA-EAE at 200 and 400 mg/kg, decreases the levels of ALP (188.50 ± 2.89 u/l and 183.83 ± 4.11 u/l) when compared to diabetic mice. After the 28 days treatment with JA-EAE at 200 and 400 mg/kg, decreases the levels of ALP (182.00 ± 5.51 u/l and 177.00 ± 6.63 u/l) when compared to diabetic mice. After the 28 days treatment with RC-EAE at 200 and 400 mg/kg, decreases the levels of ALP (157.83 ± 11.25 u/l and 149.50 ± 8.48 u/l) when compared to diabetic mice as shown in Figure 4.42.

4.10.9. Effect of ethyl acetate extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on aspartate aminotransferase

The Alloxan induced group showed significant increase in the levels of AST (26.83 ± 4.70 u/l to 91.67 ± 7.03 u/l) as compared to normal control. Glibenclamide decreases the levels of AST when compared to diabetic mice. After the 28 days treatment with MA-EAE at 200 and 400 mg/kg, decreases the levels of AST (55.00 ± 2.83 u/l and 52.33 ± 4.97 u/l) when compared to diabetic mice. After the 28 days treatment with JA-EAE at 200 and 400 mg/kg, decreases the levels of AST (51.33 ± 3.27 u/l and 49.17 ± 3.54 u/l) when compared to diabetic mice. After the 28 days treatment with RC-EAE at 200 and 400 mg/kg, decreases the levels of AST (43.00 ± 2.76 u/l and 41.83 ± 3.06 u/l) when compared to diabetic mice as shown in Figure 4.43.

4.10.10. Effect of ethyl acetate extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on urea

The Alloxan induced group showed significant increase in the levels of urea (30.67 ± 2.73 mg/dl to 75.17 ± 2.86 mg/dl) as compared to normal control. Glibenclamide decreases the levels of urea when compared to diabetic mice. After the 28 days treatment with MA-EAE at 200 and 400 mg/kg, decreases the levels of urea (56.17 ± 2.92 mg/dl and 55.33 ± 3.14 mg/dl) when compared to diabetic mice. After the 28 days treatment with JA-EAE at 200 and 400 mg/kg, decreases the levels of urea (54.00 ± 4.38 mg/dl and 52.33 ± 2.51 mg/dl) when compared to diabetic mice. After the 28 days treatment with RC-EAE at 200 and 400 mg/kg, decreases the levels of urea (44.67 ± 2.87 mg/dl and 45.17 ± 4.67 mg/dl) when compared to diabetic mice as shown in Figure 4.44.

4.10.11. Effect of ethyl acetate extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on creatinine

The Alloxan induced group showed significant increase in the levels of creatinine (0.94 ± 0.11 mg/dl to 2.17 ± 0.28 mg/dl) as compared to normal control. Glibenclamide decreases the levels of creatinine when compared to diabetic mice. After the 28 days treatment with MA-EAE at 200 and 400 mg/kg, decreases the levels of creatinine (1.57 ± 0.04 mg/dl and 1.55 ± 0.07 mg/dl) when compared to diabetic mice. After the 28 days treatment with JA-EAE at 200 and 400 mg/kg, decreases the levels of creatinine (1.55 ± 0.07 mg/dl and 1.54 ± 0.03 mg/dl) when compared to diabetic mice. After the 28 days treatment with RC-EAE at 200 and 400 mg/kg, decreases the levels of creatinine (1.45 ± 0.04 mg/dl and 1.38 ± 0.11 mg/dl) when compared to diabetic mice as shown in Figure 4.45.

4.10.12. Effect of ethyl acetate extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on uric acid

The Alloxan induced group showed significant increase in the levels of uric acid (3.87 ± 0.37 mg/dl to 9.84 ± 0.27 mg/dl) as compared to normal control. Glibenclamide decreases the levels of uric acid when compared to diabetic mice. After the 28 days treatment with MA-EAE at 200 and 400 mg/kg, decreases the levels of uric acid (6.00 ± 0.08 mg/dl and 5.73 ± 0.09 mg/dl) when compared to diabetic mice. After the 28 days treatment with JA-EAE at 200 and 400 mg/kg, decreases the levels of uric acid (5.94 ± 0.06 mg/dl and 5.64 ± 0.18 mg/dl) when compared to diabetic mice. After the 28 days treatment with RC-EAE at 200 and 400 mg/kg, decreases the levels of uric acid (5.18 ± 0.19 mg/dl and 4.88 ± 0.31 mg/dl) when compared to diabetic mice as shown in Figure 4.46.

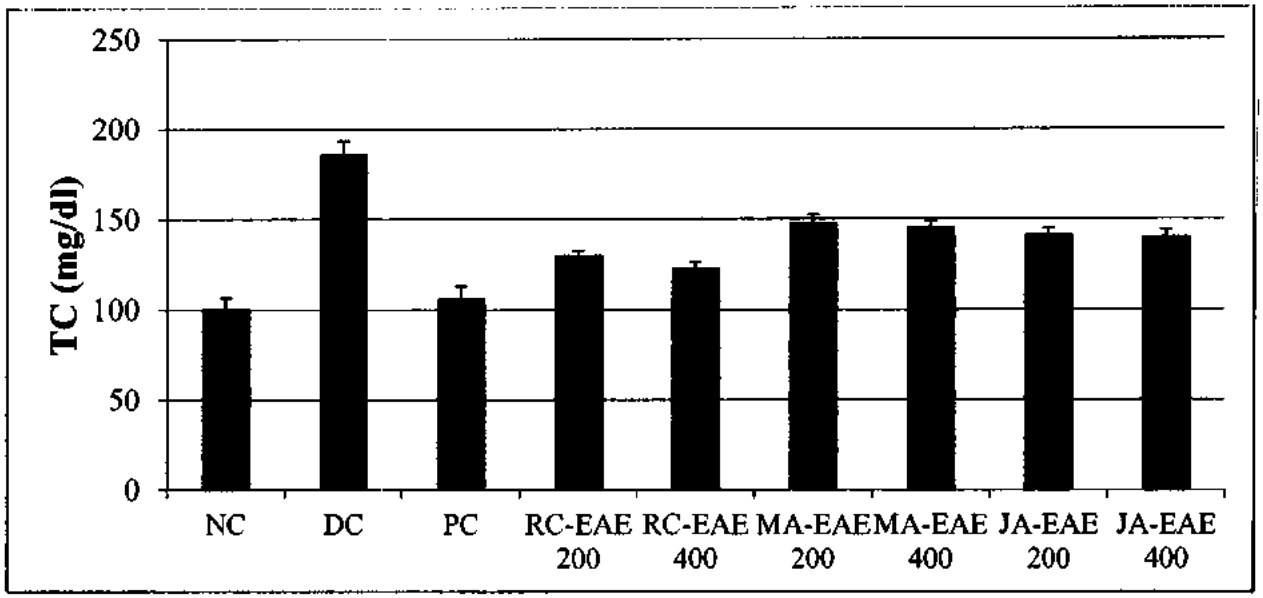


Figure 4.35: Effect of ethyl acetate extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on total cholesterol. Results are expressed as mean ± standard deviation (n=6).

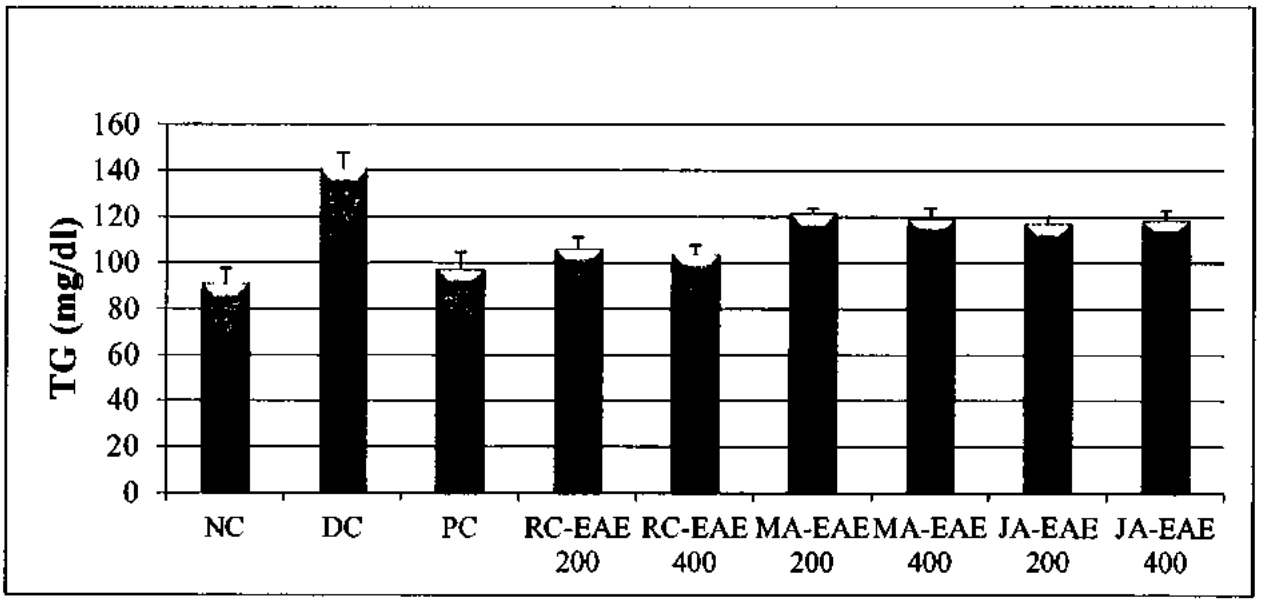


Figure 4.36: Effect of ethyl acetate extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on triglycerides. Results are expressed as mean ± standard deviation (n=6).

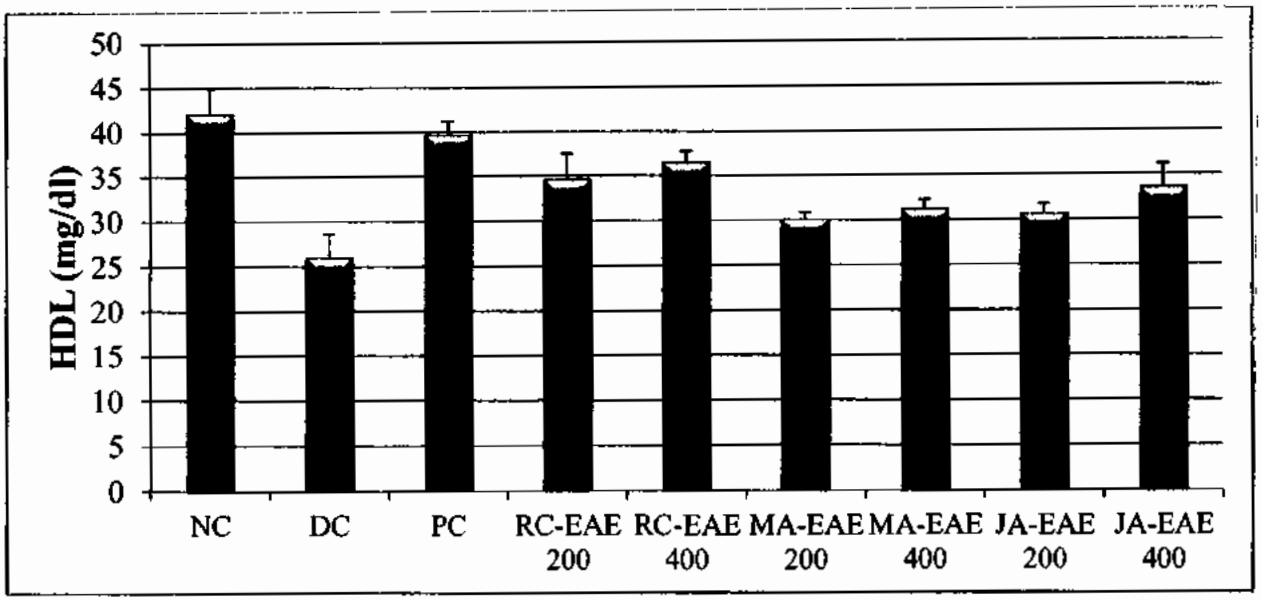


Figure 4.37: Effect of ethyl acetate extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on high-density lipoprotein. Results are expressed as mean ± standard deviation (n=6).

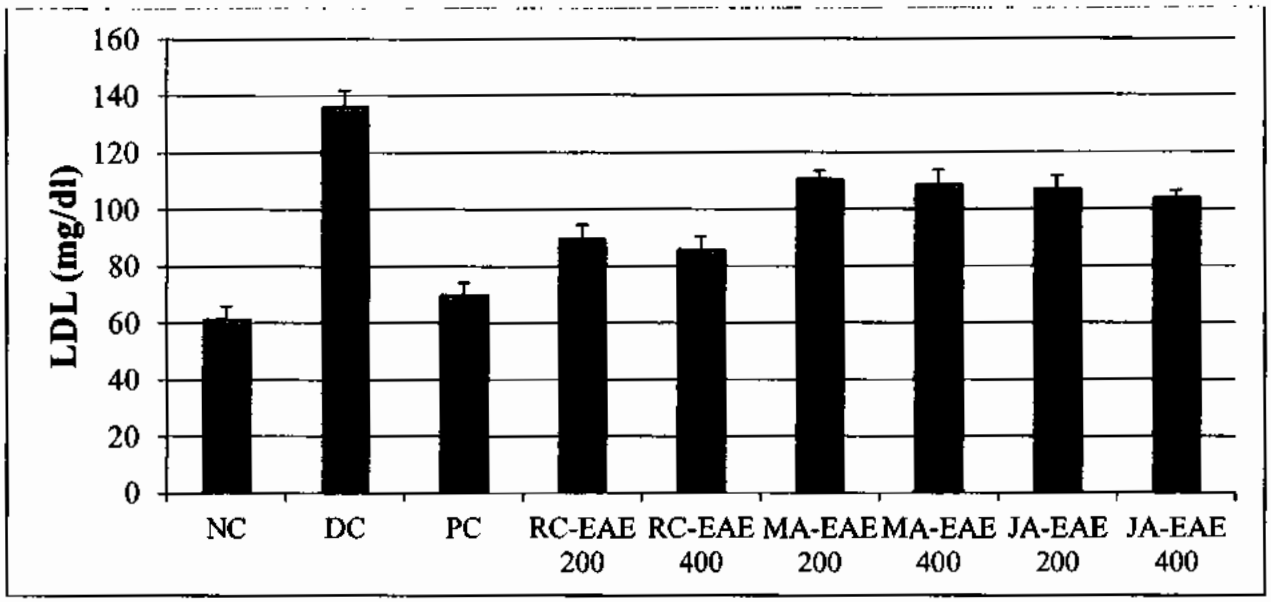


Figure 4.38: Effect of ethyl acetate extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on low-density lipoprotein. Results are expressed as mean ± standard deviation (n=6).

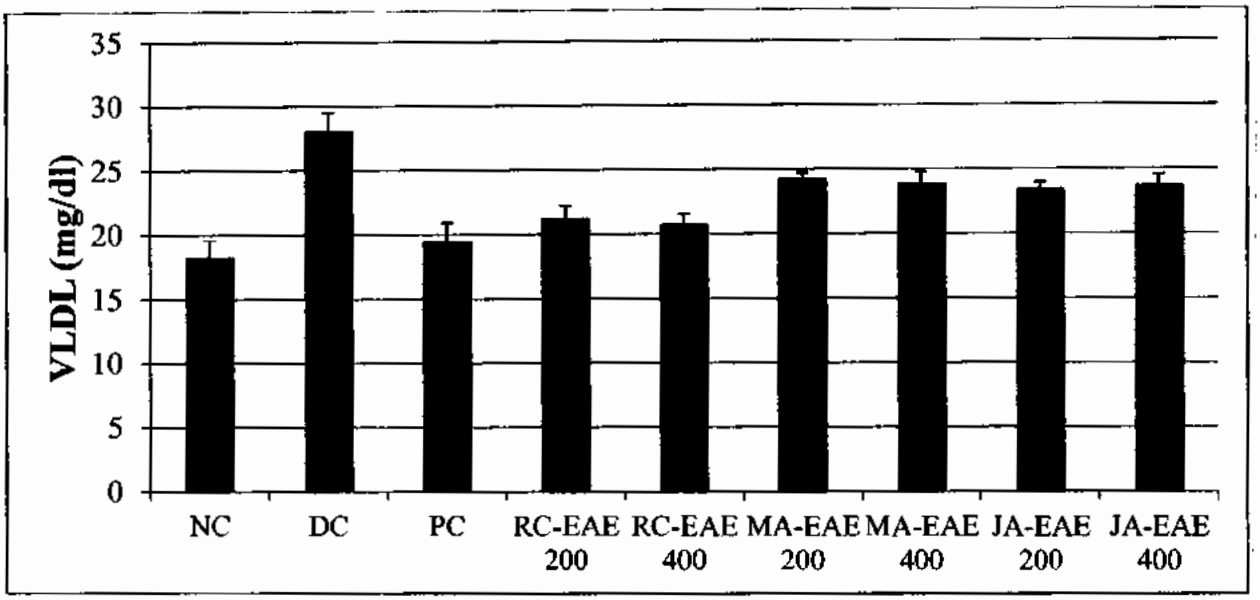


Figure 4.39: Effect of ethyl acetate extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on very-low-density lipoprotein. Results are expressed as mean ± standard deviation (n=6).

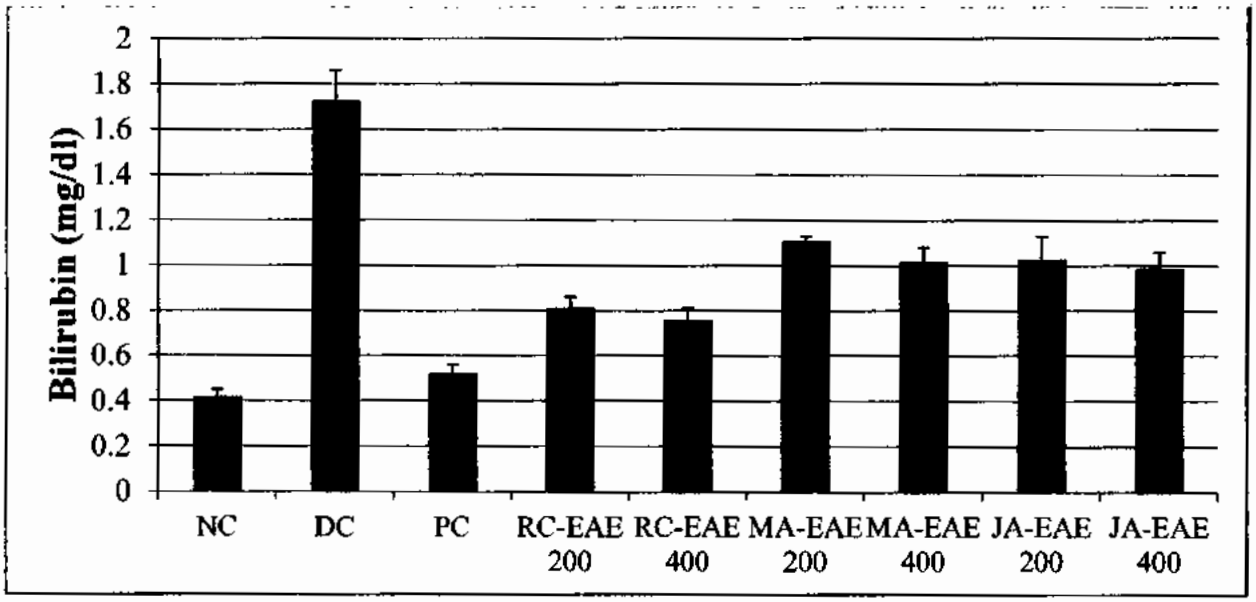


Figure 4.40: Effect of ethyl acetate extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on bilirubin. Results are expressed as mean ± standard deviation (n=6).

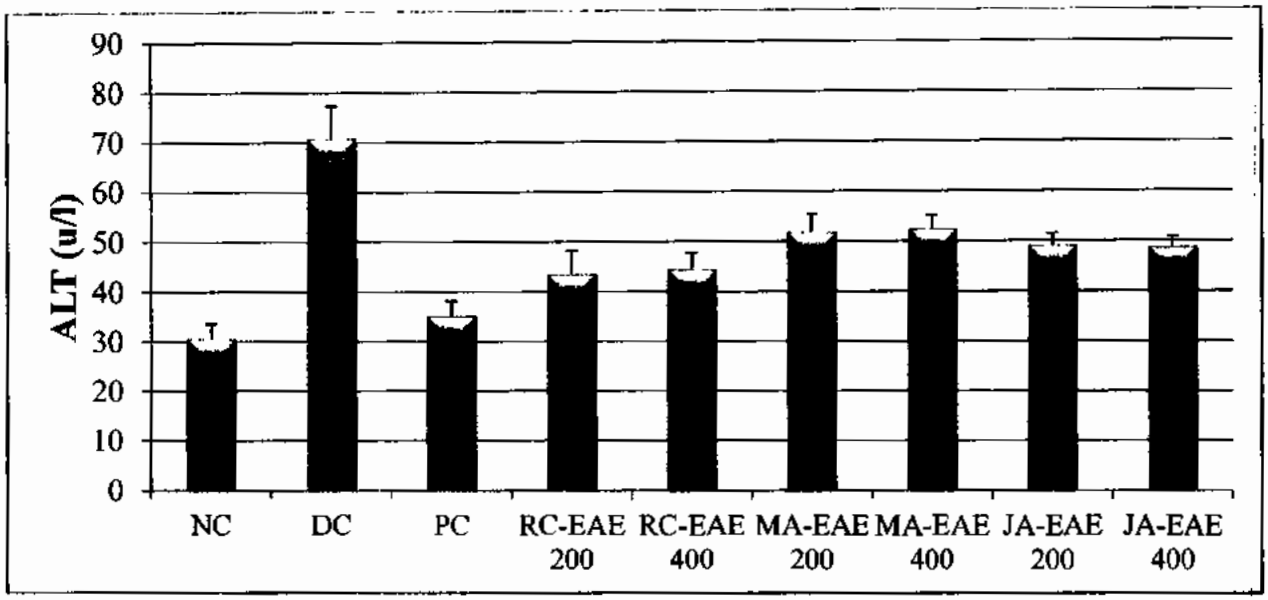


Figure 4.41: Effect of ethyl acetate extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on alanine aminotransferase. Results are expressed as mean ± standard deviation (n=6).

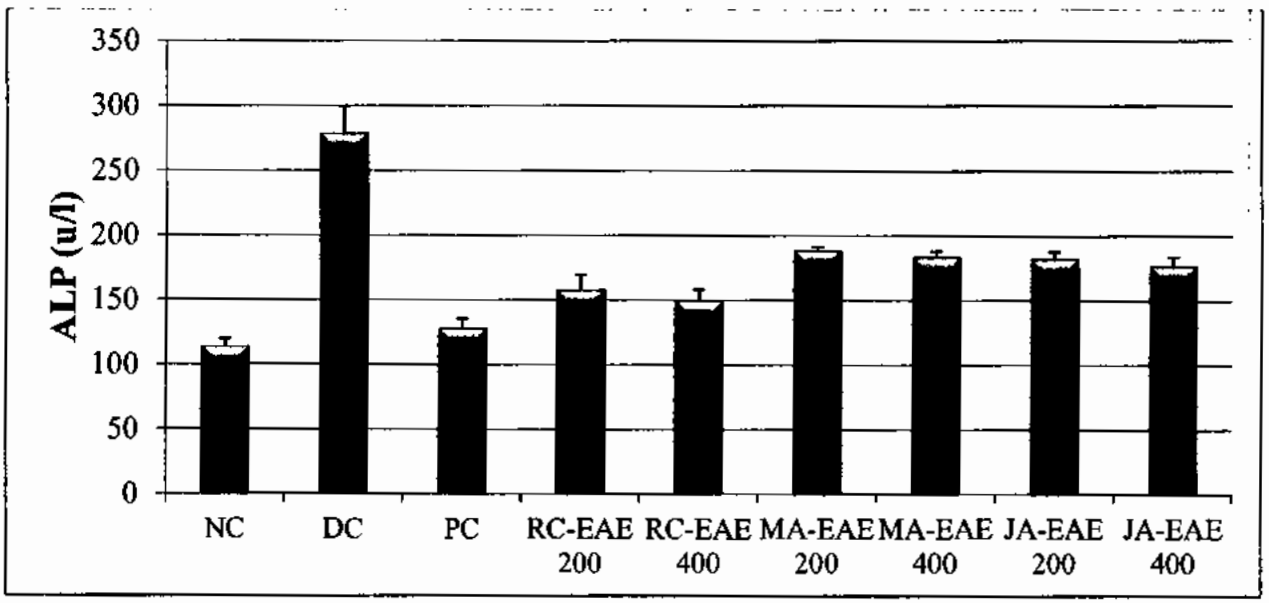


Figure 4.42: Effect of ethyl acetate extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on alkaline phosphatase. Results are expressed as mean ± standard deviation (n=6).

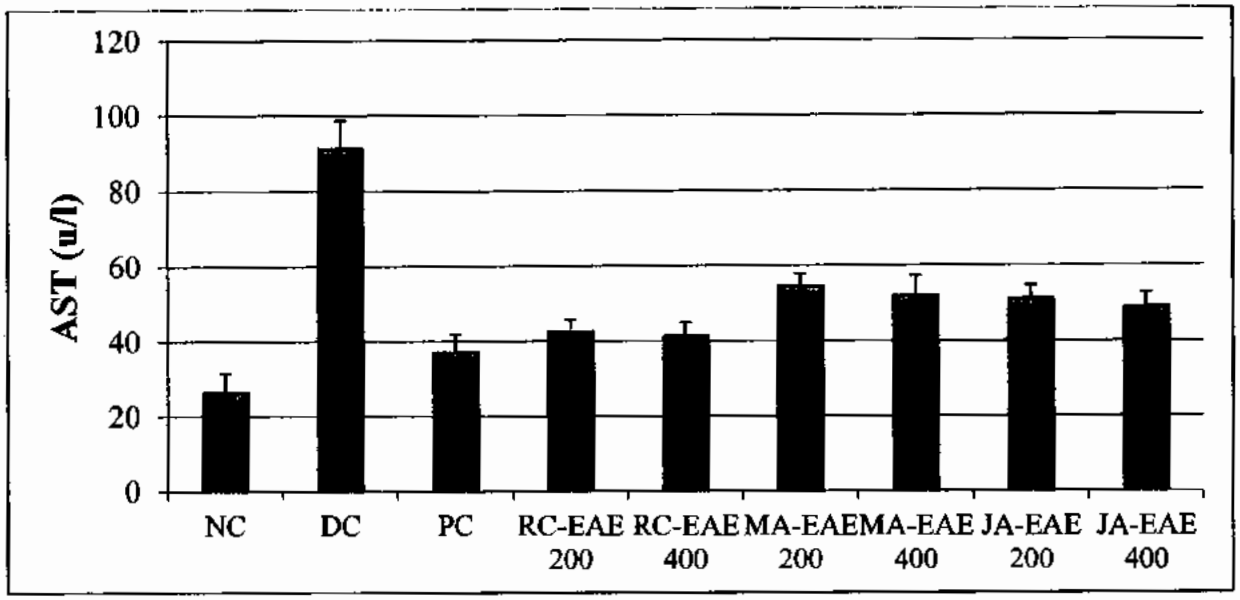


Figure 4.43: Effect of ethyl acetate extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on aspartate aminotransferase. Results are expressed as mean ± standard deviation (n=6).

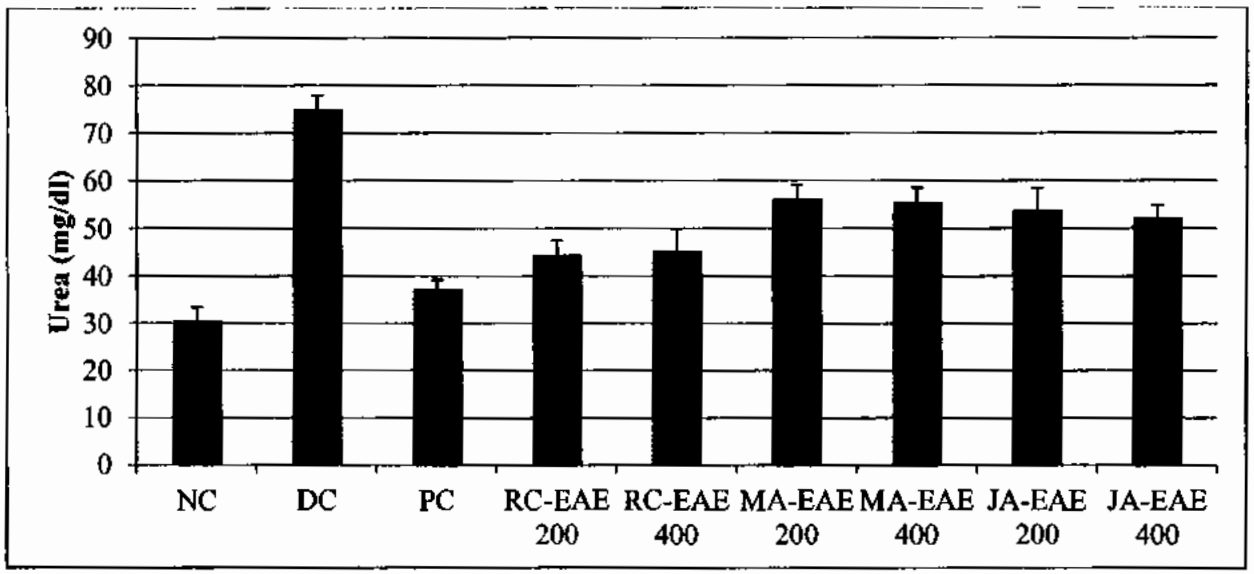


Figure 4.44: Effect of ethyl acetate extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on urea. Results are expressed as mean ± standard deviation (n=6).

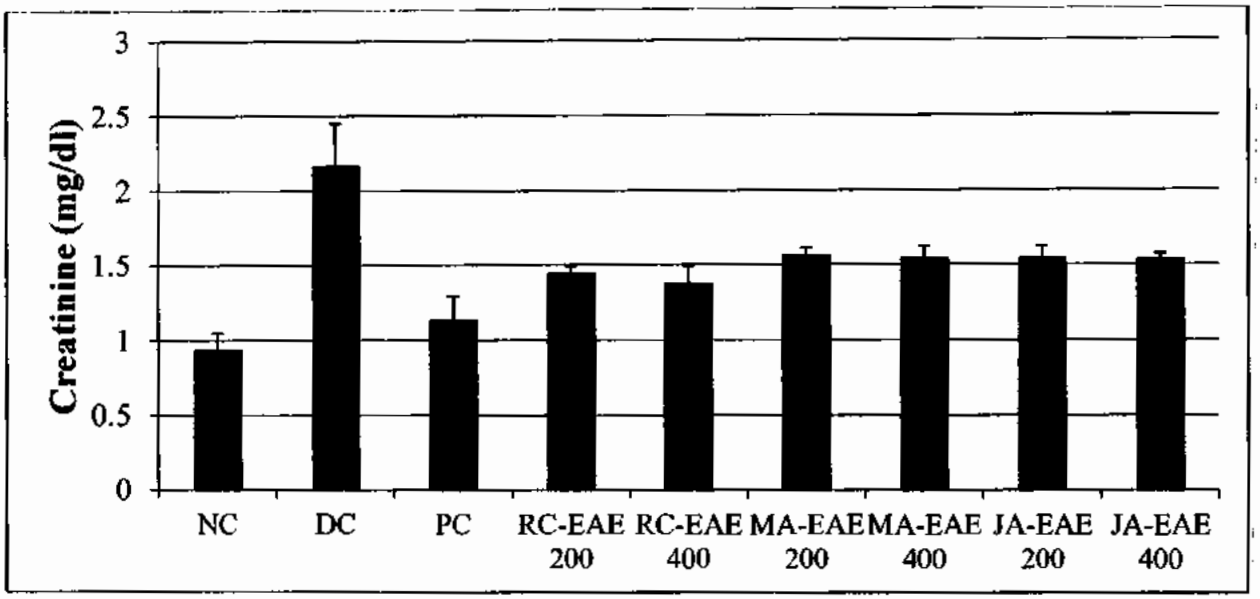


Figure 4.45: Effect of ethyl acetate extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on creatinine. Results are expressed as mean ± standard deviation (n=6).

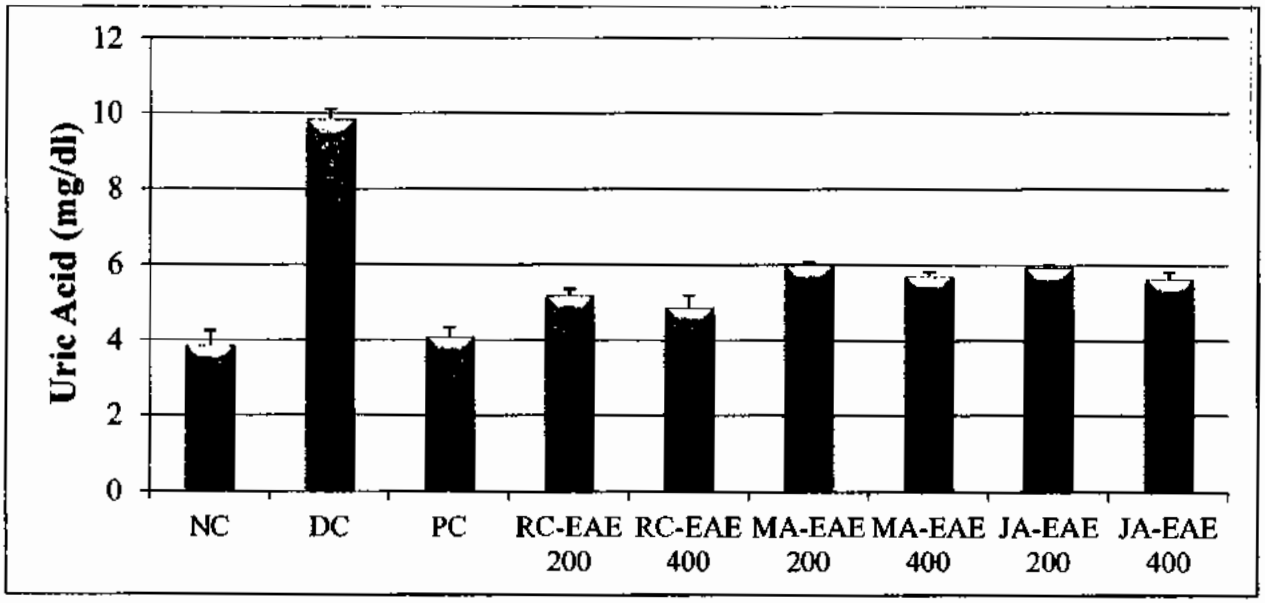


Figure 4.46: Effect of ethyl acetate extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* on uric acid. Results are expressed as mean ± standard deviation (n=6).

4.11. Antibacterial activity of *Melia azedarach*

4.11.1. Effect of methanolic extract of *Melia azedarach* against the different bacterial strains

The methanolic crude extracts of *Melia azedarach* was evaluated for *in vitro* antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* indicating different zones of inhibition as shown in Figure 4.47. When tested, the methanol leaf extracts of *Melia azedarach* showed significant activity against all the tested microorganisms. The highest antibacterial activity was recorded in MA-ME (17 ± 1 mm) against the *Staphylococcus aureus* at 1000 µg/ml, followed by MA-ME (16 ± 1 mm) against the *Bacillus subtilis*, MA-ME (16 ± 1 mm) against the *Pseudomonas aeruginosa*, MA-ME (14.33 ± 0.57 mm) against the *Escherichia coli* at 1000 µg/ml respectively, MA-ME (14.33 ± 1.15 mm) against the *Bacillus subtilis*, MA-ME (13.67 ± 0.57 mm) against the *Staphylococcus aureus*, MA-ME (13.33 ± 0.57 mm) against the *Pseudomonas aeruginosa* at 500 µg/ml respectively, MA-ME (12 ± 1 mm) against the *Bacillus subtilis* at 100 µg/ml, MA-ME (11.67 ± 0.57 mm) against the *Escherichia coli* at 500 µg/ml, MA-ME (11.67 ± 0.57 mm) against the *Pseudomonas aeruginosa*, MA-ME (11.33 ± 1.15 mm) against the *Staphylococcus aureus* and MA-ME (10 ± 1 mm) against the *Escherichia coli* at 100 µg/ml respectively. Streptomycin sulphate creates the zones of inhibition ranging from 18.33 ± 0.57 mm to 20.33 ± 0.57 mm against all of the tested bacterial strains. No inhibitory activity was observed in the DMSO. So, DMSO does not influence the susceptibility of the bacterial strains to the corresponding extracts.

4.11.2. Effect of ethanolic extract of *Melia azedarach* against the different bacterial strains

The ethanolic crude extracts of *Melia azedarach* was evaluated for *in vitro* antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* indicating different zones of inhibition as shown in Figure 4.48. When tested, the ethanol leaf extracts of *Melia azedarach* showed significant activity against all the tested microorganisms. The highest antibacterial activity was recorded in MA-EE (16.66 ± 1.15 mm) against the *Staphylococcus aureus* at 1000 µg/ml, followed by MA-EE (15.33 ± 0.57 mm) against the *Bacillus subtilis*, MA-EE (14.67 ± 0.57 mm) against the *Escherichia coli*, MA-EE

(14.33 ± 1.52 mm) against the *Pseudomonas aeruginosa* at 1000 µg/ml respectively, MA-EE (14.33 ± 0.57 mm) against the *Staphylococcus aureus*, MA-EE (13.67 ± 1.15 mm) against the *Bacillus subtilis*, MA-EE (12.33 ± 0.57 mm) against the *Pseudomonas aeruginosa* at 500 µg/ml respectively, MA-EE (11.67 ± 0.57 mm) against the *Staphylococcus aureus*, MA-EE (11.33 ± 0.57 mm) against the *Bacillus subtilis* at 100 µg/ml respectively, MA-EE (11 ± 1 mm) against the *Escherichia coli* at 500 µg/ml, MA-EE (10.67 ± 0.57 mm) against the *Pseudomonas aeruginosa* and MA-EE (10 ± 1 mm) against the *Escherichia coli* at 100 µg/ml respectively. Streptomycin sulphate creates zones of inhibition ranging from 17.66 ± 0.57 mm to 21.33 ± 1.15 mm against all of the tested bacterial strains.

4.11.3. Effect of ethyl acetate extract of *Melia azedarach* against the different bacterial strains

The ethyl acetate crude extracts of *Melia azedarach* was evaluated for *in vitro* antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* indicating different zones of inhibition as shown in Figure 4.49. When tested, the ethyl acetate leaf extracts of *Melia azedarach* showed significant activity against most of the tested microorganisms. The highest antibacterial activity was recorded in MA-EAE (13.67 ± 1.52 mm) against the *Pseudomonas aeruginosa* at 1000 µg/ml, followed by MA-EAE (12.67 ± 0.57 mm) against the *Staphylococcus aureus*, MA-EAE (11.67 ± 0.57 mm) against the *Bacillus subtilis*, MA-EAE (10.67 ± 0.57 mm) against the *Escherichia coli* at 1000 µg/ml respectively, MA-EAE (10.33 ± 0.57 mm) against the *Pseudomonas aeruginosa*, MA-EAE (9.33 ± 0.57 mm) against the *Bacillus subtilis* and MA-EAE (9 ± 1 mm) against the *Staphylococcus aureus* at 500 µg/ml respectively. No zone of inhibition was recorded in MA-EAE against the *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* at 100 µg/ml respectively and MA-EAE against the *Escherichia coli* at 500 µg/ml. Streptomycin sulphate creates zones of inhibition ranging from 17.33 ± 0.57 mm to 20.33 ± 0.57 mm against all of the tested bacterial strains.

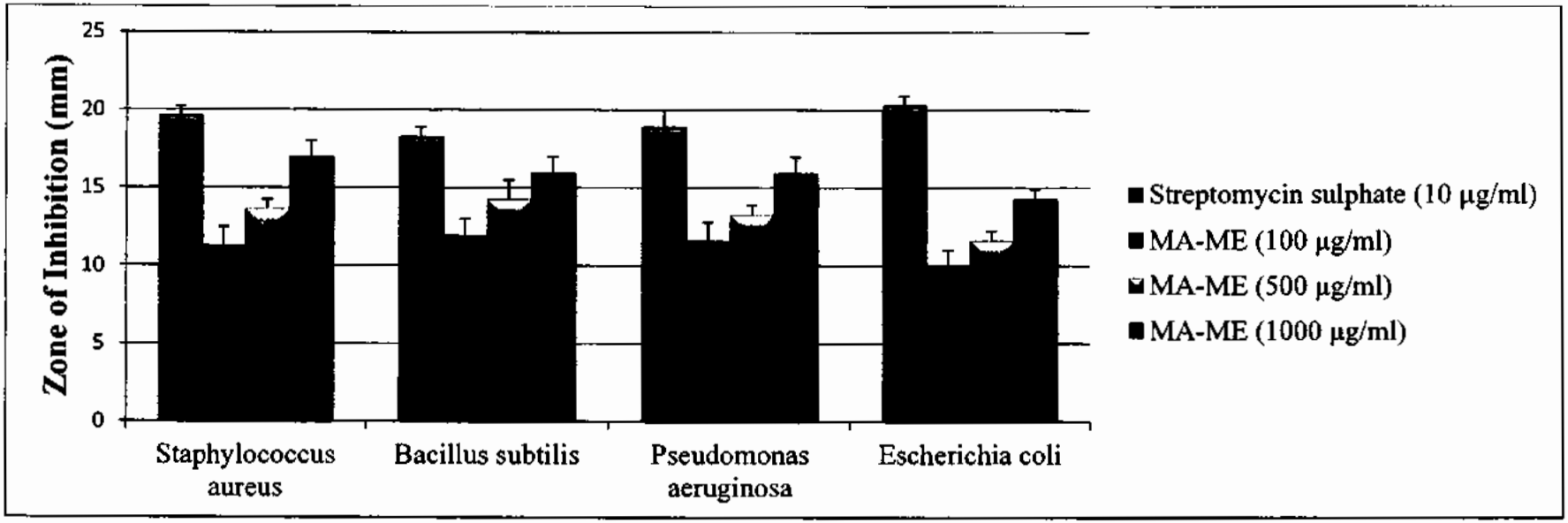


Figure 4.47: Effect of methanolic extract of *Melia azedarach* against the different bacterial strains. Results are expressed as mean ± standard deviation (n = 3).

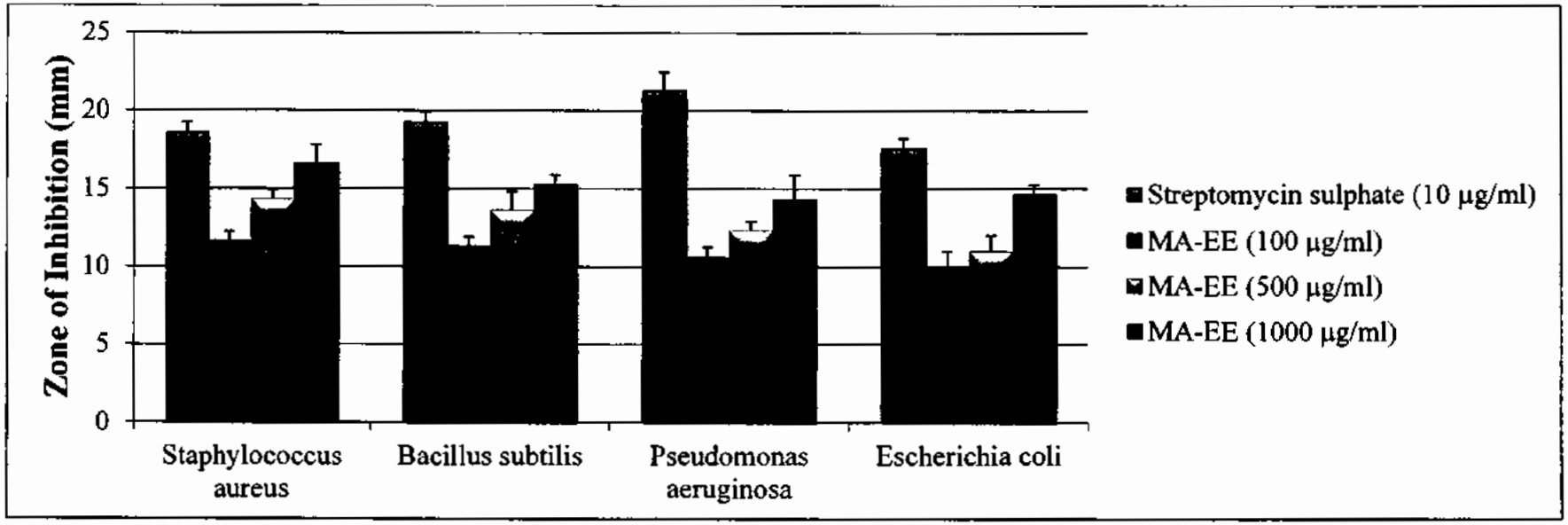


Figure 4.48: Effect of ethanolic extract of *Melia azedarach* against the different bacterial strains. Results are expressed as mean ± standard deviation (n = 3).

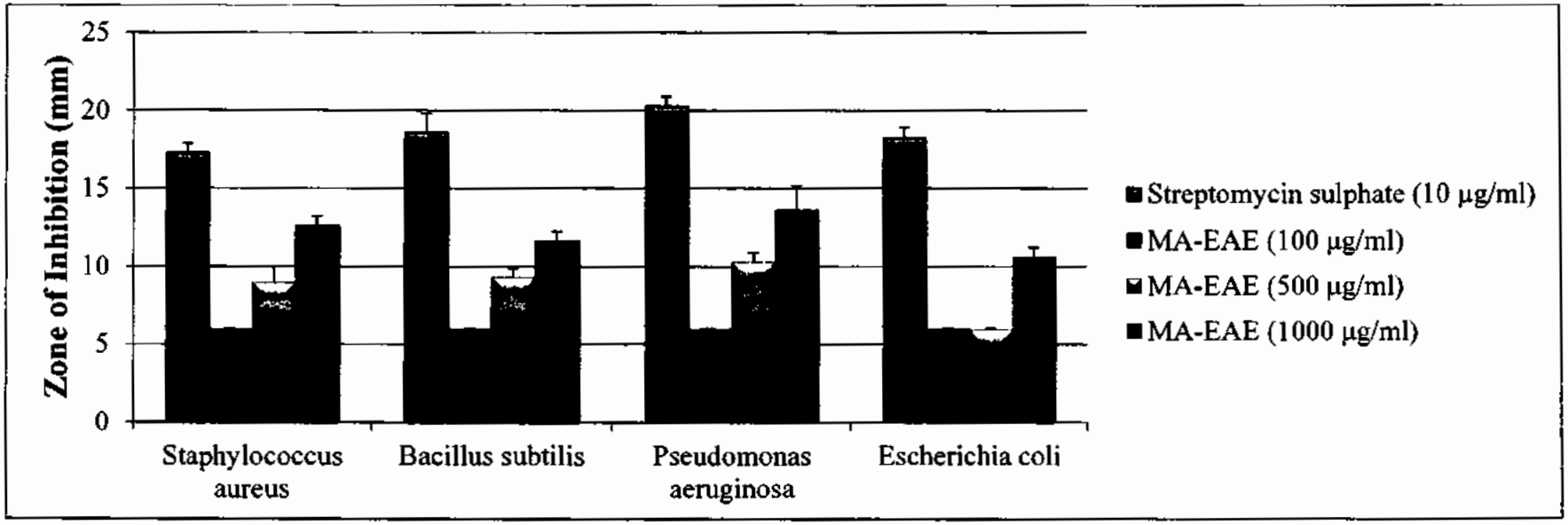


Figure 4.49: Effect of ethyl acetate extract of *Melia azedarach* against the different bacterial strains. Results are expressed as mean ± standard deviation (n = 3).

4.12. Antibacterial activity of *Justicia adhatoda*

4.12.1. Effect of methanolic extract of *Justicia adhatoda* against the different bacterial strains

The methanolic crude extracts of *Justicia adhatoda* was evaluated for *in vitro* antibacterial activity against the *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* indicating different zones of inhibition as shown in Figure 4.50. When tested, the methanol leaf extracts of *Justicia adhatoda* showed significant activity against all the tested microorganisms. The highest antibacterial activity was recorded in JA-ME (16.33 ± 0.57 mm) against the *Escherichia coli* at 1000 µg/ml, followed by JA-ME (15.66 ± 1.52 mm) against the *Pseudomonas aeruginosa*, JA-ME (15.33 ± 0.57 mm) against the *Staphylococcus aureus* at 1000 µg/ml respectively, JA-ME (13.66 ± 0.57 mm) against the *Staphylococcus aureus*, JA-ME (13 ± 1 mm) against the *Pseudomonas aeruginosa*, JA-ME (13 ± 1 mm) against the *Escherichia coli* at 500 µg/ml respectively, JA-ME (12.33 ± 0.57 mm) against the *Bacillus subtilis* at 1000 µg/ml, JA-ME (12 ± 1 mm) against the *Bacillus subtilis* at 500 µg/ml, JA-ME (11.33 ± 1.15 mm) against the *Staphylococcus aureus*, JA-ME (9.66 ± 0.57 mm) against the *Pseudomonas aeruginosa*, JA-ME (9.66 ± 0.57 mm) against the *Bacillus subtilis* and JA-ME (9.33 ± 0.57 mm) against the *Escherichia coli* at 100 µg/ml respectively. Streptomycin sulphate creates zones of inhibition ranging from 17.66 ± 0.57 mm to 21.33 ± 1.15 mm against all of the tested bacterial strains.

4.12.2. Effect of ethanolic extract of *Justicia adhatoda* against the different bacterial strains

The ethanolic crude extracts of *Justicia adhatoda* was evaluated for *in vitro* antibacterial activity against the *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli* indicating different zones of inhibition as shown in Figure 4.51. When tested, the ethanol leaf extracts of *Justicia adhatoda* showed significant activity against all the tested microorganisms. The highest antibacterial activity was recorded in JA-EE (17 ± 1 mm) against the *Escherichia coli* at 1000 µg/ml, followed by JA-EE (15.66 ± 0.57 mm) against the *Staphylococcus aureus*, JA-EE (14 ± 1 mm) against the *Bacillus subtilis* at 1000 µg/ml respectively, JA-EE (13.66 ± 0.57 mm) against the *Escherichia coli* at 500 µg/ml, JA-EE (13.33

± 0.57 mm) against the *Pseudomonas aeruginosa* at 1000 µg/ml, JA-EE (13.33 ± 0.57 mm) against the *Staphylococcus aureus*, JA-EE (13 ± 1 mm) against the *Pseudomonas aeruginosa*, JA-EE (12 ± 1 mm) against the *Bacillus subtilis* at 500 µg/ml respectively, JA-EE (11 ± 1 mm) against the *Staphylococcus aureus*, JA-EE (11 ± 1 mm) against the *Escherichia coli*, JA-EE (10.33 ± 0.57 mm) against the *Pseudomonas aeruginosa* and JA-EE (9.66 ± 1.15 mm) against the *Bacillus subtilis* at 500 µg/ml respectively. Streptomycin sulphate creates zones of inhibition ranging from 17.66 ± 0.57 mm to 22.33 ± 1.15 mm against all of the tested bacterial strains.

4.12.3. Effect of ethyl acetate extract of *Justicia adhatoda* against the different bacterial strains

The ethyl acetate crude extracts of *Justicia adhatoda* was evaluated for *in vitro* antibacterial activity against the *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli* indicating different zones of inhibition as shown in Figure 4.52. When tested, the ethyl acetate leaf extracts of *Justicia adhatoda* showed significant activity against most of the tested microorganisms. The highest antibacterial activity was recorded in JA-EAE (13 ± 1 mm) against the *Staphylococcus aureus* at 1000 µg/ml, followed by JA-EAE (11 ± 1 mm) against the *Escherichia coli*, JA-EAE (10.67 ± 0.57 mm) against the *Bacillus subtilis* at 1000 µg/ml respectively, JA-EAE (10.67 ± 0.57 mm) against the *Staphylococcus aureus*, JA-EAE (10.33 ± 0.57 mm) against the *Escherichia coli* at 500 µg/ml respectively, JA-EAE (9.33 ± 0.57 mm) against the *Pseudomonas aeruginosa* at 1000 µg/ml, JA-EAE (8.67 ± 0.57 mm) against the *Bacillus subtilis* at 500 µg/ml and JA-EAE (9 ± 1 mm) against the *Staphylococcus aureus* at 100 µg/ml. No zone of inhibition was recorded in JA-EAE against the *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* at 100 µg/ml respectively and JA-EAE against the *Pseudomonas aeruginosa* at 500 µg/ml. Streptomycin sulphate creates zones of inhibition ranging from 17.66 ± 1.15 mm to 19.66 ± 0.57 mm against all of the tested bacterial strains.

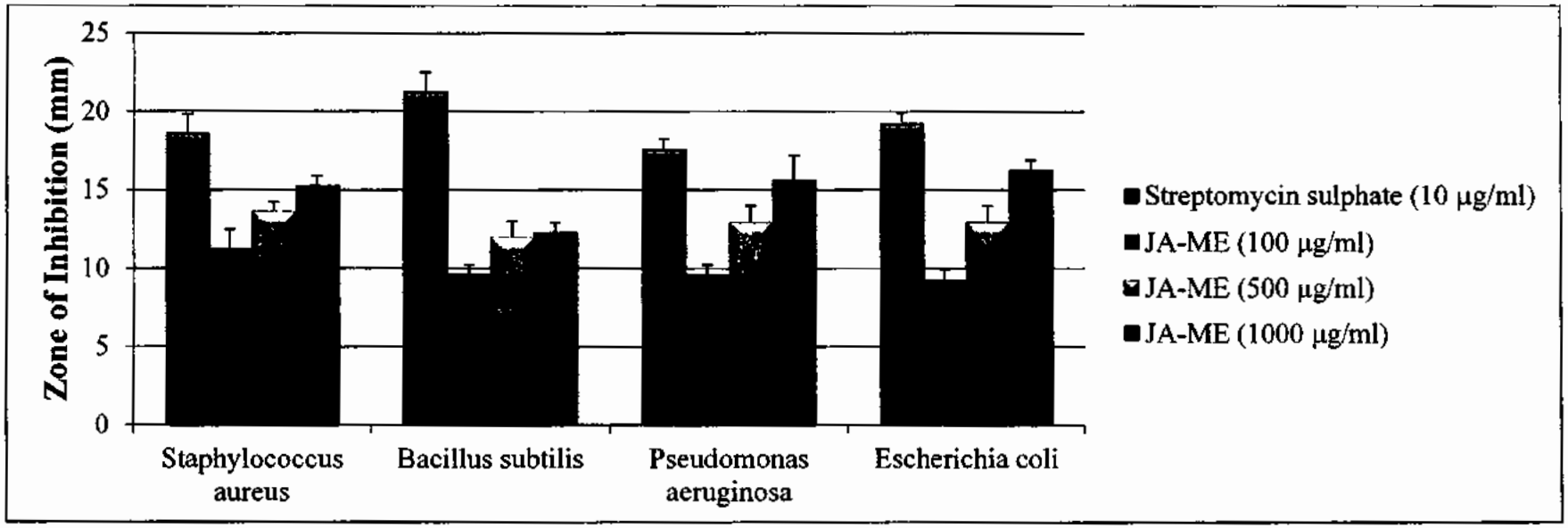


Figure 4.50: Effect of methanolic extract of *Justicia adhatoda* against the different bacterial strains. Results are expressed as mean ± standard deviation (n = 3).

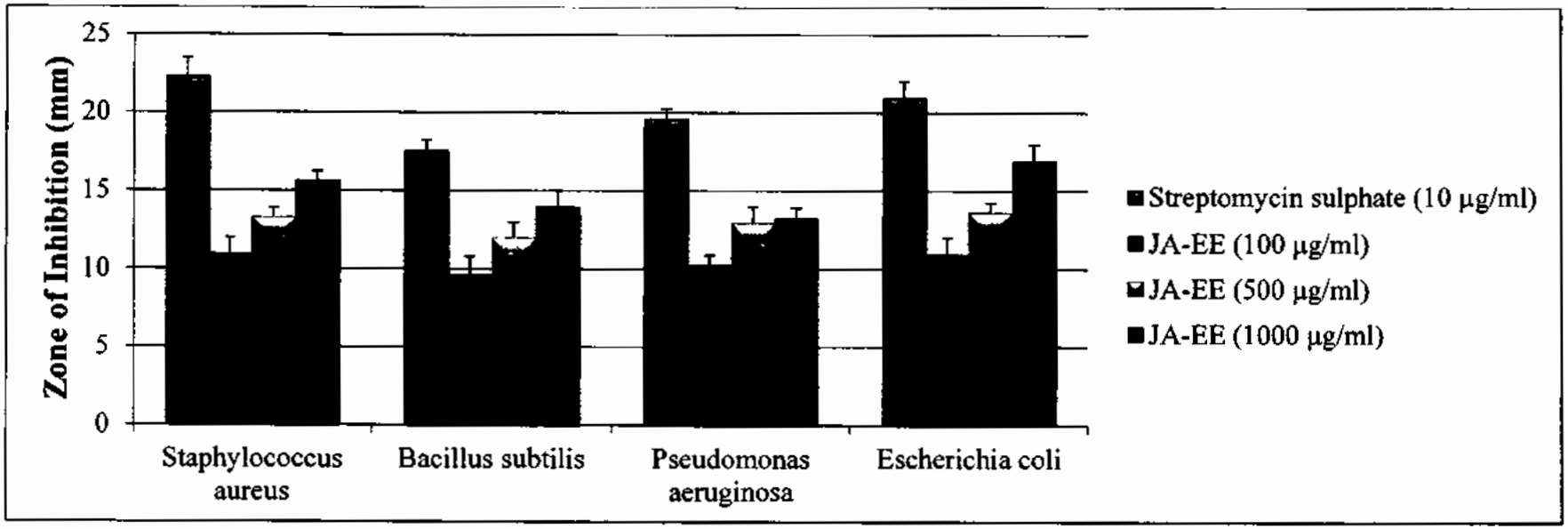


Figure 4.51: Effect of ethanolic extract of *Justicia adhatoda* against the different bacterial strains. Results are expressed as mean ± standard deviation (n = 3).

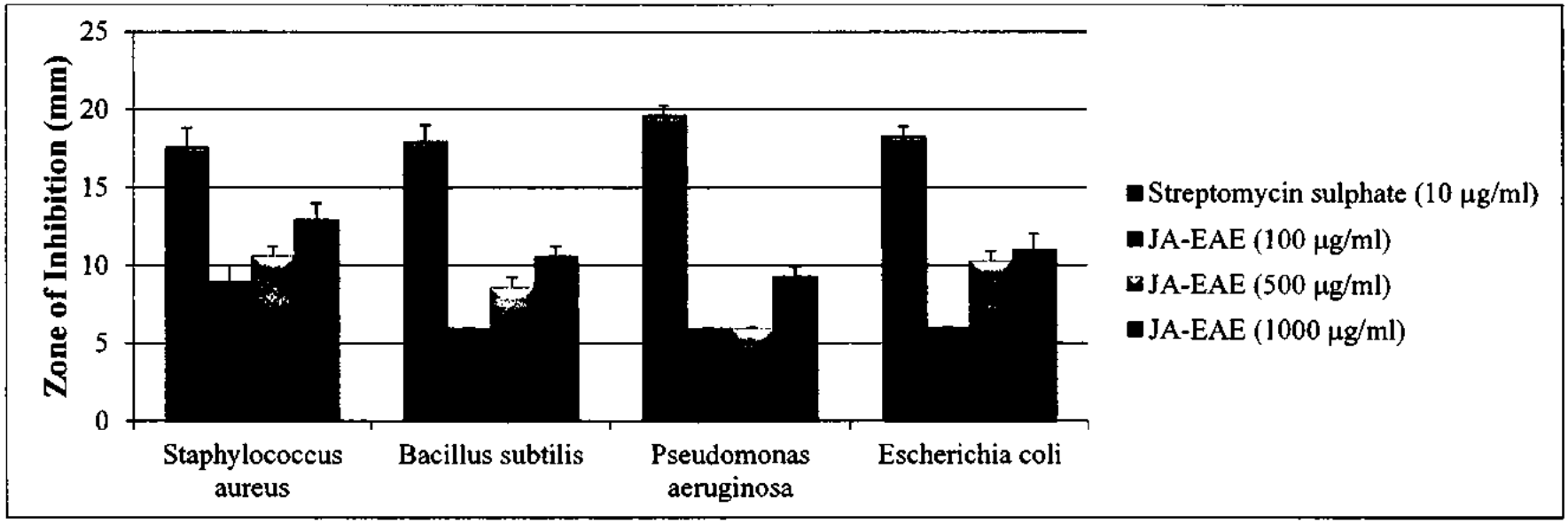


Figure 4.52: Effect of ethyl acetate extract of *Justicia adhatoda* against the different bacterial strains. Results are expressed as mean ± standard deviation (n = 3).

4.13. Antibacterial activity of *Ricinus communis*

4.13.1. Effect of methanolic extract of *Ricinus communis* against the different bacterial strains

The methanolic crude extracts of *Ricinus communis* was evaluated for *in vitro* antibacterial activity against the *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli* indicating different zones of inhibition as shown in Figure 4.53. When tested, the methanol leaf extracts of *Ricinus communis* showed significant activity against all the tested microorganisms. The highest antibacterial activity was recorded in RC-ME (18.66 ± 0.57 mm) against the *Pseudomonas aeruginosa* at 1000 µg/ml, followed by RC-ME (17.33 ± 1.15 mm) against the *Staphylococcus aureus*, RC-ME (17.33 ± 0.57 mm) against the *Bacillus subtilis,* RC-ME (16.33 ± 0.57 mm) against the *Escherichia coli* at 1000 µg/ml respectively, RC-ME (15.66 ± 1.52 mm) against the *Escherichia coli*, RC-ME (15 ± 1 mm) against the *Bacillus subtilis*, RC-ME (15 ± 1 mm) against the *Staphylococcus aureus* at 500 µg/ml respectively, RC-ME (14 ± 1 mm) against the *Bacillus subtilis*, RC-ME (14 ± 1 mm) against the *Escherichia coli,* RC-ME (13.33 ± 0.57 mm) against the *Staphylococcus aureus* at 100 µg/ml respectively, RC-ME (13 ± 1 mm) against the *Pseudomonas aeruginosa* at 500 µg/ml and RC-ME (12 ± 1 mm) against the *Pseudomonas aeruginosa* at 100 µg/ml. Streptomycin sulphate creates zones of inhibition ranging from 18 ± 1 mm to 21.33 ± 0.57 mm against all of the tested bacterial strains.

4.13.2. Effect of ethanolic extract of *Ricinus communis* against the different bacterial strains

The ethanolic crude extracts of *Ricinus communis* was evaluated for *in vitro* antibacterial activity against the *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Escherichia coli* indicating different zones of inhibition as shown in Figure 4.54. When tested, the ethanol leaf extracts of *Ricinus communis* showed significant activity against most of the tested microorganisms. The highest antibacterial activity was recorded in RC-EE (14 ± 1 mm) against the *Pseudomonas aeruginosa* and RC-EE (14 ± 1 mm) against the *Bacillus subtilis* at 1000 µg/ml, followed by RC-EE (13.33 ± 0.57 mm) against the *Staphylococcus aureus* at 1000 µg/ml, RC-EE (12.33 ± 0.57 mm) against the *Pseudomonas aeruginosa,* RC-EE (11 ± 1 mm) against the *Bacillus subtilis* at 500 µg/ml respectively, RC-EE (10 ± 1 mm) against the

Pseudomonas aeruginosa, RC-EE (9.66 ± 0.57 mm) against the *Bacillus subtilis* at 100 µg/ml respectively and RC-EE (8 ± 1 mm) against the *Staphylococcus aureus* at 500 µg/ml. No zone of inhibition was recorded in RC-EE against the *Escherichia coli* at 100 µg/ml, 500 µg/ml and 1000 µg/ml respectively and RC-EE against the *Staphylococcus aureus* at 100 µg/ml. Streptomycin sulphate creates zones of inhibition ranging from 17.66 ± 0.57 mm to 20.33 ± 0.57 mm against all of the tested bacterial strains

4.13.3. Effect of ethyl acetate extract of *Ricinus communis* against the different bacterial strains

The ethyl acetate crude extracts of *Ricinus communis* was evaluated for *in vitro* antibacterial activity against the *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* indicating different zones of inhibition as shown in Figure 4.55. When tested, the ethyl acetate leaf extracts of *Ricinus communis* showed significant activity against all the tested microorganisms. The highest antibacterial activity was recorded in RC-EAE (17.66 ± 0.57 mm) against the *Staphylococcus aureus* at 1000 µg/ml, followed by RC-EAE (16.66 ± 0.57 mm) against the *Bacillus subtilis*, RC-EAE (16 ± 1 mm) against the *Pseudomonas aeruginosa,* RC-EAE (15.33 ± 0.57 mm) against the *Escherichia coli* at 1000 µg/ml respectively, RC-EAE (15.33 ± 0.57 mm) against the *Bacillus subtilis*, RC-EAE (14.33 ± 0.57 mm) against the *Staphylococcus aureus,* RC-EAE (14 ± 1 mm) against the *Escherichia coli* at 500 µg/ml respectively, RC-EAE (14 ± 1 mm) against the *Staphylococcus aureus* at 100 µg/ml, RC-EAE (13 ± 1 mm) against the *Pseudomonas aeruginosa* at 500 µg/ml, RC-EAE (12.33 ± 0.57 mm) against the *Pseudomonas aeruginosa*, RC-EAE (11 ± 1 mm) against the *Bacillus subtilis* and RC-EAE (10 ± 1 mm) against the *Escherichia coli* at 100 µg/ml respectively. Streptomycin sulphate creates zones of inhibition ranging from 17.66 ± 0.57 mm to 22.33 ± 0.57 mm against all of the tested bacterial strains.

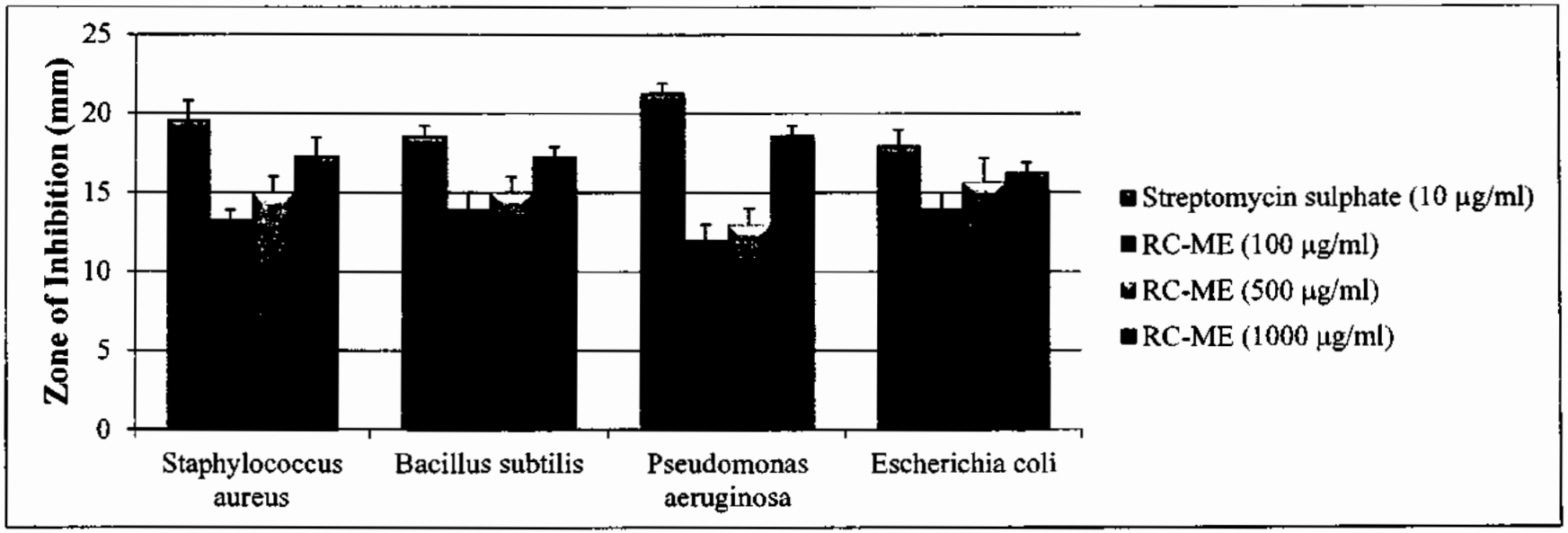


Figure 4.53: Effect of methanolic extract of *Ricinus communis* against different bacterial strains. Results are expressed as mean ± standard deviation (n = 3).

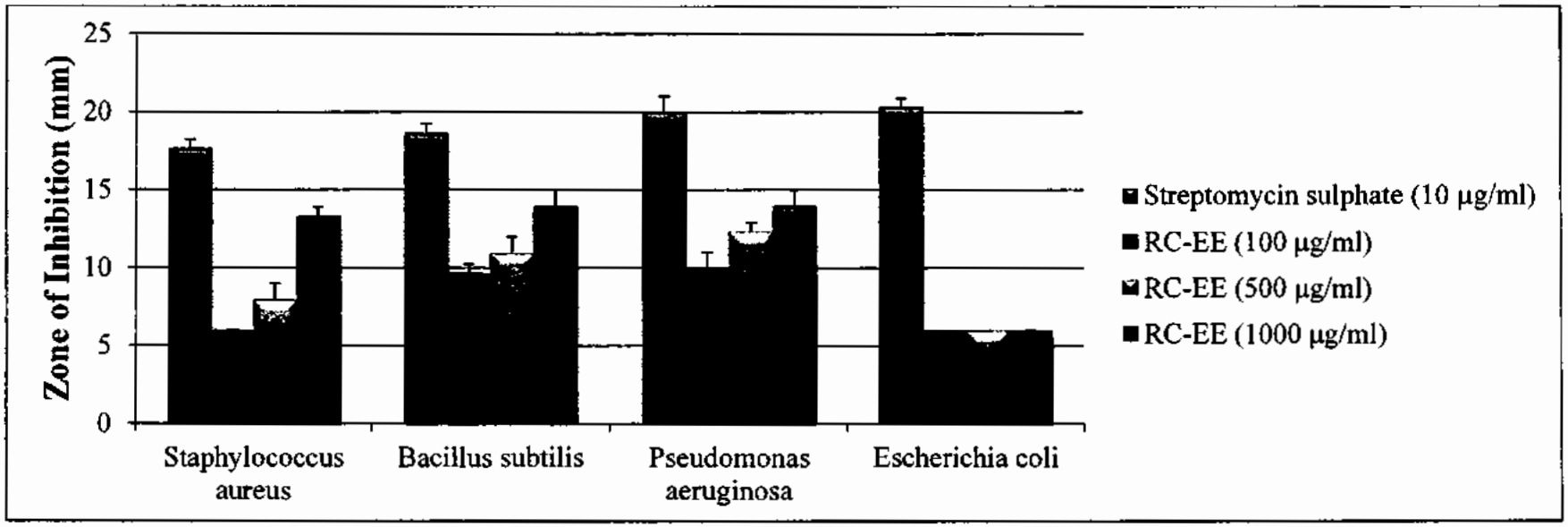


Figure 4.54: Effect of ethanolic extract of *Ricinus communis* against the different bacterial strains. Results are expressed as mean ± standard deviation (n = 3).

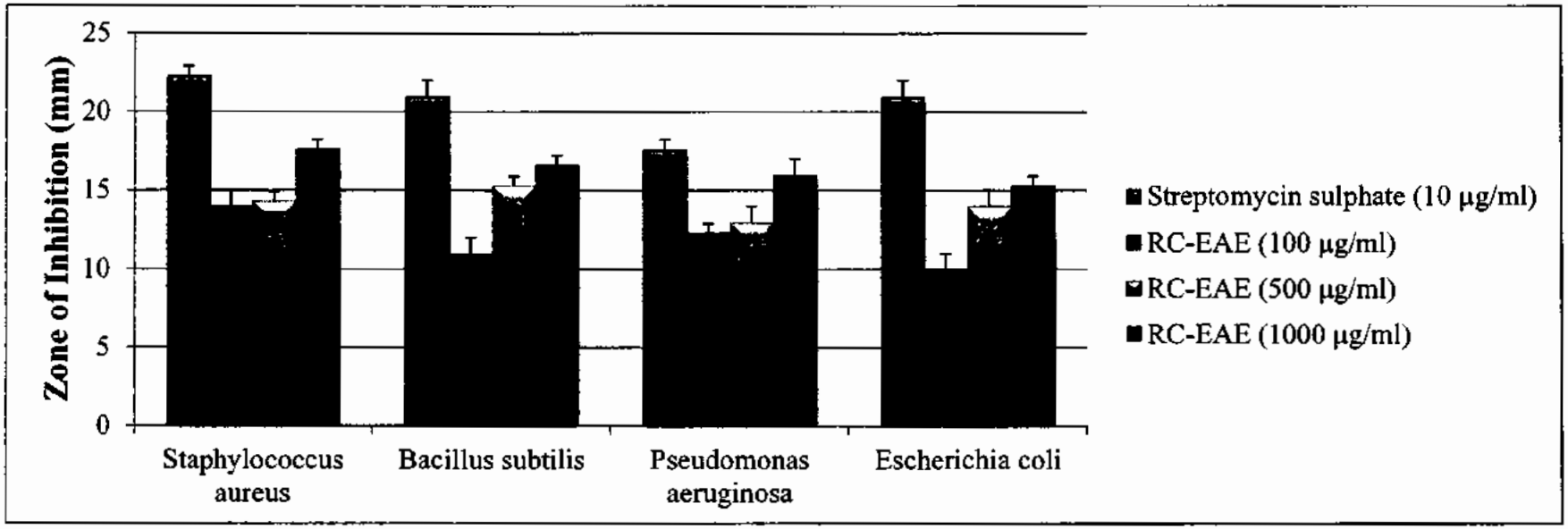


Figure 4.55: Effect of ethyl acetate extract of *Ricinus communis* against the different bacterial strains. Results are expressed as mean ± standard deviation (n = 3).

4.14. Antifungal activity of *Melia azedarach*

4.14.1. Effect of methanolic extract of *Melia azedarach* against the different fungal strains

The methanolic crude extracts of *Melia azedarach* was evaluated for *in vitro* antifungal activity against the *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans* and *Fusarium oxysporum* indicating different zones of inhibition as shown in Figure 4.56. When tested, the methanol leaf extracts of *Melia azedarach* showed significant activity against some of the tested microorganisms. The highest antifungal activity was recorded in MA-ME (13.66 ± 0.57 mm) against the *Aspergillus flavus* at 1000 µg/ml, followed by MA-ME (12.33 ± 0.57 mm) against the *Fusarium oxysporum*, MA-ME (11.33 ± 0.57 mm) against the *Aspergillus niger* at 1000 µg/ml respectively, MA-ME (10.33 ± 1.15 mm) against the *Aspergillus flavus* at 500 µg/ml. No zone of inhibition was recorded in MA-ME against the *Candida albicans* at 1000 µg/ml, MA-ME against the *Aspergillus niger*, *Candida albicans* and *Fusarium oxysporum* at 500 µg/ml respectively and MA-ME against the *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans* and *Fusarium oxysporum* at 100 µg/ml respectively. The positive control (Nystatin) creates zones of inhibition ranging from 18.33 ± 0.57 mm to 20.33 ± 0.57 mm against all of the tested fungal strains.

4.14.2. Effect of ethanolic extract of *Melia azedarach* against the different fungal strains

The ethanolic crude extracts of *Melia azedarach* was evaluated for *in vitro* antifungal activity against *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans* and *Fusarium oxysporum* indicating different zones of inhibition as shown in Figure 4.57. When tested, the ethanol leaf extracts of *Melia azedarach* showed significant activity against some of the tested microorganisms. The highest antifungal activity was recorded in MA-EE (14.33 ± 0.57 mm) against the *Fusarium oxysporum* at 1000 µg/ml, followed by MA-EE (14 ± 1 mm) against the *Aspergillus flavus*, MA-EE (13.33 ± 0.57 mm) against the *Aspergillus niger* at 1000 µg/ml respectively, MA-EE (12.66 ± 1.15 mm) against the *Aspergillus flavus,* MA-EE (11 ± 1 mm) against the *Aspergillus niger,* MA-EE (11 ± 1 mm) against the *Fusarium oxysporum* at 500 µg/ml respectively. No zone of inhibition was recorded in MA-EE against the *Candida albicans* at 1000 µg/ml, MA-EE against the *Candida albicans* at 500 µg/ml and MA-EE against the

Aspergillus niger, *Candida albicans* and *Fusarium oxysporum* at 100 µg/ml respectively. The positive control (Nystatin) creates zones of inhibition ranging from 18.33 ± 0.57 mm to 21.33 ± 0.57 mm against all of the tested fungal strains.

4.14.3. Effect of ethyl acetate extract of *Melia azedarach* against the different fungal strains

The ethyl acetate crude extracts of *Melia azedarach* was evaluated for *in vitro* antifungal activity against *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans* and *Fusarium oxysporum* indicating different zones of inhibition as shown in Figure 4.58. When tested, the ethyl acetate leaf extracts of *Melia azedarach* showed significant activity against few of the tested microorganisms. The highest antifungal activity was recorded in MA-EAE (12.33 ± 0.57 mm) against the *Aspergillus flavus* at 1000 µg/ml, followed by MA-EAE (11.66 ± 1.15 mm) against the *Fusarium oxysporum* at 1000 µg/ml, MA-EAE (11 ± 1 mm) against the *Aspergillus flavus* at 500 µg/ml. No zone of inhibition was recorded in MA-EAE against the *Aspergillus niger* and *Candida albicans* at 1000 µg/ml respectively, MA-EAE against the *Aspergillus niger*, *Candida albicans* and *Fusarium oxysporum* at 500 µg/ml respectively and MA-EAE against the *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans* and *Fusarium oxysporum* at 100 µg/ml respectively. The positive control (Nystatin) creates zones of inhibition ranging from 18.33 ± 0.57 mm to 20.33 ± 1.15 mm against all of the tested fungal strains.

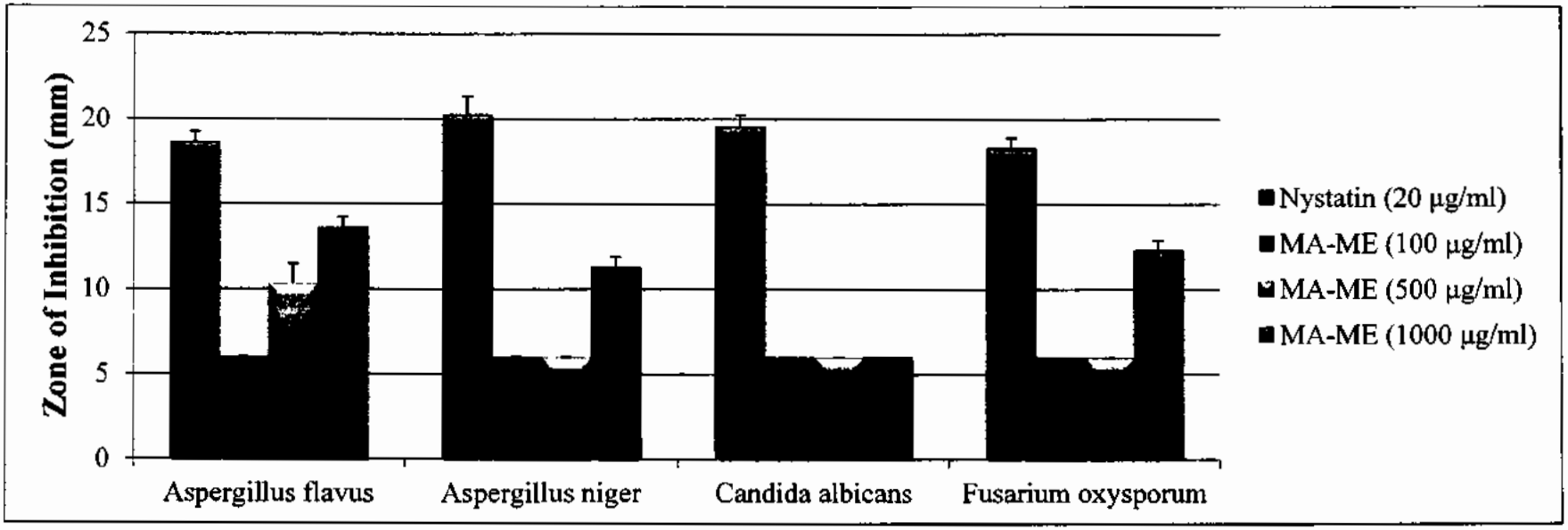


Figure 4.56: Effect of methanolic extract of *Melia azedarach* against the different fungal strains. Results are expressed as mean ± standard deviation (n = 3).

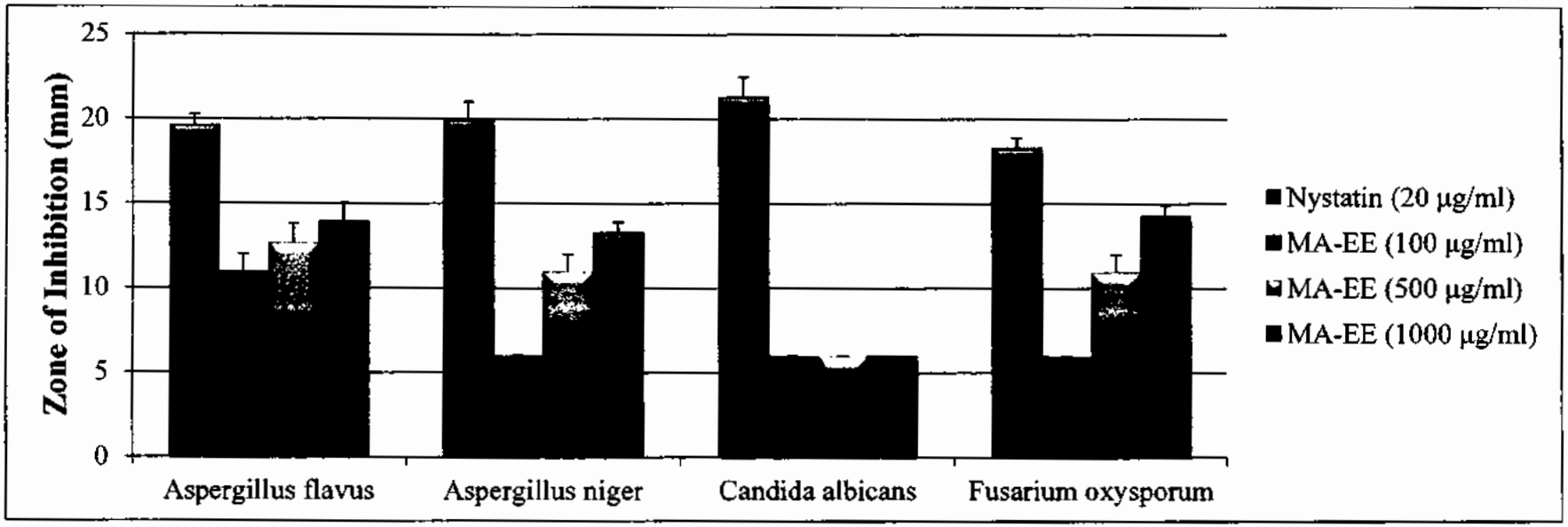


Figure 4.57: Effect of ethanolic extract of *Melia azedarach* against the different fungal strains. Results are expressed as mean ± standard deviation (n = 3).

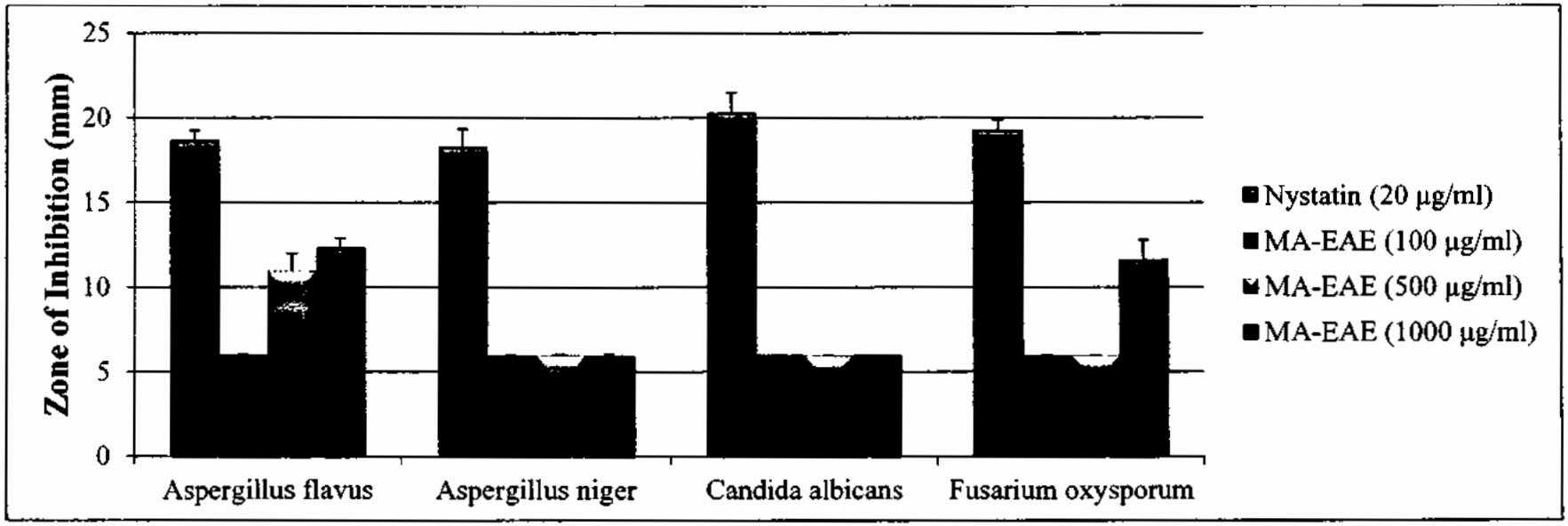


Figure 4.58: Effect of ethyl acetate extract of *Melia azedarach* against the different fungal strains. Results are expressed as mean ± standard deviation (n = 3).

4.15. Antifungal activity of *Justicia adhatoda*

4.15.1. Effect of methanolic extract of *Justicia adhatoda* against the different fungal strains

The methanolic crude extracts of *Justicia adhatoda* was evaluated for *in vitro* antifungal activity against *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans* and *Fusarium oxysporum* indicating different zones of inhibition as shown in Figure 4.59. When tested, the methanol leaf extracts of *Justicia adhatoda* showed significant activity against some of the tested microorganisms. The highest antifungal activity was recorded in JA-ME (15.66 ± 0.57 mm) against the *Aspergillus niger* at 1000 µg/ml, followed by JA-ME (14.33 ± 0.57 mm) against the *Candida albicans* at 1000 µg/ml, JA-ME (13 ± 1 mm) against the *Aspergillus niger*, JA-ME (13 ± 1 mm) against *Candida albicans* at 500 µg/ml respectively, JA-ME (11.33 ± 1.15 mm) against the *Fusarium oxysporum* at 1000 µg/ml, JA-ME (11.33 ± 1 mm) against *Aspergillus niger*, JA-ME (9.66 ± 0.57 mm) against the *Candida albicans* at 100 µg/ml respectively. No zone of inhibition was recorded in JA-ME against the *Aspergillus flavus* at 1000 µg/ml, JA-ME against the *Aspergillus flavus* and *Fusarium oxysporum* at 500 µg/ml respectively and JA-ME against the *Aspergillus flavus* and *Fusarium oxysporum* at 100 µg/ml respectively. The positive control (Nystatin) creates zones of inhibition ranging from 18.66 ± 1.15 mm to 19.66 ± 0.57 mm against all of the tested fungal strains.

4.15.2. Effect of ethanolic extract of *Justicia adhatoda* against the different fungal strains

The ethanolic crude extracts of *Justicia adhatoda* was evaluated for *in vitro* antifungal activity against *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans* and *Fusarium oxysporum* indicating different zones of inhibition as shown in Figure 4.60. When tested, the ethanol leaf extracts of *Justicia adhatoda* showed significant activity against most of the tested microorganisms. The highest antifungal activity was recorded in JA-EE (14 ± 1 mm) against the *Aspergillus niger* at 1000 µg/ml, followed by JA-EE (12.33 ± 0.57 mm) against the *Aspergillus niger* at 500 µg/ml, JA-EE (10.66 ± 0.57 mm) against the *Fusarium oxysporum,* JA-EE (10.33 ± 0.57 mm) against the *Candida albicans* at 1000 µg/ml respectively, JA-EE (10.33 ± 0.57 mm) against the *Aspergillus niger* at 100 µg/ml, JA-EE (9.66 ± 1.15 mm) against the *Aspergillus*

flavus at 1000 µg/ml and JA-EE (8.66 ± 0.57 mm) against the *Candida albicans* at 500 µg/ml. No zone of inhibition was recorded in JA-EE against the *Aspergillus flavus* and *Fusarium oxysporum* at 500 µg/ml respectively, JA-EE against the *Aspergillus flavus Candida albicans* and *Fusarium oxysporum* at 100 µg/ml respectively. The positive control (Nystatin) creates zones of inhibition ranging from 17.33 ± 0.57 mm to 20.66 ± 1.15 mm against all of the tested fungal strains.

4.15.3. Effect of ethyl acetate extract of *Justicia adhatoda* against the different fungal strains

The ethyl acetate crude extracts of *Justicia adhatoda* was evaluated for *in vitro* antifungal activity against the *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans* and *Fusarium oxysporum* indicating different zones of inhibition as shown in Figure 4.61. When tested, the ethyl acetate leaf extracts of *Justicia adhatoda* showed significant activity against some of the tested microorganisms. The highest antifungal activity was recorded in JA-EAE (14.33 ± 1.15 mm) against the *Aspergillus niger* at 1000 µg/ml, followed by JA-EAE (13.66 ± 0.57 mm) against the *Candida albicans* at 1000 µg/ml, JA-EAE (10.33 ± 0.57 mm) against the *Candida albicans* and JA-EAE (8.66 ± 0.57 mm) against the *Aspergillus niger* at 500 µg/ml respectively. No zone of inhibition was recorded in JA-EAE against the *Aspergillus flavus* and *Fusarium oxysporum* at 1000 µg/ml respectively, JA-EAE against the *Aspergillus flavus* and *Fusarium oxysporum* at 500 µg/ml respectively and JA-EAE against the *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans* and *Fusarium oxysporum* at 100 µg/ml respectively. The positive control (Nystatin) creates zones of inhibition ranging from 18.66 ± 0.57 mm to 21 ± 1 mm against all of the tested fungal strains.

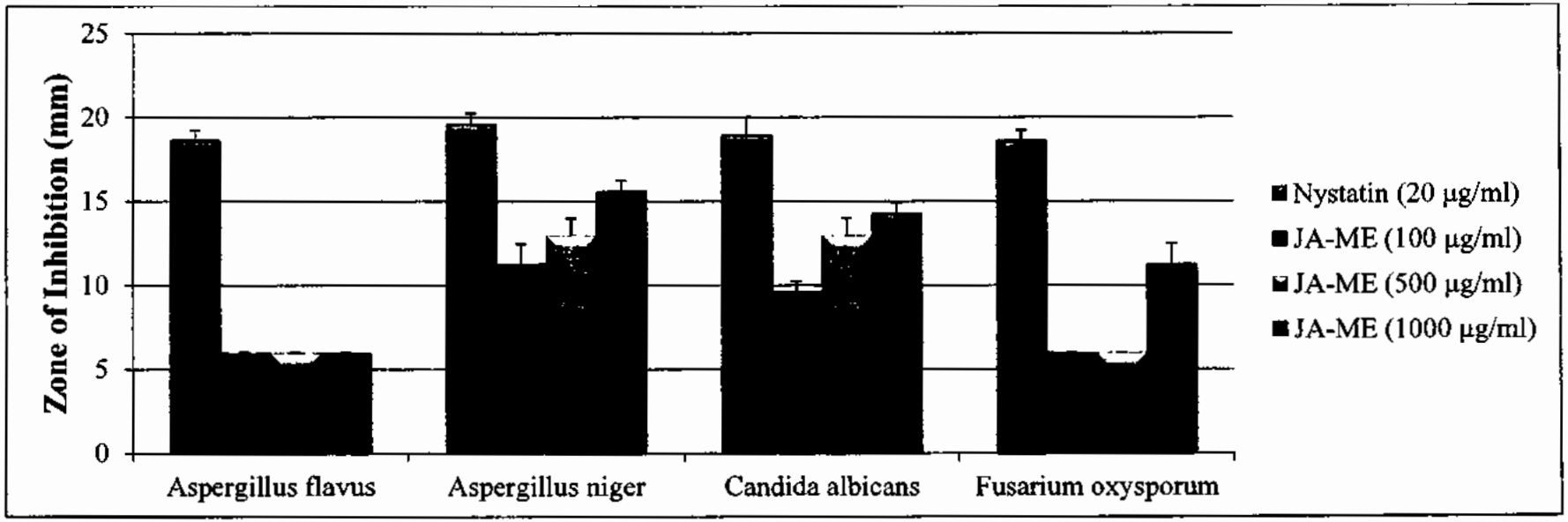


Figure 4.59: Effect of methanolic extract of *Justicia adhatoda* against the different fungal strains. Results are expressed as mean ± standard deviation (n = 3).

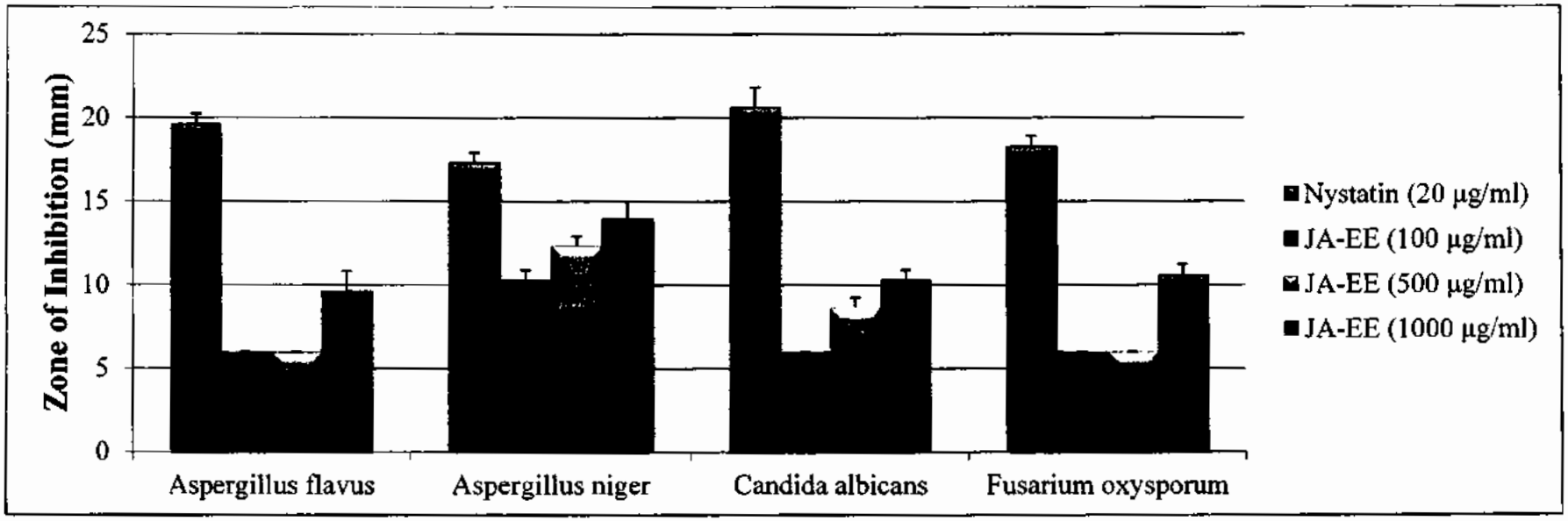


Figure 4.60: Effect of ethanolic extract of *Justicia adhatoda* against the different fungal strains. Results are expressed as mean ± standard deviation (n = 3).

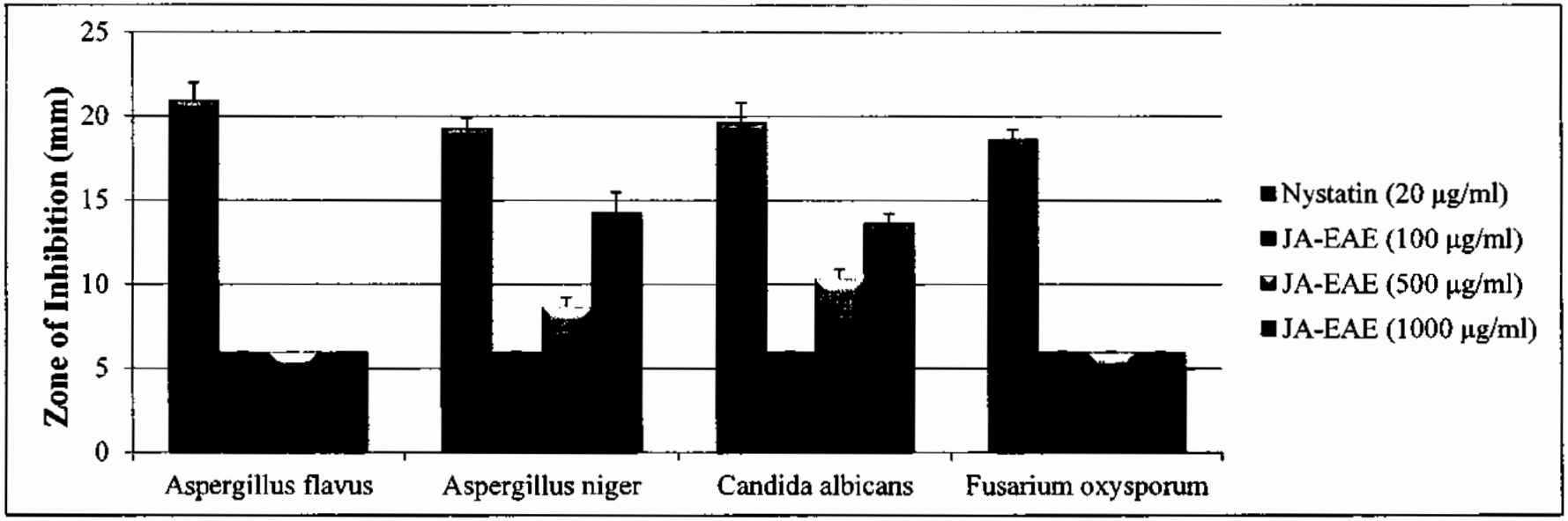


Figure 4.61: Effect of ethyl acetate extract of *Justicia adhatoda* against the different fungal strains. Results are expressed as mean ± standard deviation (n = 3).

4.16. Antifungal activity of *Ricinus communis*

4.16.1. Effect of methanolic extract of *Ricinus communis* against the different fungal strains

The methanolic crude extracts of *Ricinus communis* was evaluated for *in vitro* antifungal activity against the *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans* and *Fusarium oxysporum* indicating different zones of inhibition as shown in Figure 4.62. When tested, the methanol leaf extracts of *Ricinus communis* showed significant activity against most of the tested microorganisms. The highest antifungal activity was recorded in RC-ME (16 ± 1 mm) against the *Aspergillus flavus* at 1000 µg/ml, followed by RC-ME (14 ± 1 mm) against the *Aspergillus flavus* at 500 µg/ml, RC-ME (13.66 ± 0.57 mm) against the *Aspergillus niger*, RC-ME (12.33 ± 0.57 mm) against the *Fusarium oxysporum* at 1000 µg/ml respectively, RC-ME (12 ± 1 mm) against the *Aspergillus niger* at 500 µg/ml, RC-ME (11.33 ± 0.57 mm) against the *Candida albicans* at 1000 µg/ml, RC-ME (10 ± 1 mm) against the *Aspergillus flavus* and RC-ME (9.66 ± 0.57 mm) against the *Aspergillus niger* at 100 µg/ml respectively. No zone of inhibition was recorded in RC-ME against the *Candida albicans* and *Aspergillus flavus* at 500 µg/ml respectively and RC-ME against the *Candida albicans* and *Aspergillus flavus* at 100 µg/ml respectively. The positive control (Nystatin) creates zones of inhibition ranging from 18 ± 1 mm to 20 ± 1 mm against all of the tested fungal strains.

4.16.2. Effect of ethanolic extract of *Ricinus communis* against the different fungal strains

The ethanolic crude extracts of *Ricinus communis* was evaluated for *in vitro* antifungal activity against the *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans* and *Fusarium oxysporum* indicating different zones of inhibition as shown in Figure 4.63. When tested, the ethanol leaf extracts of *Ricinus communis* showed significant activity against most of the tested microorganisms. The highest antifungal activity was recorded in RC-EE (12.66 ± 1.15 mm) against the *Aspergillus flavus* at 1000 µg/ml, followed by RC-EE (11.33 ± 0.57 mm) against the *Fusarium oxysporum* at 1000 µg/ml, RC-EE (11 ± 1 mm) against the *Aspergillus flavus* at 500 µg/ml, RC-EE (9.66 ± 0.57 mm) against the *Aspergillus niger* at 1000 µg/ml, RC-EE (8.66 ± 0.57 mm) against the *Fusarium oxysporum,* RC-EE (8.33 ± 0.57 mm) against the *Aspergillus*

niger at 500 µg/ml respectively and RC-EE (7.66 ± 0.57 mm) against the *Aspergillus flavus* at 100 µg/ml respectively. No zone of inhibition was recorded in RC-EE against the *Candida albicans* at 1000 µg/ml, RC-EE against the *Candida albicans* at 500 µg/ml and RC-EE against the *Aspergillus niger*, *Candida albicans* and *Fusarium oxysporum* at 500 µg/ml respectively. The positive control (Nystatin) creates zones of inhibition ranging from 18.33 ± 1.15 mm to 22.33 ± 1.15 mm against all of the tested fungal strains.

4.16.3. Effect of ethyl acetate extract of *Ricinus communis* against the different fungal strains

The ethyl acetate crude extracts of *Ricinus communis* was evaluated for *in vitro* antifungal activity against the *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans* and *Fusarium oxysporum* indicating different zones of inhibition as shown in Figure 4.64. When tested, the ethyl acetate leaf extracts of *Ricinus communis* showed significant activity against most of the tested microorganisms. The highest antifungal activity was recorded in RC-EAE (15 ± 1 mm) against the *Aspergillus flavus* at 1000 µg/ml, followed by RC-EAE (12.66 ± 0.57 mm) against the *Aspergillus flavus* at 500 µg/ml, RC-EAE (12 ± 1 mm) against the *Aspergillus niger* at 1000 µg/ml, RC-EAE (12 ± 1 mm) against the *Aspergillus niger* at 100 µg/ml, RC-EAE (11.66 ± 1.15 mm) against the *Fusarium oxysporum* at 1000 µg/ml, RC-EAE (11.33 ± 0.57 mm) against the *Aspergillus niger*, RC-EAE (9.33 ± 0.57 mm) against the *Fusarium oxysporum* at 500 µg/ml respectively and RC-EAE (8.66 ± 0.57 mm) against the *Candida albicans* at 1000 µg/ml. No zone of inhibition was recorded in RC-EAE against the *Candida albicans* at 500 µg/ml and RC-EAE against the *Aspergillus niger*, *Candida albicans* and *Fusarium oxysporum* at 100 µg/ml respectively. The positive control (Nystatin) creates zones of inhibition ranging from 17.33 ± 0.57 mm to 20.66 ± 1.15 mm against all of the tested fungal strains.

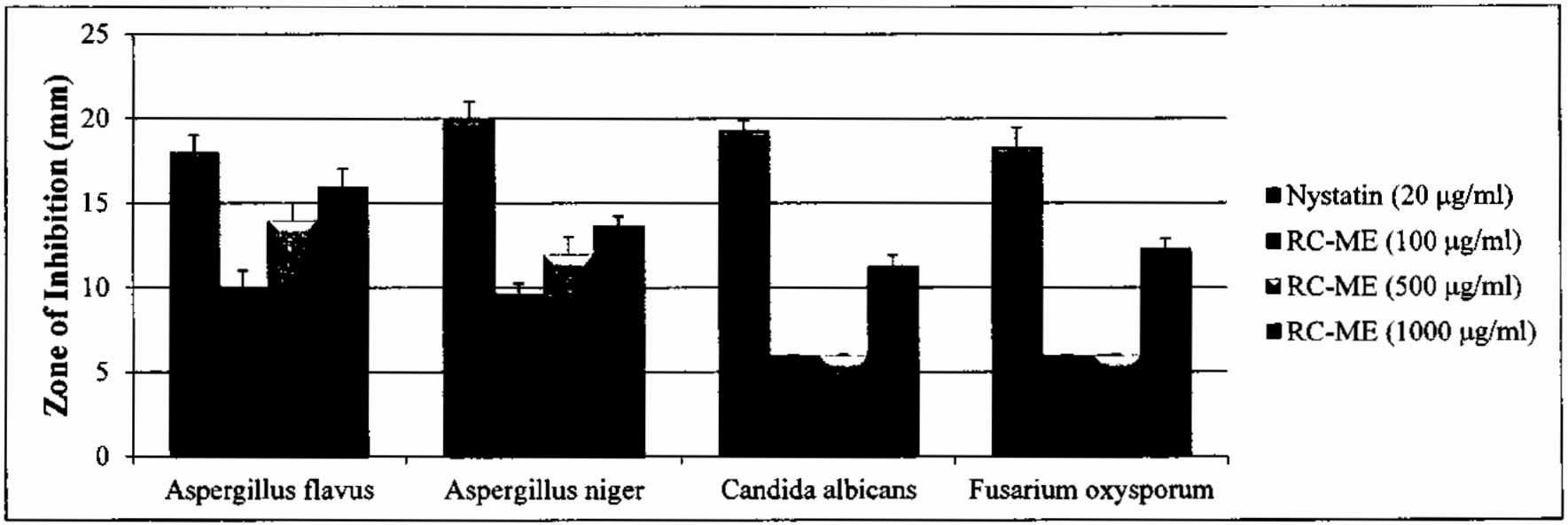


Figure 4.62: Effect of methanolic extract of *Ricinus communis* against the different fungal strains. Results are expressed as mean ± standard deviation (n = 3).

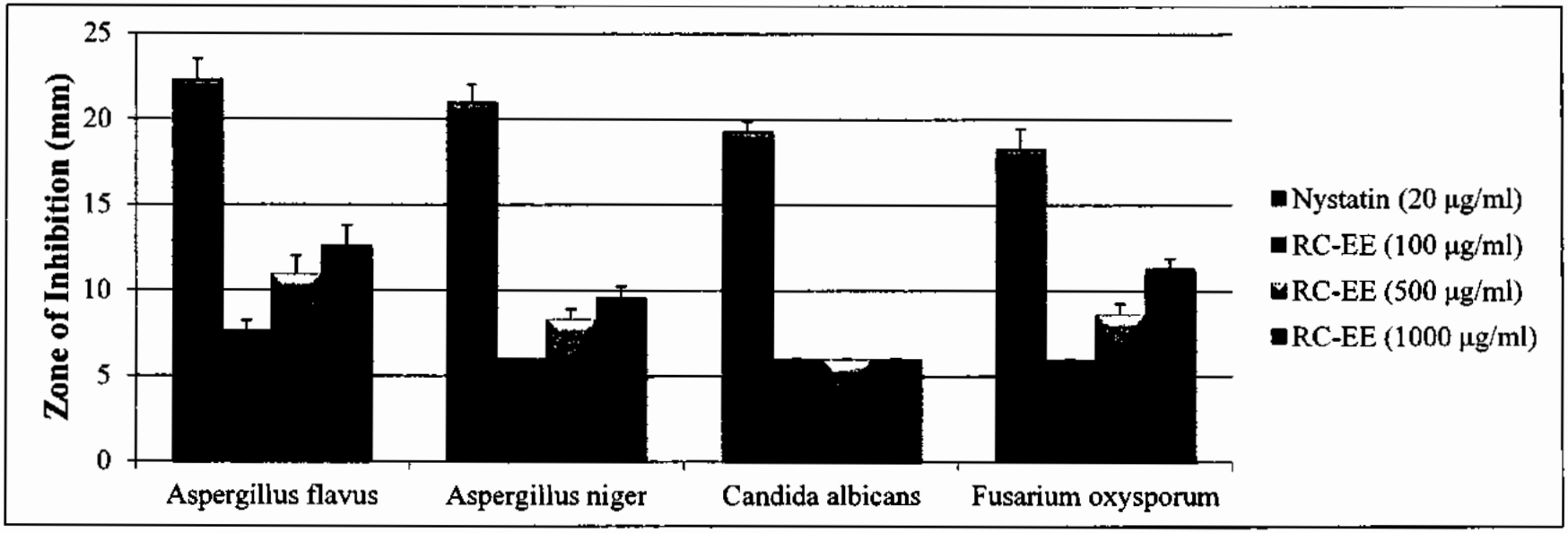


Figure 4.63: Effect of ethanolic extract of *Ricinus communis* against the different fungal strains. Results are expressed as mean ± standard deviation (n = 3).

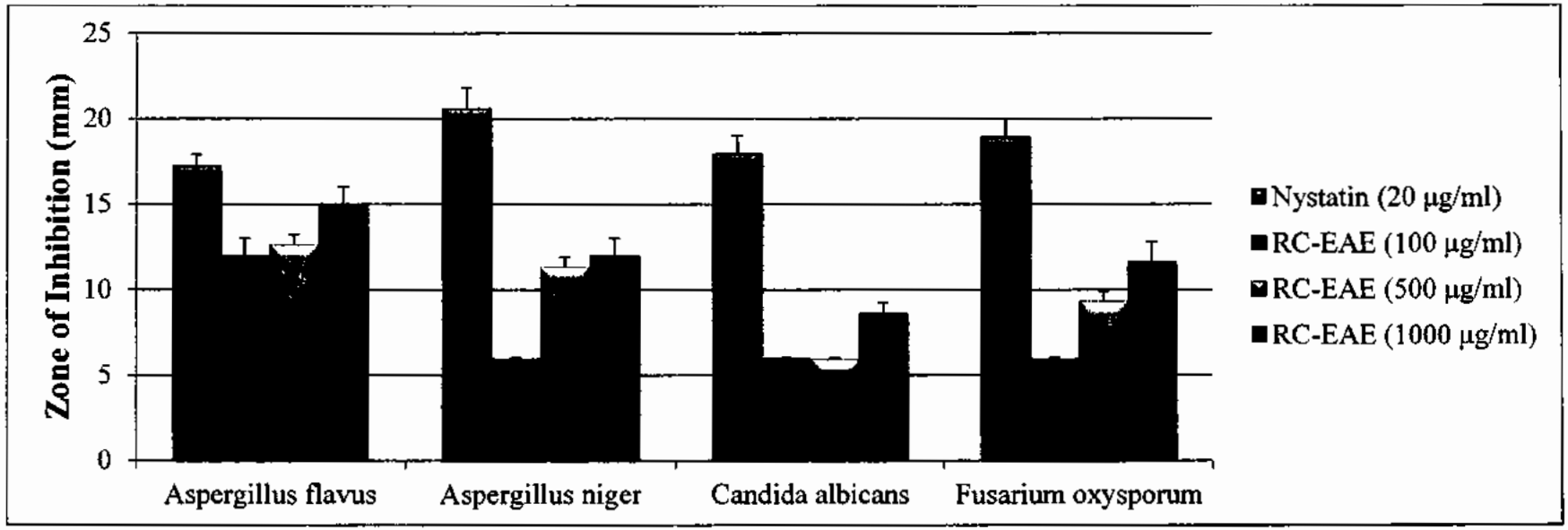


Figure 4.64: Effect of ethyl acetate extract of *Ricinus communis* against the different fungal strains. Results are expressed as mean ± standard deviation (n = 3).

4.17. Anticancer activity of *Melia azedarach*

4.17.1. Inhibition of the Proliferation of HepG2 cell line by *Melia azedarach* extracts

The anticancer activity of *Melia azedarach* extracts was detected by MTT assay at 48 hours and 72 h as shown in Figure 4.65 and 4.66. All the three *Melia azedarach* extracts showed good antiproliferative activity at both time intervals. The MA-ME demonstrated more effective results with percent viability of 12.33% and 6.55% at 48 h on both higher concentrations of 500 µg/ml and 1000 µg/ml respectively. The MA-ME demonstrated more effective results with percent viability of 19.72% at 72 h on higher concentration of 1000 µg/ml. The MA-EE demonstrated more effective results with percent viability of 16.79% at 48 h on higher concentration of 1000 µg/ml. The MA-EE demonstrated more effective results with percent viability of 15.53% at 72 h on higher concentration of 1000 µg/ml. The MA-EAE demonstrated more effective results with percent viability of 20.01% at 48 h on higher concentration of 1000 µg/ml. The MA-EAE demonstrated more effective results with percent viability of 24.70% at 72 h on higher concentration of 1000 µg/ml.

4.17.2. Inhibition of the Proliferation of HCCLM3 cell line by *Melia azedarach* extracts

The anticancer activity of *Melia azedarach* extracts was detected by MTT assay at 48 h and 72 h as shown in Figure 4.67 and 4.68. All the three *Melia azedarach* extracts showed good antiproliferative activity at both time intervals. The MA-ME demonstrated more effective results with percent viability of 22.83% at 48 h on higher concentration of 1000 µg/ml. The MA-ME demonstrated more effective results with percent viability of 10.84% at 72 h on higher concentration of 1000 µg/ml. The MA-EE demonstrated more effective results with percent viability of 20.31% at 48 h on higher concentration of 1000 µg/ml. The MA-EE demonstrated more effective results with percent viability of 19.69% at 72 h on higher concentration of 1000 µg/ml. The MA-EAE demonstrated more effective results with percent viability of 26.03% at 48 h on higher concentration of 1000 µg/ml. The MA-EAE demonstrated more effective results with percent viability of 20.57% at 72 h on higher concentration of 1000 µg/ml.

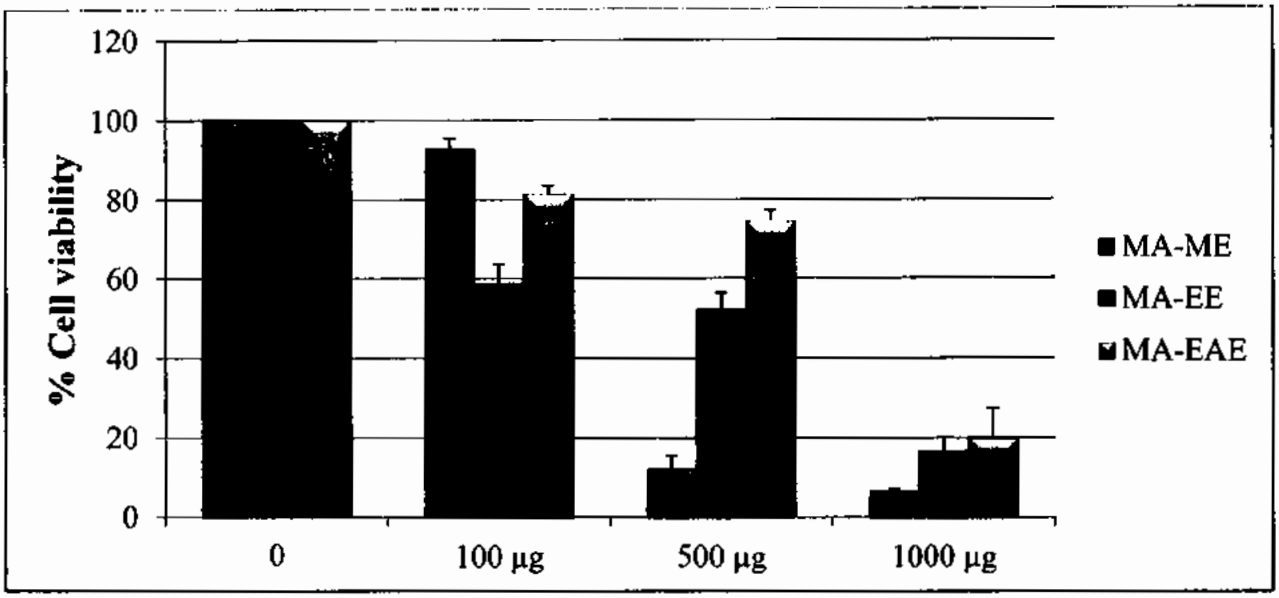


Figure 4.65: Inhibition of the Proliferation of HepG2 cell line by *Melia azedarach* extracts at 48 hours. Results are expressed as mean ± standard deviation (n = 3).

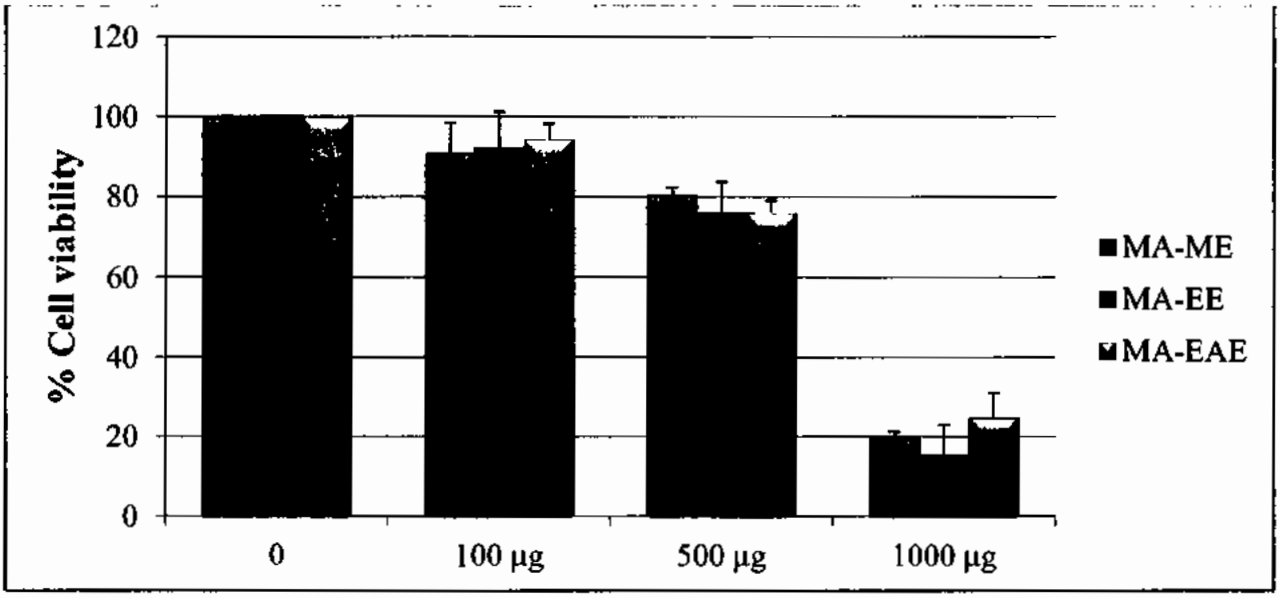


Figure 4.66: Inhibition of the Proliferation of HepG2 cell line by *Melia azedarach* extracts at 72 hours. Results are expressed as mean ± standard deviation (n = 3).

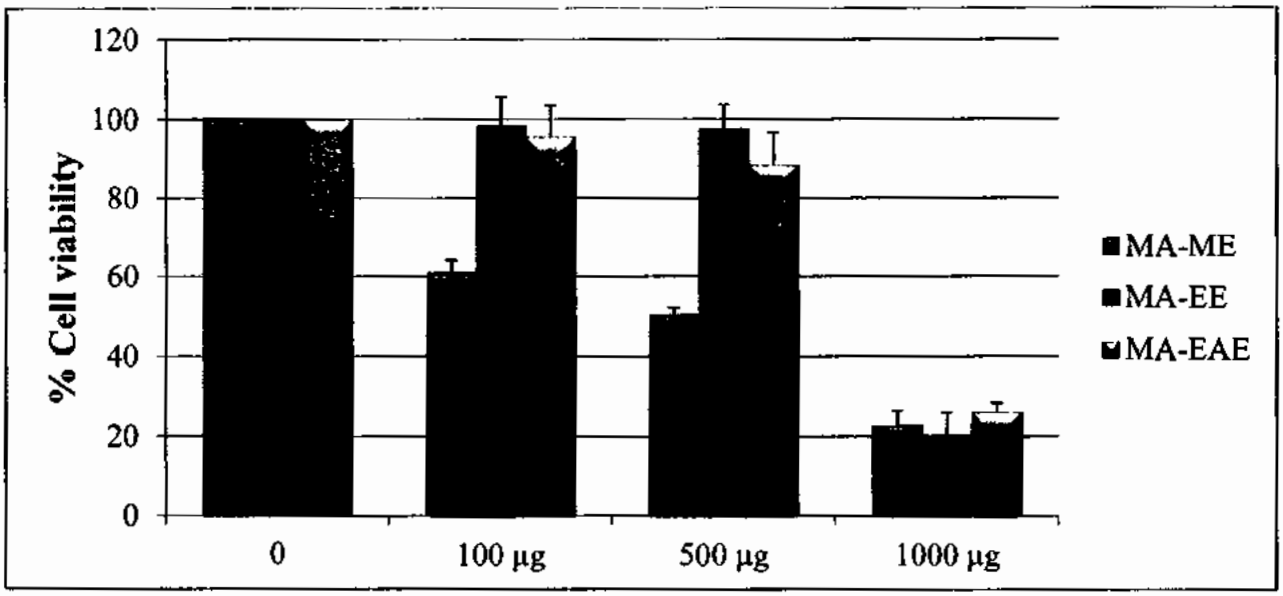


Figure 4.67: Inhibition of the Proliferation of HCCLM3 cell line by *Melia azedarach* extracts at 48 hours. Results are expressed as mean ± standard deviation (n = 3).

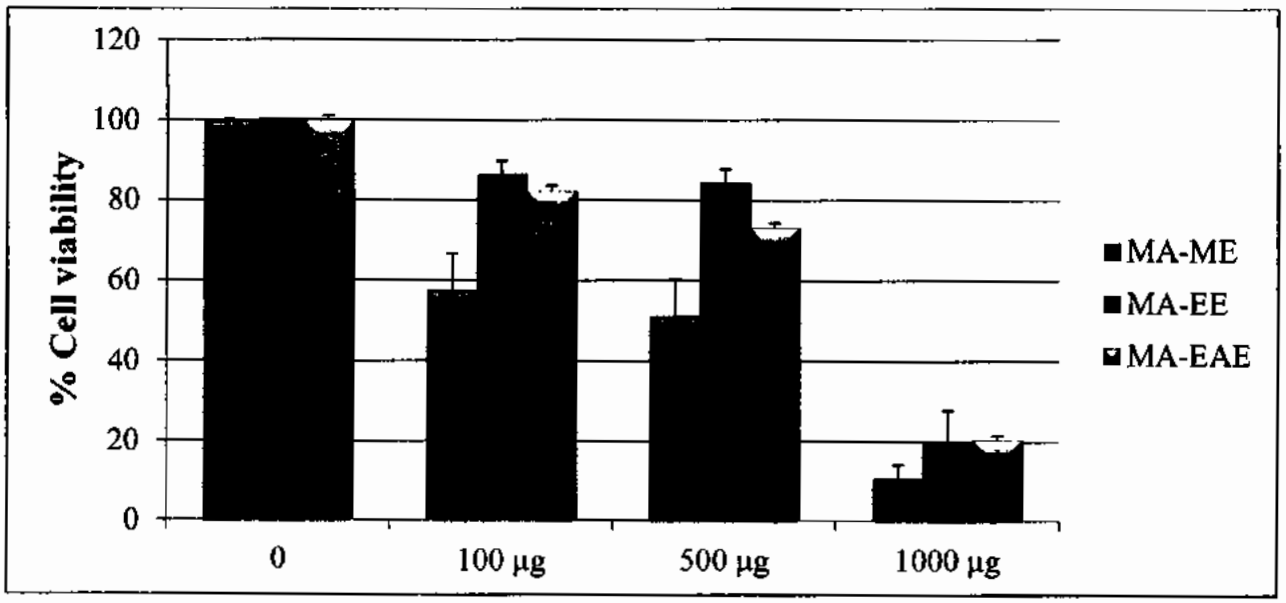


Figure 4.68: Inhibition of the Proliferation of HCCLM3 cell line by *Melia azedarach* extracts at 72 hours. Results are expressed as mean ± standard deviation (n = 3).

4.18. Anticancer activity of *Justicia adhatoda*

4.18.1. Inhibition of the Proliferation of HepG2 cell line by *Justicia adhatoda* extracts

The anticancer activity of *Justicia adhatoda* extracts was detected by MTT assay at 48 h and 72 h as shown in Figure 4.69 and 4.70. All the three *Justicia adhatoda* extracts showed good antiproliferative activity at both time intervals. The JA-ME demonstrated more effective results with percent viability of 20.31% and 16.52% at 48 h on both higher concentrations of 500 µg/ml and 1000 µg/ml respectively. The JA-ME demonstrated more effective results with percent viability of 31.06% at 72 h on higher concentration of 1000 µg/ml. The JA-EE demonstrated more effective results with percent viability of 20.13% at 48 h on higher concentration of 1000 µg/ml. The JA-EAE demonstrated more effective results with percent viability of 18.13%, 9.84% and 6.14% at 48 h on all the three concentrations of 100 µg/ml, 500 µg/ml and 1000 µg/ml respectively. The JA-EAE demonstrated more effective results with percent viability of 17.42% and 10.89% at 72 h on both higher concentrations of 500 µg/ml and 1000 µg/ml respectively.

4.18.2. Inhibition of the Proliferation of HCCLM3 cell line by *Justicia adhatoda* extracts

The anticancer activity of *Justicia adhatoda* extracts was detected by MTT assay at 48 hours and 72 hours as shown in Figure 4.71 and 4.72. All the three *Justicia adhatoda* extracts showed good antiproliferative activity at both time intervals. The JA-ME demonstrated more effective results with percent viability of 32.03% at 48 h on higher concentration of 1000 µg/ml. The JA-EE demonstrated more effective results with percent viability of 23.99% and 19.43% at 48 h on both higher concentrations of 500 µg/ml and 1000 µg/ml respectively. The JA-EAE demonstrated more effective results with percent viability of 15.86%, 13.30% and 11.73% at 48 h on all the three concentrations of 100 µg/ml, 500 µg/ml and 1000 µg/ml respectively. The JA-EAE demonstrated more effective results with percent viability of 20.48%, 14.33% and 9.91% at 72 h on all the three concentrations of 100 µg/ml, 500 µg/ml and 1000 µg/ml respectively.

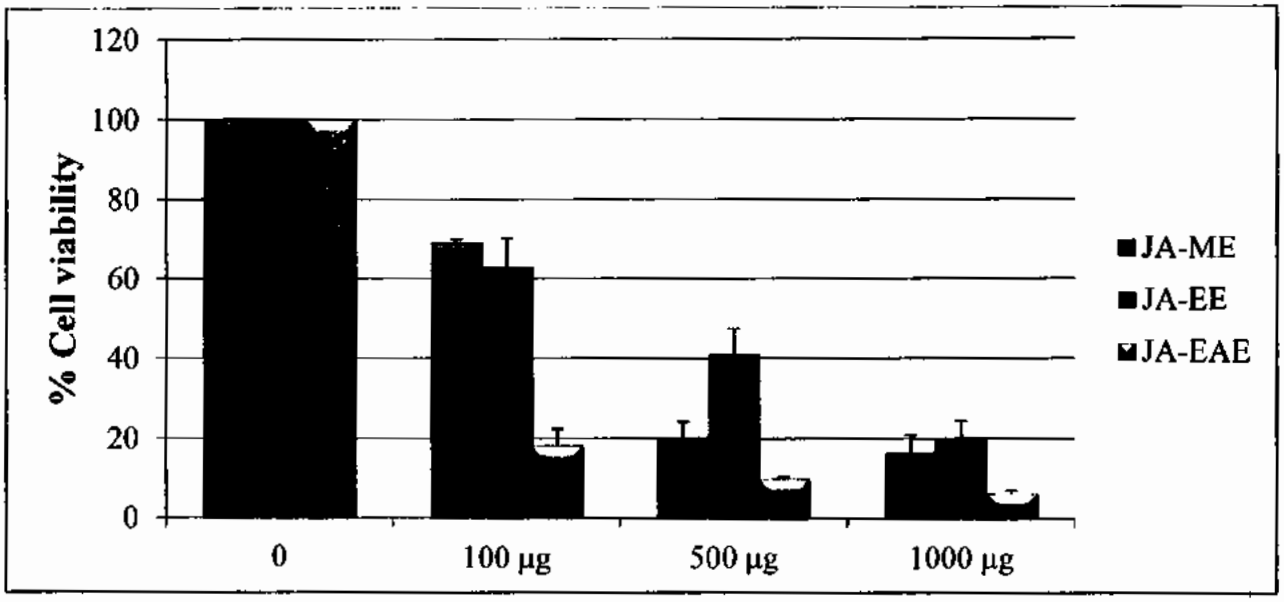


Figure 4.69: Inhibition of the Proliferation of HepG2 cell line by *Justicia adhatoda* extracts at 48 hours. Results are expressed as mean ± standard deviation (n = 3).

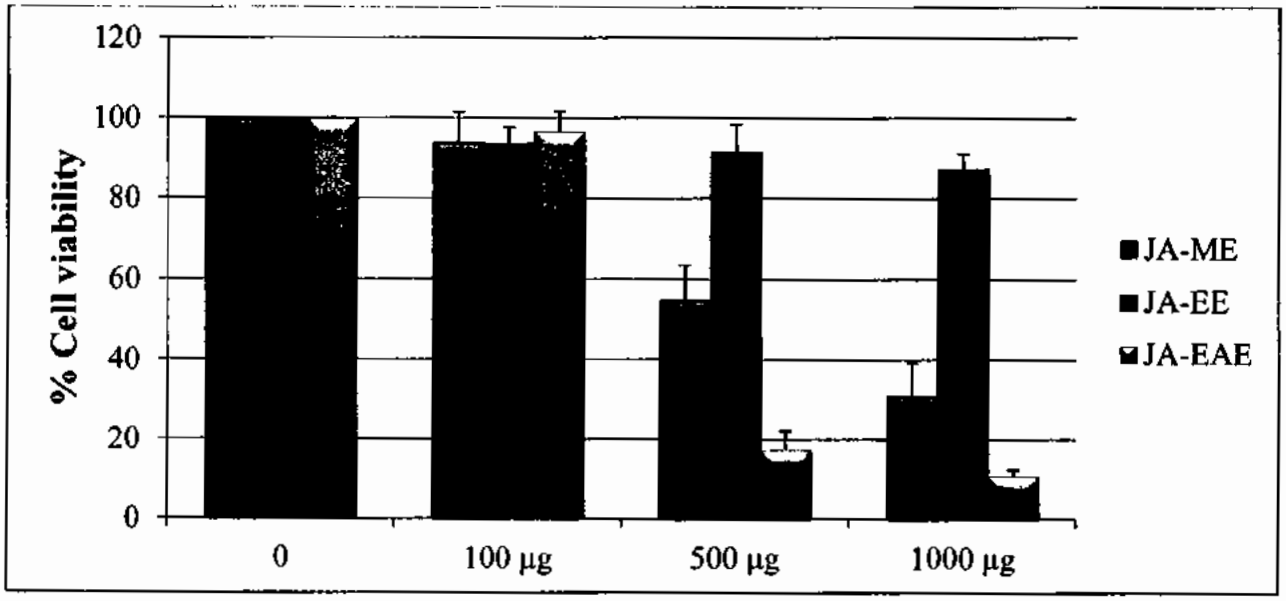


Figure 4.70: Inhibition of the Proliferation of HepG2 cell line by *Justicia adhatoda* extracts at 72 hours. Results are expressed as mean ± standard deviation (n = 3).

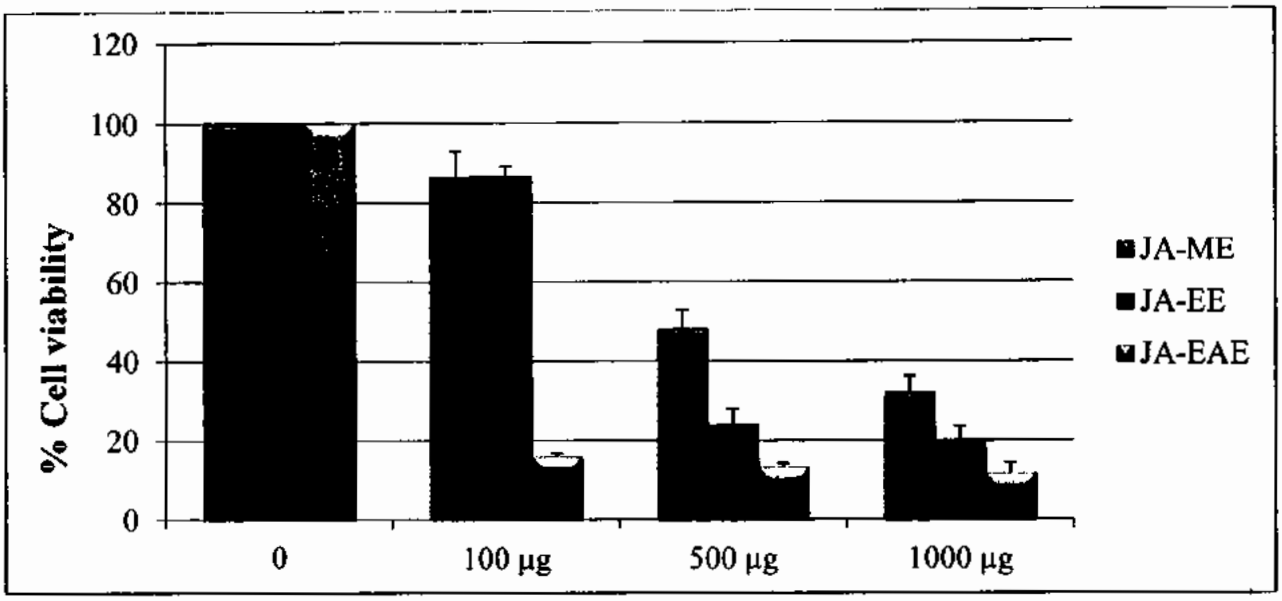


Figure 4.71: Inhibition of the Proliferation of HCCLM3 cell line by *Justicia adhatoda* extracts at 48 hours. Results are expressed as mean ± standard deviation (n = 3).

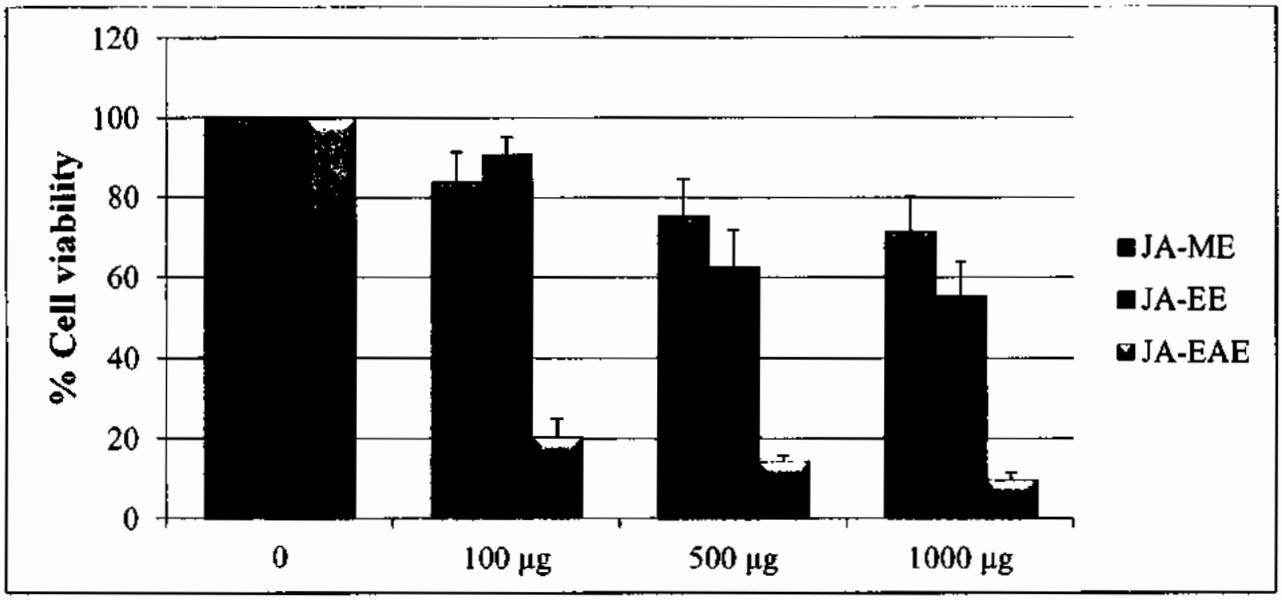


Figure 4.72: Inhibition of the Proliferation of HCCLM3 cell line by *Justicia adhatoda* extracts at 72 hours. Results are expressed as mean ± standard deviation (n = 3).

4.19. Anticancer activity of *Ricinus communis*

4.19.1. Inhibition of the Proliferation of HepG2 cell line by *Ricinus communis* extracts

The anticancer activity of *Ricinus communis* extracts was detected by MTT assay at 48 h and 72 h as shown in Figure 4.73 and 4.74. All the three *Ricinus communis* extracts showed good antiproliferative activity at both time intervals. The RC-EE demonstrated more effective results with percent viability of 21.14% at 48 h on higher concentration of 1000 µg/ml. The RC-EAE demonstrated more effective results with percent viability of 12.81%, 9.17% and 8.33% at 48 h on all the three concentrations of 100 µg/ml, 500 µg/ml and 1000 µg/ml respectively. The RC-EAE demonstrated more effective results with percent viability of 17.71% and 13.87% at 72 h on both higher concentrations of 500 µg/ml and 1000 µg/ml respectively.

4.19.2. Inhibition of the Proliferation of HCCLM3 cell line by *Ricinus communis* extracts

The anticancer activity of *Ricinus communis* extracts was detected by MTT assay at 48 h and 72 h as shown in Figure 4.75 and 4.76. All the three *Ricinus communis* extracts showed good antiproliferative activity at both time intervals. The RC-EE demonstrated more effective results with percent viability of 36.20% and 9.75% at 48 h on both higher concentrations of 500 µg/ml and 1000 µg/ml respectively. The RC-EE demonstrated more effective results with percent viability of 34.18% at 72 h on higher concentration of 1000 µg/ml. The RC-EAE demonstrated more effective results with percent viability of 9.42%, 6.15% and 5.97% at 48 h on all the three concentrations of 100 µg/ml, 500 µg/ml and 1000 µg/ml respectively. The RC-EAE demonstrated more effective results with percent viability of 21.62%, 18.33% and 13.15% at 72 h on all the three concentrations of 100µg/ml, 500 µg/ml and 1000 µg/ml respectively.

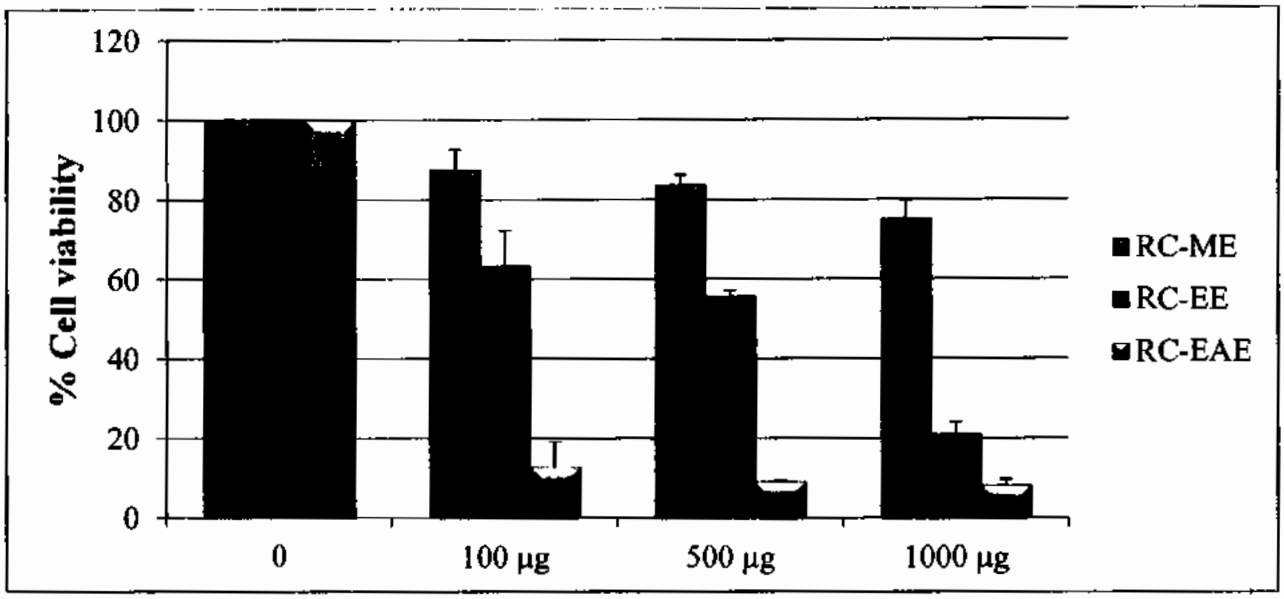


Figure 4.73: Inhibition of the Proliferation of HepG2 cell line by *Ricinus communis* extracts at 48 hours. Results are expressed as mean ± standard deviation (n = 3).

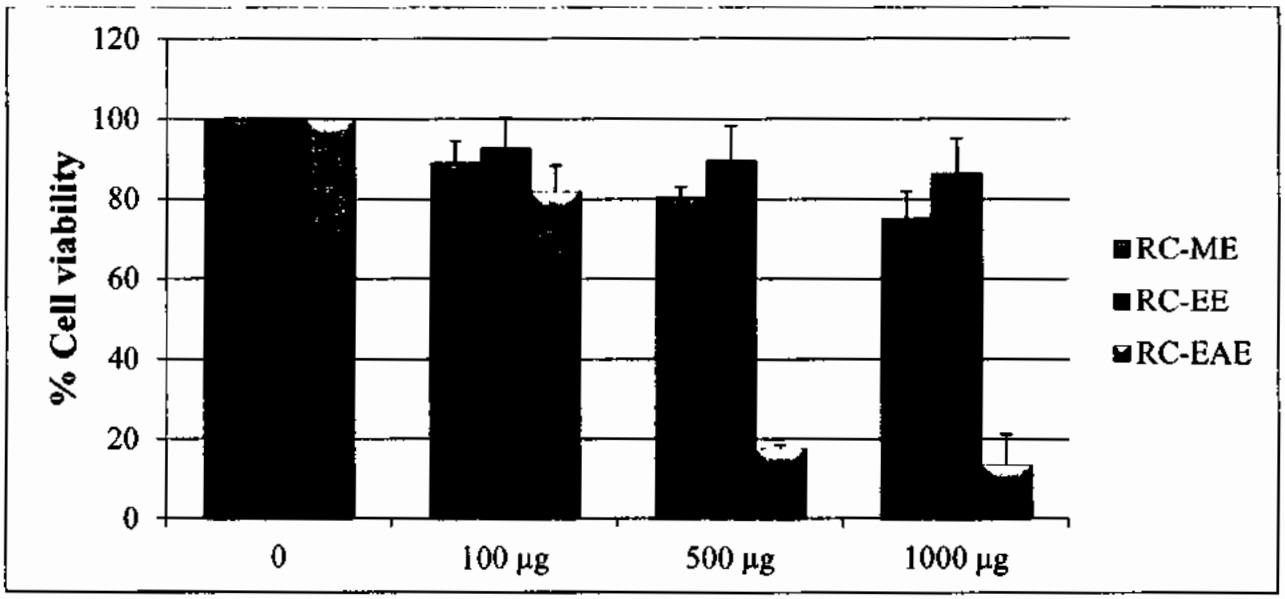


Figure 4.74: Inhibition of the Proliferation of HepG2 cell line by *Ricinus communis* extracts at 72 hours. Results are expressed as mean ± standard deviation (n = 3).

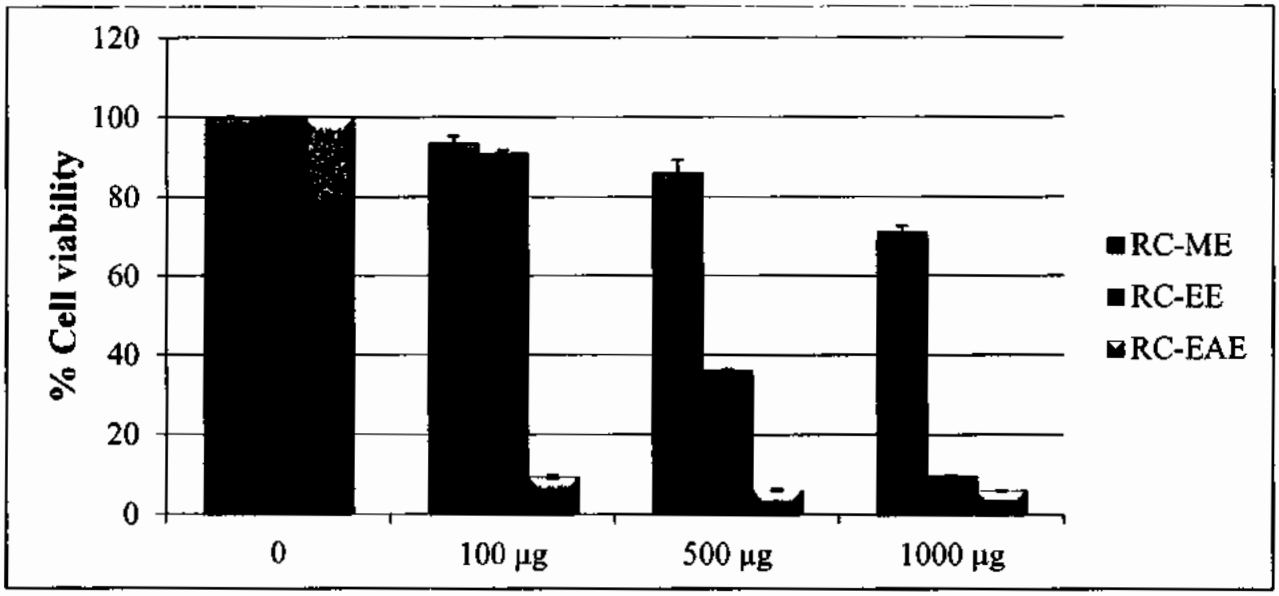


Figure 4.75: Inhibition of the Proliferation of HCCLM3 cell line by *Ricinus communis* extracts at 48 hours. Results are expressed as mean ± standard deviation (n = 3).

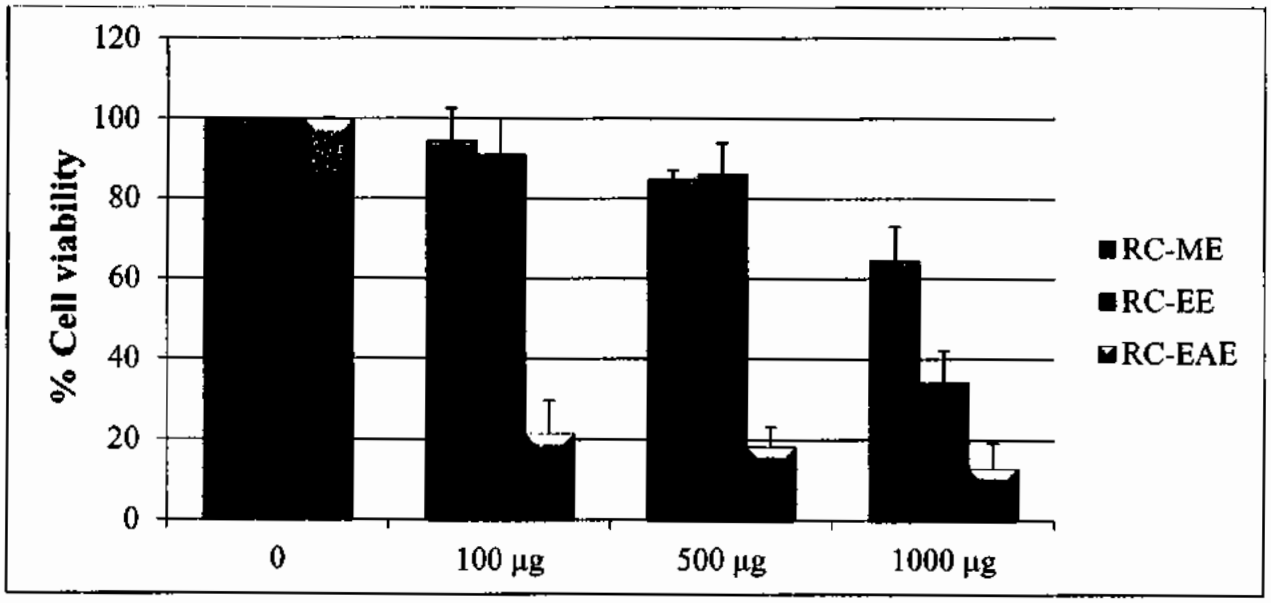


Figure 4.76: Inhibition of the Proliferation of HCCLM3 cell line by *Ricinus communis* extracts at 72 hours. Results are expressed as mean ± standard deviation (n = 3).

4.20. Flow cytometric analysis of *Melia azedarach*

4.20.1. *Melia azedarach* methanolic extract induced G0/G1 Phase Arrest in HepG2 Cells

Based upon the results of MTT assay, the cell cycle arrest was performed on HepG2 cell line at 72 h, as most of the extracts showed more promising results on HepG2 cell line. Flow cytometric analysis was conducted at 72 h as shown in Figure 4.77. The methanolic extract of *Melia azedarach* showed significant accumulation of cells in sub-G1 phase, dose dependently with 11.24%, 64.81% and 87.21% cell cycle arrest at 100 µg/ml, 500 µg/ml and 1000 µg/ml respectively.

4.20.2. *Melia azedarach* ethanolic extract induced G0/G1 Phase Arrest in HepG2 Cells

Based upon the results of MTT assay, the cell cycle arrest was performed on HepG2 cell line at 72 h, as most of the extracts showed more promising results on HepG2 cell line. Flow cytometric analysis was conducted at 72 h as shown in Figure 4.78. The ethanolic extract of *Melia azedarach* showed significant accumulation of cells in sub-G1 phase, dose dependently with 26.73%, 66.14% and 81.12% cell cycle arrest at 100 µg/ml, 500 µg/ml and 1000 µg/ml respectively.

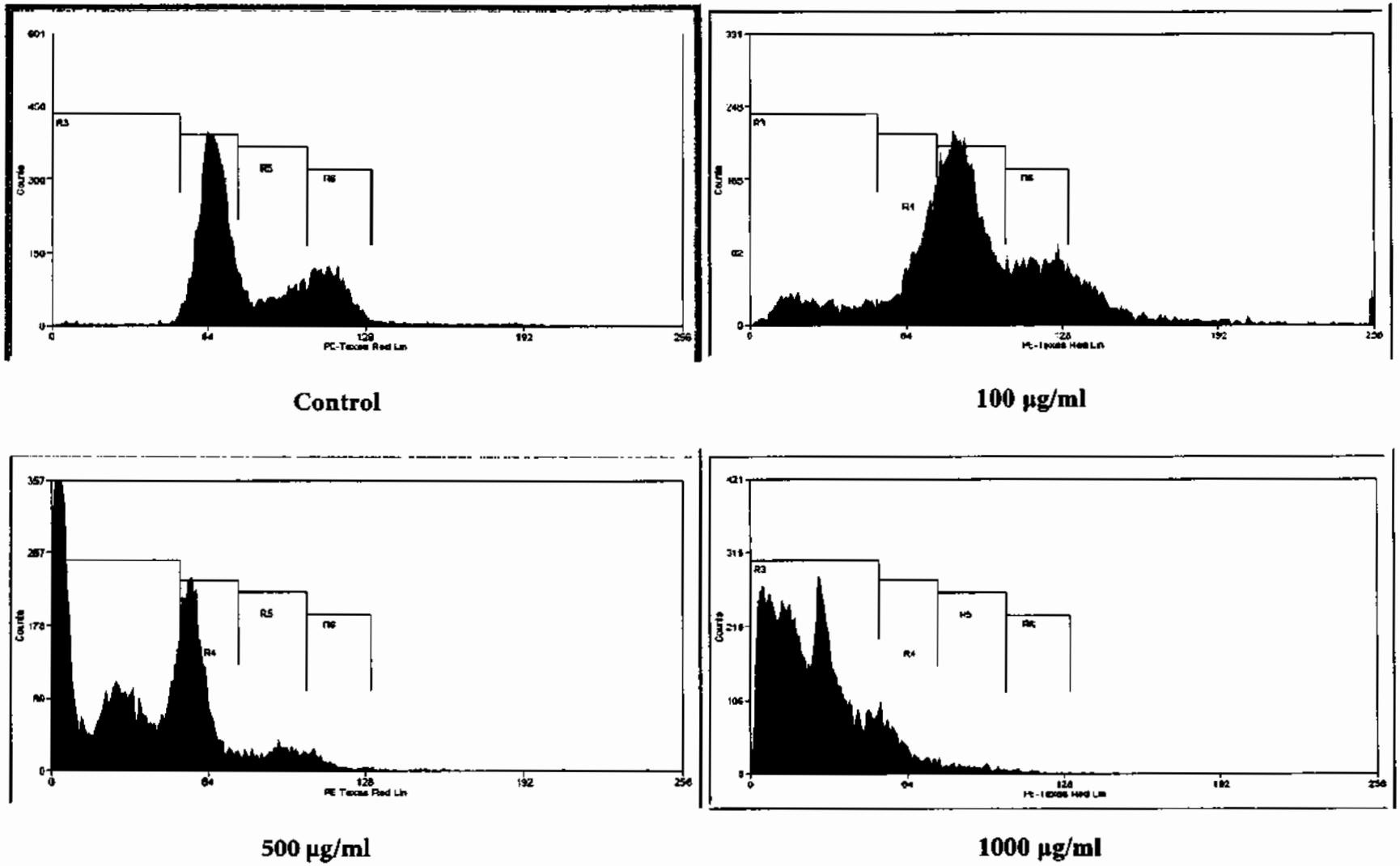
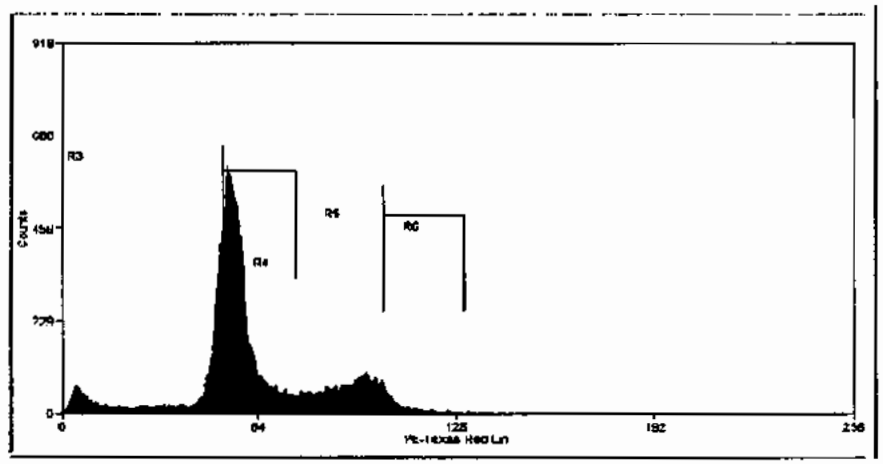
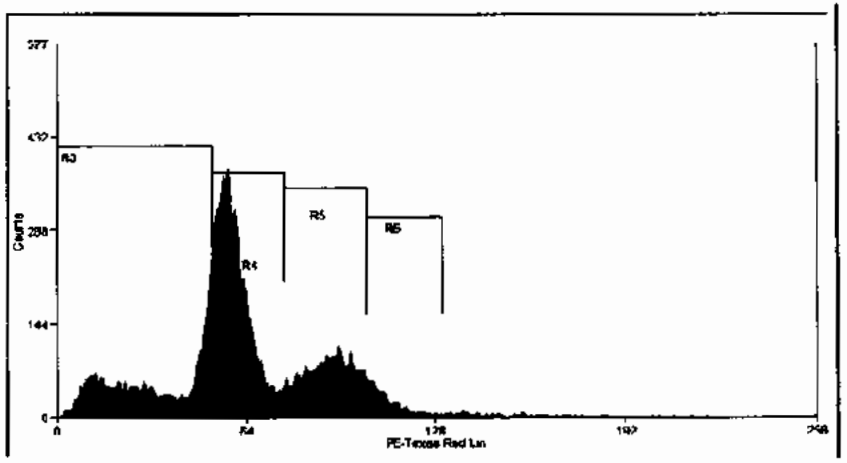


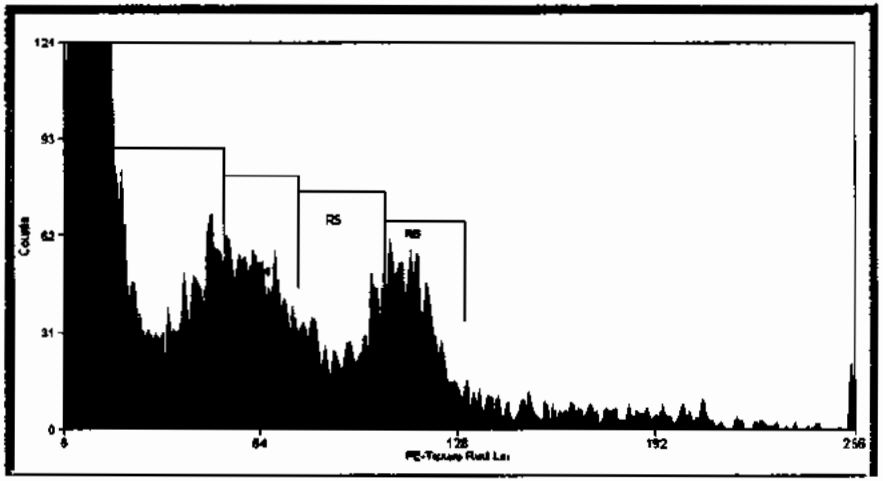
Figure 4.77: *Melia azedarach* methanolic extract induced G0/G1 Phase Arrest in HepG2 Cells at 72 hours. R3 region is Sub-G1 phase, R4 is G1 phase, R5 is S phase and R6 is G2/M phase.



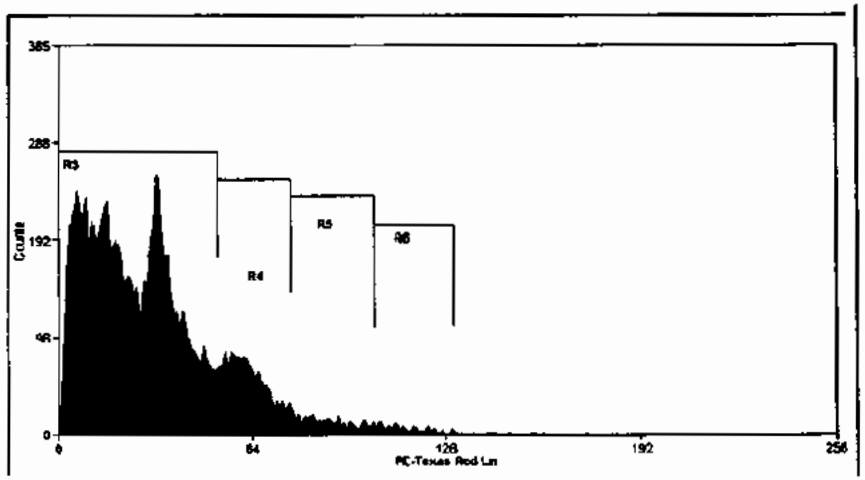
Control



100 µg/ml



500 µg/ml



1000 µg/ml

Figure 4.78: *Melia azedarach* ethanolic extract induced G0/G1 Phase Arrest in HepG2 Cells at 72 hours. R3 region is Sub-G1 phase, R4 is G1 phase, R5 is S phase and R6 is G2/M phase.

4.21. Flow cytometric analysis of *Justicia adhatoda*

4.21.1. *Justicia adhatoda* ethyl acetate extract induced G0/G1 Phase Arrest in HepG2 Cells

Based upon the results of MTT assay, the cell cycle arrest was performed on HepG2 cell line at 72 h, as most of the extracts showed more promising results on HepG2 cell line. Flow cytometric analysis was conducted at 72 h as shown in Figure 4.79. The ethyl acetate extract of *Justicia adhatoda* showed significant accumulation of cells in sub-G1 phase, dose dependently with 13.39%, 55.63% and 92.29% cell cycle arrest at 100 µg/ml, 500 µg/ml and 1000 µg/ml respectively.

4.22. Flow cytometric analysis of *Ricinus communis*

4.22.1. *Ricinus communis* ethyl acetate extract induced G0/G1 Phase Arrest in HepG2 Cells

Based upon the results of MTT assay, the cell cycle arrest was performed on HepG2 cell line at 72 h, as most of the extracts showed more promising results on HepG2 cell line. Flow cytometric analysis was conducted at 72 h as shown in Figure 4.80. The ethyl acetate extract of *Ricinus communis* showed significant accumulation of cells in sub-G1 phase, dose dependently with 17.38%, 70.95% and 98.86% cell cycle arrest at 100 µg/ml, 500 µg/ml and 1000 µg/ml respectively.

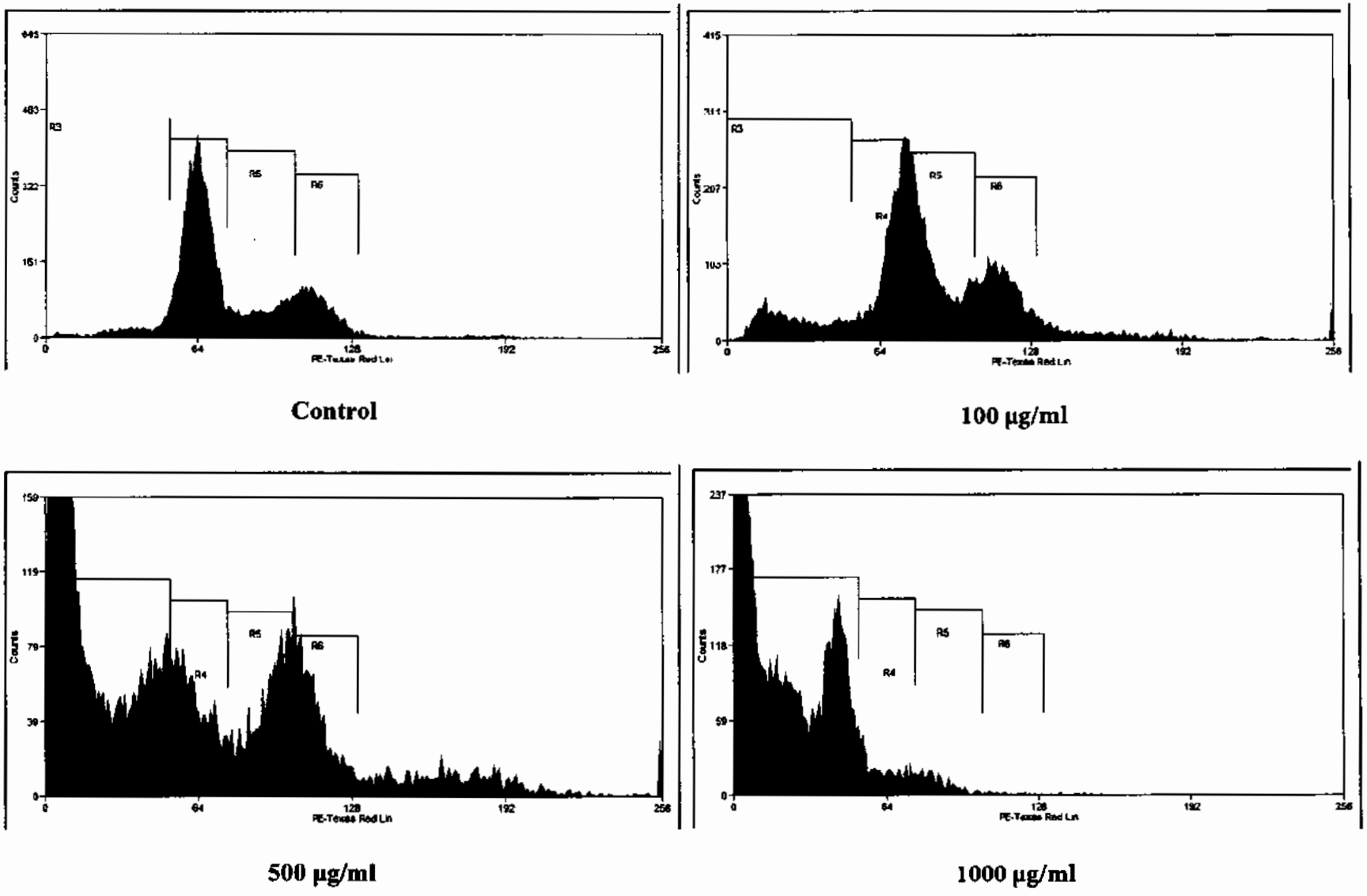


Figure 4.79: *Justicia adhatoda* ethyl acetate extract induced G0/G1 Phase Arrest in HepG2 Cells at 72 hours. R3 region is Sub-G1 phase, R4 is G1 phase, R5 is S phase and R6 is G2/M phase.

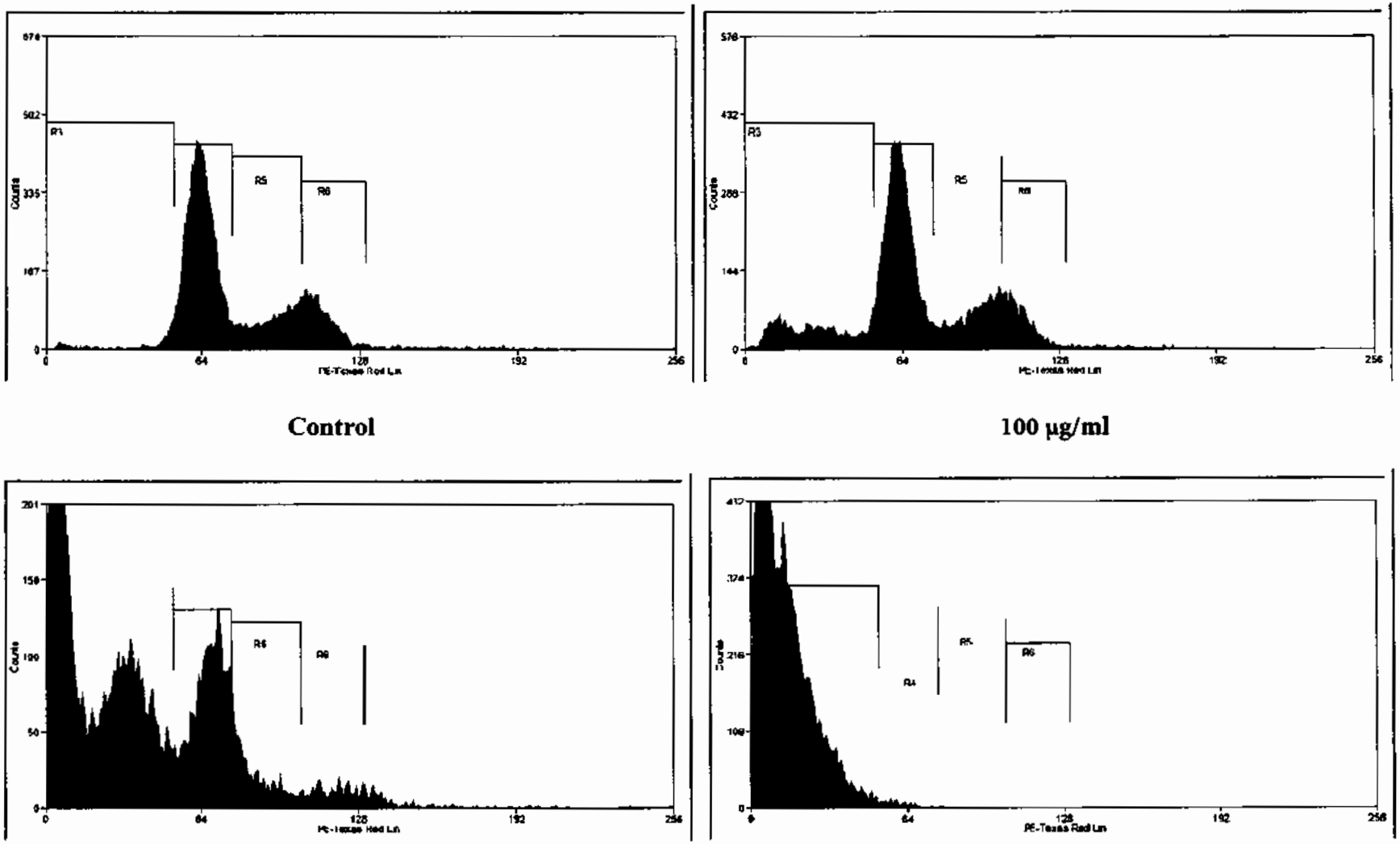


Figure 4.80: *Ricinus communis* ethyl acetate extract induced G0/G1 Phase Arrest in HepG2 Cells at 72 hours. R3 region is Sub-G1 phase, R4 is G1 phase, R5 is S phase and R6 is G2/M phase.

4.23. Elemental Analysis of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis*

Table No. 4.23 reveals wide variation in elemental concentrations in *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis*. The concentrations of Phosphorus, Potassium, Calcium and Magnesium are major elements and have been found 1600.3 ppm, 13769.3 ppm, 27898.6 ppm and 3745.3 ppm respectively in *Melia azedarach* leaves. The concentrations of Phosphorus, Potassium, Calcium and Magnesium have been found 1427 ppm, 22562.7 ppm, 23559.3 ppm and 5991 ppm respectively in *Justicia adhatoda* leaves. The concentrations of Phosphorus, Potassium, Calcium and Magnesium have been found 1924 ppm, 16776.6 ppm, 24327 ppm and 3605.3 ppm respectively in *Ricinus communis* leaves. Phosphorus concentration has been reported maximum in *Ricinus communis*, followed by *Melia azedarach* and *Justicia adhatoda*. Potassium concentration has been reported maximum in *Justicia adhatoda*, followed by *Ricinus communis* and *Melia azedarach*. Calcium concentration has been reported maximum in *Melia azedarach*, followed by *Ricinus communis* and *Justicia adhatoda*. Magnesium concentration has been reported maximum in *Justicia adhatoda*, followed by *Melia azedarach* and *Ricinus communis*.

The concentrations of Chromium, Manganese, Iron and Cobalt have been found 0.6 ppm, 41 ppm, 200 ppm and 0.5 ppm respectively in *Melia azedarach* leaves. The concentrations of Chromium, Manganese, Iron and Cobalt have been found 0.5 ppm, 26.7 ppm, 166.7 ppm and 0.6 ppm respectively in *Justicia adhatoda* leaves. The concentrations of Chromium, Manganese, Iron and Cobalt have been found 0.6 ppm, 145 ppm, 290 ppm and 0.7 ppm respectively in *Ricinus communis* leaves. Chromium concentration has been reported maximum in *Melia azedarach* and *Ricinus communis*, followed by *Justicia adhatoda*. Manganese concentration has been reported maximum in *Ricinus communis*, followed by *Melia azedarach* and *Justicia adhatoda*. Iron concentration has been reported maximum in *Ricinus communis*, followed by *Melia azedarach* and *Justicia adhatoda*. Cobalt concentration has been reported maximum in *Ricinus communis*, followed by *Justicia adhatoda* and *Melia azedarach*.

The concentrations of Copper, Zinc, Boron and Aluminum have been found 7.6 ppm, 50.3 ppm, 57 ppm and 274.6 ppm respectively in *Melia azedarach* leaves. The concentrations of Copper, Zinc, Boron and Aluminum have been found 8 ppm, 37.7 ppm, 33.7 ppm and 269 ppm

respectively in *Justicia adhatoda* leaves. The concentrations of Copper, Zinc, Boron and Aluminum have been found 9 ppm, 38 ppm, 67.3 ppm and 373.3 ppm respectively in *Ricinus communis* leaves. Copper concentration has been reported maximum in *Ricinus communis*, followed by *Justicia adhatoda* and *Melia azedarach*. Zinc concentration has been reported maximum in *Melia azedarach*, followed by *Justicia adhatoda* and *Ricinus communis*. Boron concentration has been reported maximum in *Ricinus communis*, followed by *Melia azedarach* and *Justicia adhatoda*. Aluminum concentration has been reported maximum in *Ricinus communis*, followed by *Melia azedarach* and *Justicia adhatoda*.

The concentrations of Sodium and Barium have been found 117.3 ppm and 19.6 ppm respectively in *Melia azedarach* leaves. The concentrations of Sodium and Barium have been found 87.3 ppm and 12.3 ppm respectively in *Justicia adhatoda* leaves. The concentrations of Sodium and Barium found 103 ppm and 13.6 ppm respectively in *Ricinus communis* leaves. Sodium concentration has been reported maximum in *Melia azedarach*, followed by *Justicia adhatoda* and *Ricinus communis*. Barium concentration has been reported maximum in *Melia azedarach*, followed by *Justicia adhatoda* and *Ricinus communis*.

Nickel was absent in *Melia azedarach* but detected in *Justicia adhatoda* and *Ricinus communis* with concentrations of 0.5 ppm and 0.6 ppm respectively. Nickel concentration has been reported maximum in *Ricinus communis* than *Justicia adhatoda*. Molybdenum was absent in *Melia azedarach* but detected in *Justicia adhatoda* and *Ricinus communis* with concentrations of 0.5 ppm and 2.2 ppm respectively. Molybdenum concentration has been reported maximum in *Ricinus communis* than *Justicia adhatoda*. Lithium was absent in *Melia azedarach* and *Justicia adhatoda* but detected in *Ricinus communis* with concentration of 1 ppm.

Table 4.23: Elemental Analysis of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis*

S.NO.	Name of Elements	*Melia azedarach* (Conc. ppm)	*Justicia adhatoda* (Conc. ppm)	*Ricinus communis* (Conc. ppm)
1	Fe (Iron)	200 ± 20.30	166.7 ± 12.66	290 ± 10.39
2	Zn (Zinc)	50.3 ± 1.15	37.7 ± 0.58	38 ± 1
3	Cu (Copper)	7.6 ± 2.05	8 ± 1.60	9 ± 0
4	Cr (Chromium)	0.6 ± 0.11	0.5 ± 0	0.6 ± 0.12
5	Mn (Manganese)	41 ± 1	26.7 ± 0.58	145 ± 4.58
6	Co (Cobalt)	0.5 ± 0.05	0.6 ± 0	0.7 ± 0.1
7	Ni (Nickel)	ND	0.5 ± 0.05	0.6 ± 0.12
8	Mg (Magnesium)	3745.3 ± 117.67	5991 ± 68.98	3605.3 ± 63.52
9	Al (Aluminum)	274.6 ± 11.59	269 ± 9.64	373.3 ± 7.02
10	Ca (Calcium)	27898.6 ± 1188.86	23559.3 ± 687.93	24327 ± 142.52
11	Li (Lithium)	ND	ND	1 ± 0.05
12	Na (Sodium)	117.3 ± 10.97	87.3 ± 1.53	103 ± 5.57
13	K (Potassium)	13769.3 ± 595.49	22562.7 ± 299.20	16776.6 ± 417.19
14	Ba (Barium)	19.6 ± 1.15	12.3 ± 0.58	13.6 ± 0.58
15	B (Boron)	57 ± 1	33.7 ± 0.58	67.3 ± 0.58
16	P (Phosphorous)	1600.3 ± 174.01	1427 ± 13.89	1924 ± 0
17	Mo (Molybdenum)	ND	0.5 ± 0.05	2.2 ± 0.26

4.24. Anionic Ion-Exchange Chromatography of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis*

Table No. 4.24 reveals the anions concentrations in *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis*. The concentrations of Chloride, Fluoride, Nitrate, Phosphate and Sulfate have been found 115.83 ppm, 207.20 ppm, 67.87 ppm, 477.27 ppm and 2643.33 ppm respectively in *Melia azedarach* leaves. The concentrations of Chloride, Fluoride, Nitrate, Phosphate and Sulfate have been found 1019 ppm, 22.13 ppm, 139.17 ppm, 183.97 ppm and 1057.57 ppm respectively in *Justicia adhatoda* leaves. The concentrations of Chloride, Fluoride, Nitrate, Phosphate and Sulfate have been found 593.27 ppm, 138.7 ppm, 69.97 ppm, 366.77 ppm and 3176.37 ppm respectively in *Ricinus communis* leaves. Chloride concentration has been reported maximum in *Justicia adhatoda*, followed by *Ricinus communis* and *Melia azedarach*. Fluoride concentration has been reported maximum in *Melia azedarach*, followed by *Ricinus communis* and *Justicia adhatoda*. Nitrate concentration has been reported maximum in *Justicia adhatoda*, followed by *Ricinus communis* and *Melia azedarach*. Phosphate concentration has been reported maximum in *Melia azedarach*, followed by *Ricinus communis* and *Justicia adhatoda*. Sulfate concentration has been reported maximum in *Ricinus communis*, followed by *Melia azedarach* and *Justicia adhatoda*. Bromide was absent in *Melia* azedarach, Justicia *adhatoda* and *Ricinus communis*.

Table 4.24: Anionic Ion-Exchange Chromatography of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis*

S.NO.	Anions	*Melia azedarach* (Conc. ppm)	*Justicia adhatoda* (Conc. ppm)	*Ricinus communis* (Conc. ppm)
1	Cl^- (Chloride)	115.83	1019	593.27
2	Br^- (Bromide)	ND	ND	ND
3	F^- (Fluoride)	207.20	22.13	138.7
4	NO_3^- (Nitrate)	67.87	139.17	69.97
5	PO_4^{3-} (Phosphate)	477.27	183.97	366.77
6	SO_4^{2-} (Sulfate)	2643.33	1057.57	3176.37

4.25. CHNS Analysis of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis*

Table No. 4.25 reveals the Carbon, Hydrogen, Nitrogen and Sulphur concentrations in *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis*. The percentage of Carbon, Hydrogen and Nitrogen have been found 43.82%, 5.91% and 3.20% respectively in *Melia azedarach* leaves. The percentage of Carbon, Hydrogen and Nitrogen have been found 43.37%, 6.47% and 5.24% respectively in *Justicia adhatoda* leaves. The percentage of Carbon, Hydrogen and Nitrogen have been found 44.01%, 6.37% and 5.55% respectively in *Ricinus communis* leaves. Carbon percentage has been reported maximum in *Ricinus communis*, followed by *Melia azedarach* and *Justicia adhatoda*. Hydrogen percentage has been reported maximum in *Justicia adhatoda* followed by *Ricinus communis* and *Melia azedarach*. Nitrogen percentage has been reported maximum in *Ricinus communis*, followed by *Justicia adhatoda* and *Melia azedarach*. Sulphur was absent in *Melia azedarach* and *Justicia adhatoda* but detected in *Ricinus communis* with percentage of less than 0.50%.

Table 4.25: CHNS Analysis of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis*

S.NO.	CHNS	*Melia azedarach* (% age)	*Justicia adhatoda* (% age)	*Ricinus communis* (% age)
1	C	43.82	43.37	44.01
2	H	5.91	6.47	6.37
3	N	3.20	5.24	5.55
4	S	ND	ND	< 0.50

4.26. Gas chromatography-Mass spectrometry (GC-MS) analysis of methanolic leaves extract of *Melia azedarach*

The methanolic leaves extract of *Melia azedarach* was selected for GC-MS analysis. Upon GC-MS analysis, the extract contains 50 chemical constituents eluted between 5.70 and 52.21 minutes as shown in Figure 4.81. These compounds belong to different chemical classes and most of them are reported to exhibit important biological activities. The identified compounds with their peak number, retention time (RT), peak area (%), molecular formula and weight and structure are presented in Table 4.26. The major compounds identified were Chloroacetic acid allyl ester (19.92%), D-Galactose (6.82%), 1H-Indene, 2,3-dihydro-4-methyl- (5.52%), 3,4-Dimethylthiophene-2-thiol (4.99%), 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (3.79%), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (3.70%), n-Hexadecanoic acid (3.54%), Isophytol, acetate (2.89%), Cyclohexane, propyl- (2.68%), 2-Hexen-1-ol, acetate, (E)- (2.57%), Thiazole, 2,5-diethyl- (2.44%), Neophytadiene (2.03), .beta.-Sitosterol (1.85%), Benzenemethanol, 3-fluoro- (1.70%), Benzenemethanol, 3-fluoro- (1.65%), 2-Methoxy-4-vinylphenol (1.63%), Heptanoic acid, 6-oxo- (1.52%), Furan, 2,5-dihydro-3-methyl- (1.44%), Piperazine, 2-methyl- (1.30%), Phenol, 3,5-bis(1,1-dimethylethyl)- (1.28%) and 2,4(1H,3H)-Pyrimidinedione, 1,3-dimethyl- (1.08%).

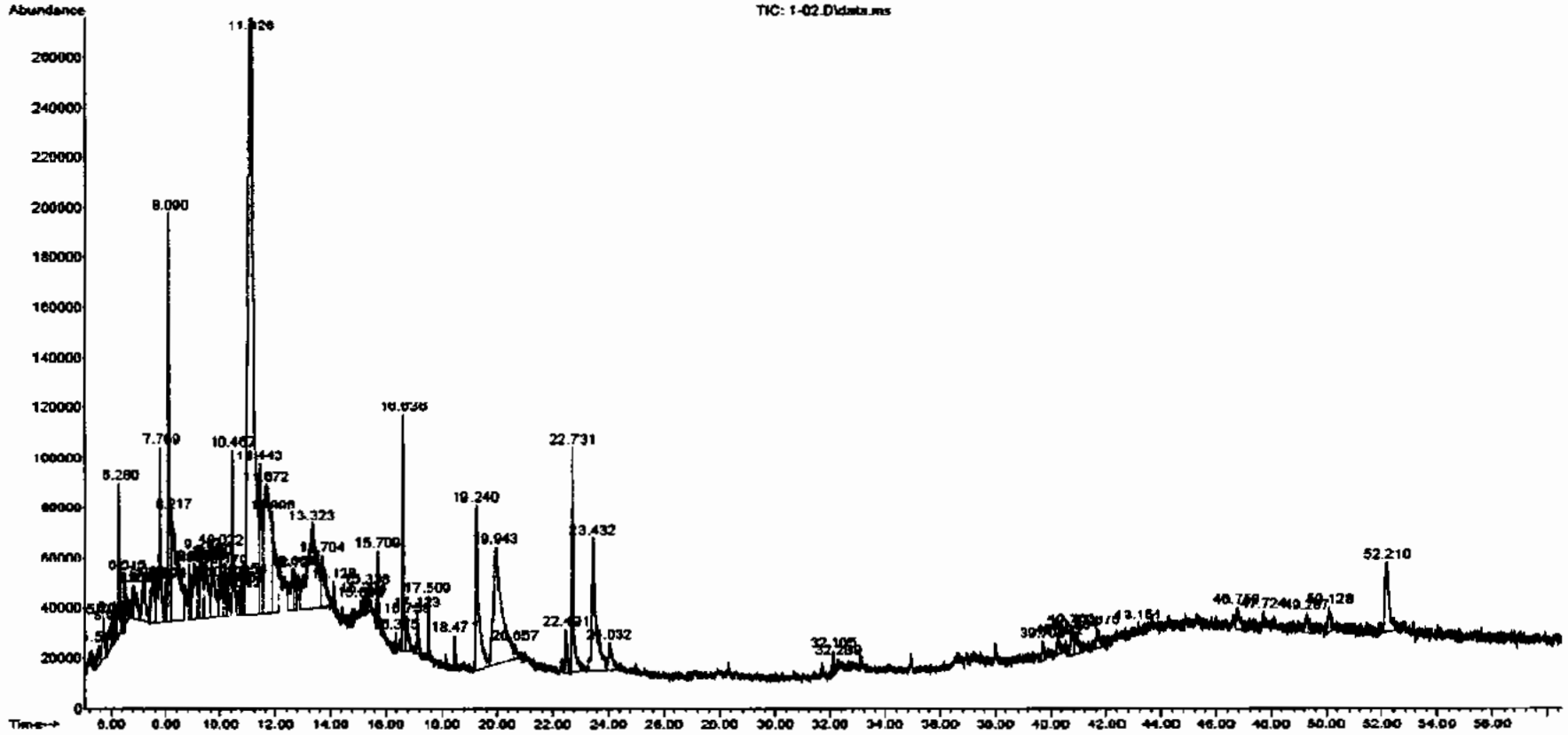
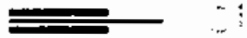
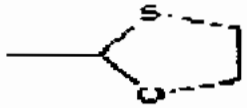
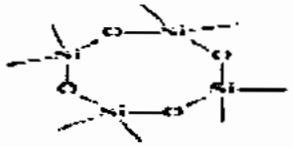
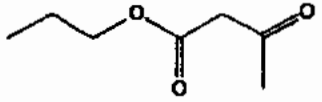
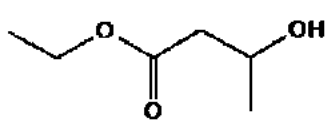
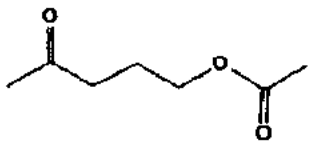
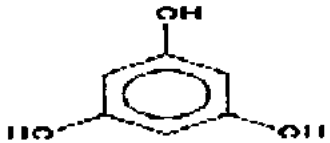
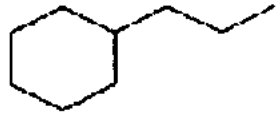
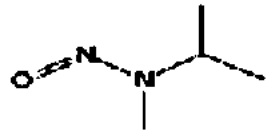
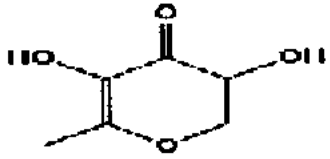
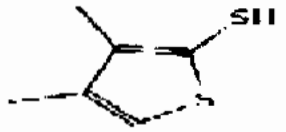
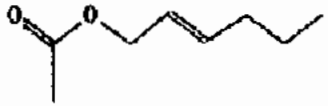
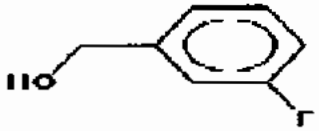
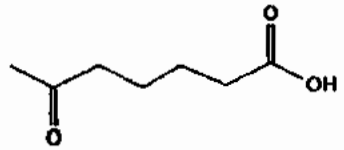
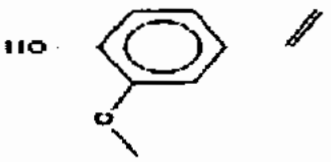


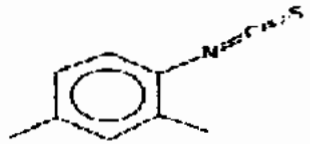
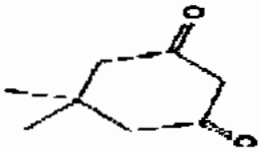
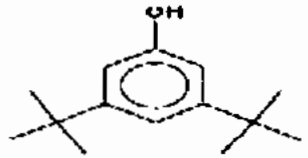
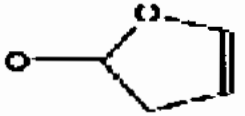
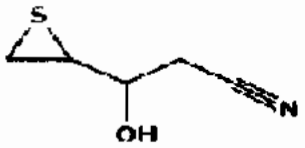
Figure 4.81: GC-MS chromatogram of methanolic leaf extract of *Melia azedarach*

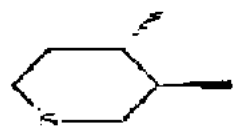
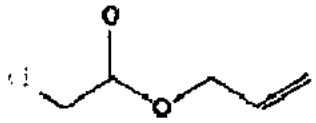
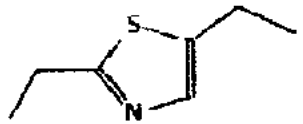
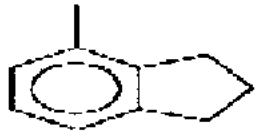
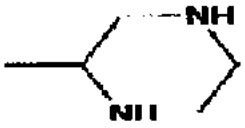
Table 4.26: Phytoconstituents identified in the methanol leaves extract of *Melia azedarach* by GC-MS

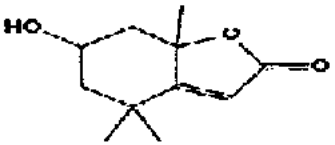
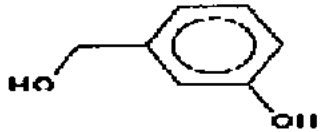
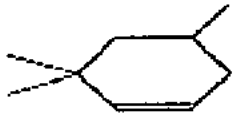
Sr. No.	RT	Compound	Molecular Formula	Molecular Weight	Peak Area %	Structure
1	5.705	Styrene	C_8H_8	104	0.66	
2	6.046	Ethyne, chloro-	C_2HCl	60	0.37	
3	6.109	2-Methyl-1,3-oxathiolane	C_4H_8OS	104	0.26	
4	6.280	Cyclotetrasiloxane, octamethyl-	$C_8H_{24}O_4Si_4$	96	1.65	
5	6.515	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	144	0.67	
6	6.794	Butanoic acid, 3-oxo-, propyl ester	$C_7H_{12}O_3$	144	0.32	

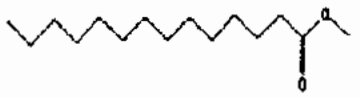
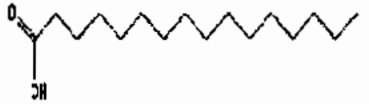
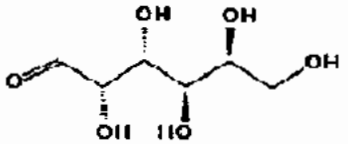
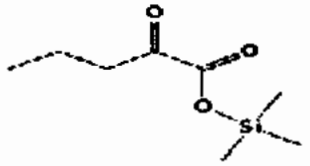
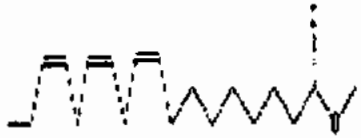
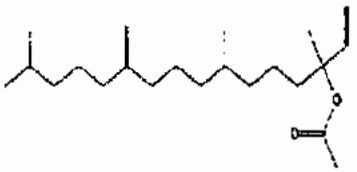
7	6.878	Butanoic acid, 3-hydroxy-, ethyl ester	$C_6H_{12}O_3$	132	0.68	
8	7.206	2-Pentanone, 5-(acetyloxy)-	$C_7H_{12}O_3$	144	0.90	
9	7.490	1,3,5-Benzenetriol	$C_6H_6O_3$	126	0.95	
10	7.769	Cyclohexane, propyl-	C_9H_{18}	126	2.68	
11	7.994	2-Propanamine, N-methyl-N-nitroso-	$C_4H_{10}N_2O$	102	0.38	
12	8.090	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	3.70	

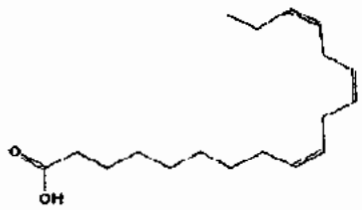
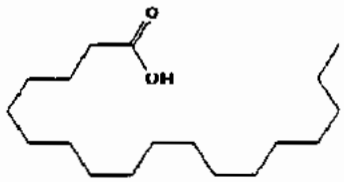
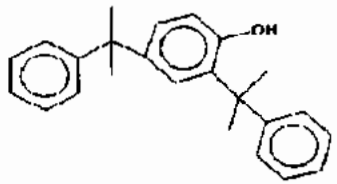
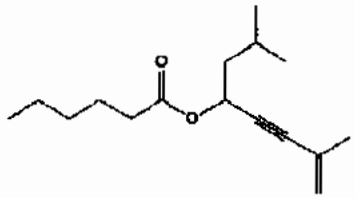
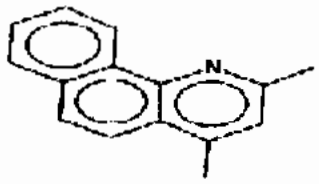
13	8.217	3,4-Dimethylthiophene-2-thiol	$C_6H_8S_2$	144	4.99	
14	8.891	2-Hexen-1-ol, acetate, (E)-	$C_8H_{14}O_2$	142	0.57	
15	9.049	Furan, 2,5-dihydro-3-methyl-	C_5H_8O	84	1.44	
16	9.295	Benzenemethanol, 3-fluoro-	C_7H_7FO	126	1.70	
17	9.446	Heptanoic acid, 6-oxo-	$C_7H_{12}O_3$	144	1.52	
18	9.679	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	1.63	

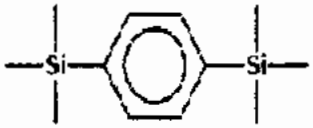
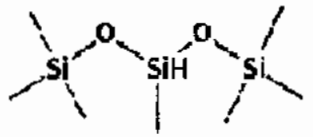
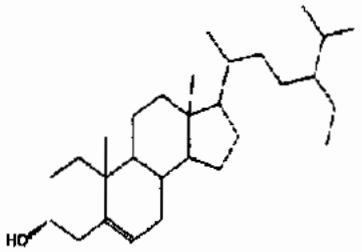
19	10.022	2,4(1H,3H)-Pyrimidinedione, 1,3-dimethyl-	$C_6H_8N_2O_2$	140	1.08	
20	10.179	2,4-Dimethylphenyl isothiocyanate	C_9H_9NS	163	0.47	
21	10.298	1,3-Cyclohexanedione, 5,5-dimethyl-	$C_8H_{12}O_2$	140	0.46	
22	10.467	Phenol, 3,5-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206	1.28	
23	10.642	2(3H)-Furanone	$C_4H_4O_2$	84	0.34	
24	10.763	Episulfide isomer 2	C_5H_7NOS	129	0.32	

25	10.854	Trans-3,4-dimethylthiane	$C_7H_{14}S$	130	0.34	
26	11.124	Chloroacetic acid allyl ester	$C_5H_7ClO_2$	134	19.92	
27	11.443	Thiazole, 2,5-diethyl-	$C_7H_{11}NS$	141	2.44	
28	11.896	2-Hexen-1-ol, acetate, (E)-	$C_8H_{14}O_2$	142	2.57	
29	13.323	1H-Indene, 2,3-dihydro-4-methyl-	$C_{10}H_{12}$	132	5.52	
30	13.704	Piperazine, 2-methyl-	$C_5H_{12}N_2$	100	1.30	

31	15.338	Tridecanoic acid	$C_{13}H_{26}O_2$	214	0.30	
32	15.709	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	$C_{11}H_{16}O_3$	196	0.71	
33	16.636	Neophytadiene	$C_{20}H_{38}$	278	2.03	
34	16.758	Benzenemethanol, 3-hydroxy-	$C_7H_8O_2$	124	0.60	
35	17.123	Cyclohexene, 3,3,5-trimethyl-	C_9H_{16}	124	0.42	
36	17.509	9-Octadecyne	$C_{18}H_{34}$	250	0.55	

37	18.471	Tetradecanoic acid, 10,13-dimethyl-, methyl ester	$C_{17}H_{34}O_2$	270	0.42	
38	19.240	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	3.54	
39	19.943	D-Galactose	$C_6H_{12}O_6$	180	6.82	
40	20.657	2-Oxopentanoic acid, TMS derivative	$C_8H_{16}O_3Si$	188	0.26	
41	22.491	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292	0.61	
42	22.731	Isophytol, acetate	$C_{22}H_{42}O_2$	338	2.89	

43	23.432	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	3.79	
44	24.032	Octadecanoic acid	$C_{18}H_{36}O_2$	284	0.71	
45	32.105	Phenol, 2,4-bis(1-methyl-1-phenylethyl)-	$C_{24}H_{26}O$	330	0.28	
46	40.882	Hexanoic acid, 2,7-dimethyloct-7-en-5-yn-4-yl ester	$C_{16}H_{26}O_2$	250	0.48	
47	46.750	Benzo[h]quinoline, 2,4-dimethyl-	$C_{15}H_{13}N$	207	0.51	

48	49.287	1,4-Bis(trimethylsilyl)benzene	$C_{12}H_{22}Si_2$	222	0.48	
49	50.128	1,1,1,3,5,5,5-Heptamethyltrisiloxane	$C_7H_{22}O_2Si_3$	222	0.54	
50	52.210	.beta.-Sitosterol	$C_{29}H_{50}O$	414	1.85	

4.27. Gas chromatography-Mass spectrometry (GC-MS) analysis of ethanolic leaves extract of *Melia azedarach*

The ethanolic leaves extract of *Melia azedarach* was selected for GC-MS analysis. Upon GC-MS analysis, the extract contains 53 chemical constituents eluted between 5.73 and 52.76 minutes as shown in Figure 4.82. These compounds belong to different chemical classes and most of them are reported to exhibit important biological activities. The identified compounds with their peak number, retention time (RT), peak area (%), molecular formula and weight and structure are presented in Table 4.27. The major compounds identified were 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (10.78%), Vitamin E (8.11%), 9,12,15-Octadecatrienoic acid, (Z,Z,Z) (6.12%), 4-Methylheptane-3,5-dione (5.54%), .beta.-Sitosterol (4.96%), Neophytadiene (4.04%), n-Hexadecanoic acid (3.61%), 11-Bromoundecanoic acid (2.19%), 2-Amino-4,6-dimethoxypyrimidine (1.75%), Phenol, 3,5-bis(1,1-dimethylethyl) (1.62%), Heptacosane (1.60%), Thymol (1.59%), Phytol, acetate (1.48%), 1,2-Propanediol diformate (1.42%), Methyleugenol (1.37%), 8-Aminocaprylic acid (1.33%), 3-Hydroxymandelic acid, methyl ester (1.29%), 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R) (1.22%), 1,4-Bis(trimethylsilyl) benzene (1.22%), Eicosane (1.14%), 2,2-Dichloroethyl methyl ether (1.13%), Triethyl borate (1.13%), Caryophyllene (1.13%), Ethanol, 2-[2-(2-ethoxyethoxy)ethoxy] (1.10%), Phytol (0.99%) and Hexadecanoic acid, ethyl ester (0.99%).

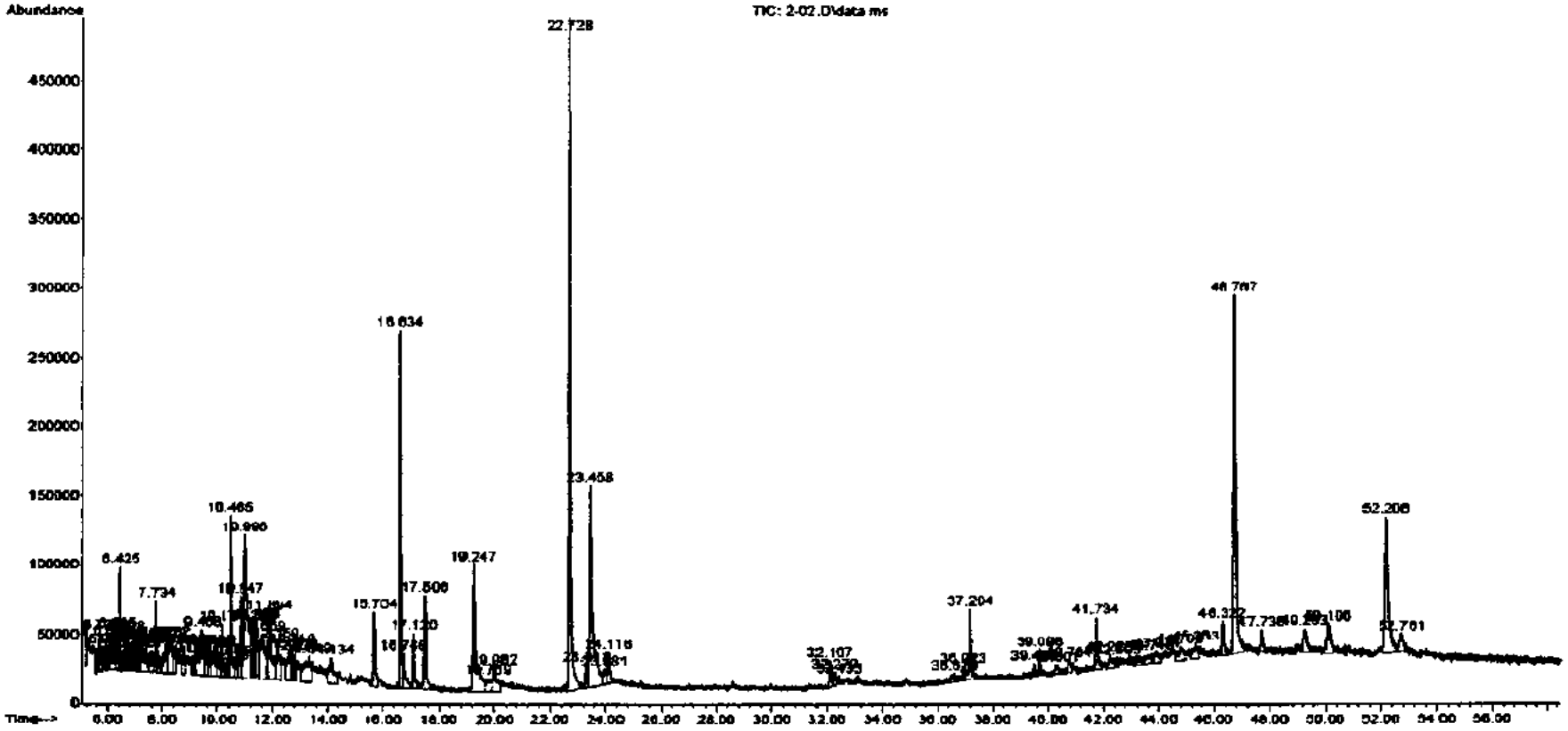
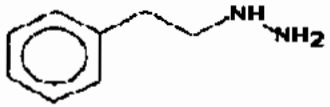
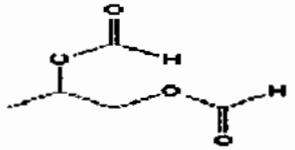
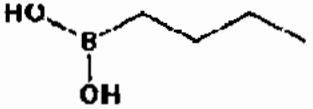
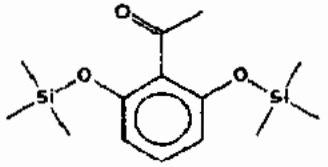
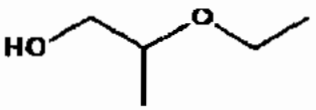
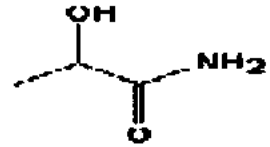
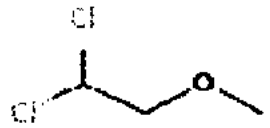
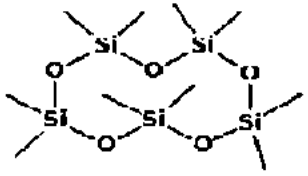
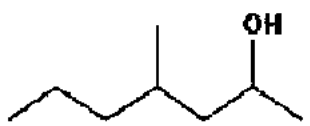
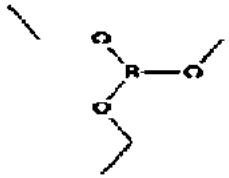
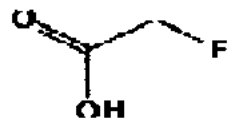
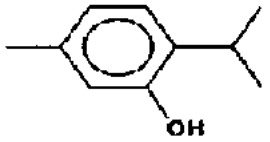
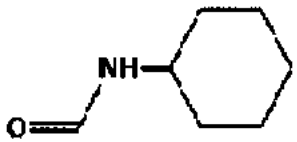


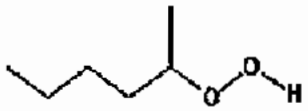
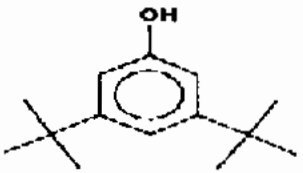
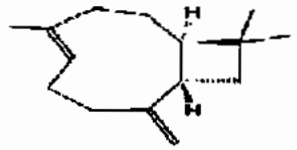
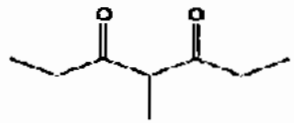
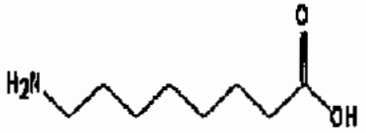
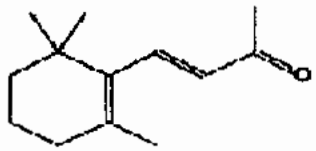
Figure 4.82: GC-MS chromatogram of ethanolic leaf extract of *Melia azedarach*

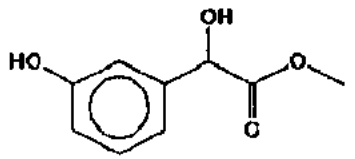
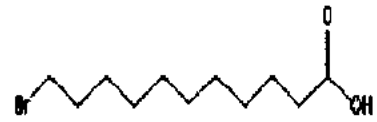
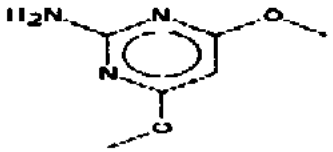
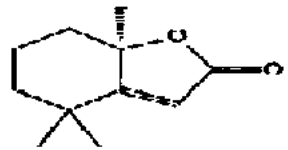
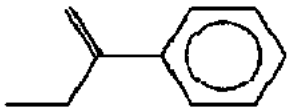
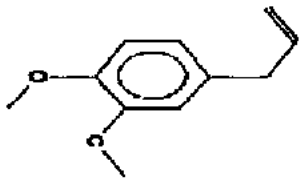
Table 4.27: Phytoconstituents identified in the ethanol leaves extract of *Melia azedarach* by GC-MS

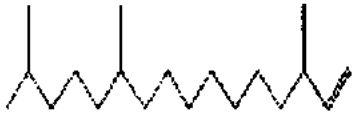
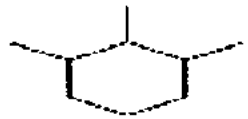
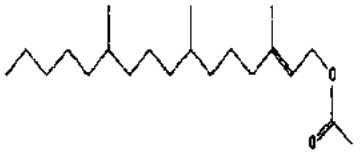
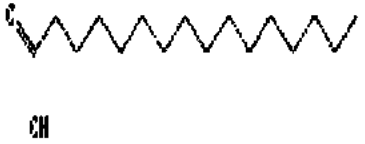
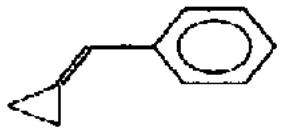
Sr. No.	RT	Compound	Molecular Formula	Molecular Weight	Peak Area %	Structure
1	5.737	Phenelzine	$C_8H_{12}N_2$	136	0.58	
2	5.926	1-Butene, 4-methoxy	$C_5H_{10}O$	86	0.41	
3	6.096	1,2-Propanediol diformate	$C_5H_8O_4$	132	1.42	
4	6.292	1-Butaneboronic acid	$C_4H_{11}BO_2$	101	0.52	
5	6.425	2,6-Dihydroxyacetophenone, 2TMS derivative	$C_{14}H_{24}O_3Si_2$	296	0.74	
6	6.620	1-Propanol, 2-ethoxy	$C_5H_{12}O_2$	104	0.44	

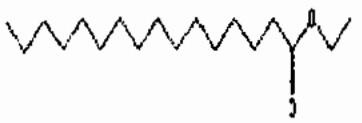
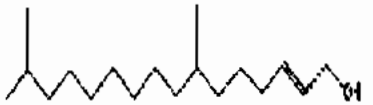
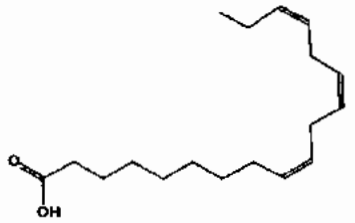
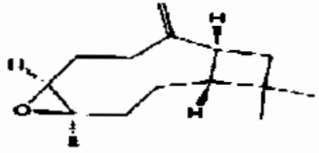
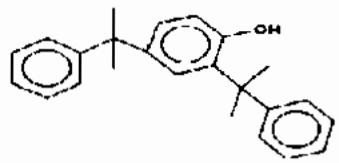
7	6.765	2-Octanol	$C_8H_{18}O$	130	0.64	
8	7.043	Propanamide, 2-hydroxy	$C_3H_7NO_2$	89	0.52	
9	7.169	Isoxazolidine	C_3H_7NO	73	0.41	
10	7.232	2,2-Dichloroethyl methyl ether	$C_3H_6Cl_2O$	128	1.13	
11	7.572	2-Hexadecanol	$C_{16}H_{34}O$	242	0.54	
12	7.734	Cyclopentasiloxane, decamethyl	$C_{10}H_{30}O_5Si_5$	370	0.55	

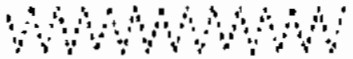
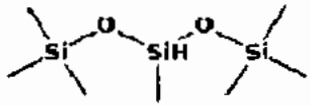
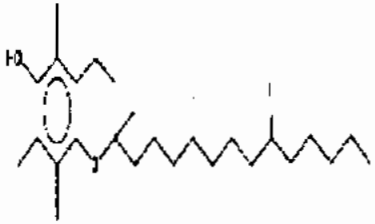
13	7.793	2-Heptanol, 4-methyl	$C_8H_{18}O$	130	0.62	
14	8.128	Ethanol, 2-[2-(2-ethoxyethoxy)ethoxy]	$C_8H_{18}O_4$	178	1.10	
15	8.260	Triethyl borate	$C_6H_{15}BO_3$	145	1.13	
16	8.405	Fluoroacetic acid	$C_2H_3FO_2$	78	0.59	
17	9.408	Thymol	$C_{10}H_{14}O$	150	1.59	
18	9.522	Formamide, N-cyclohexyl	$C_7H_{13}NO$	127	0.62	

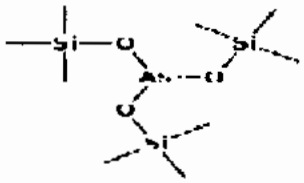
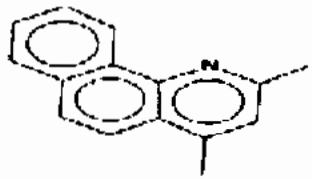
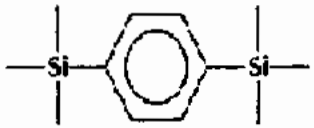
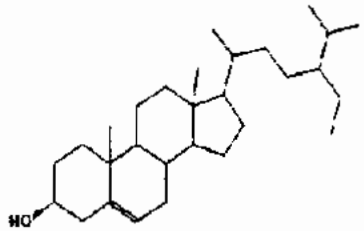
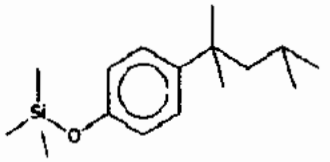
19	9.724	Hydroperoxide, 1-methylpentyl	$C_6H_{14}O_2$	118	0.53	
20	10.465	Phenol, 3,5-bis(1,1-dimethylethyl)	$C_{14}H_{22}O$	206	1.62	
21	10.847	Caryophyllene	$C_{15}H_{24}$	204	1.13	
22	10.996	4-Methylheptane-3,5-dione	$C_8H_{14}O_2$	142	5.54	
23	11.288	8-Aminocaprylic acid	$C_8H_{17}NO_2$	159	1.33	
24	11.370	trans-.beta.-Ionone	$C_{13}H_{20}O$	192	0.85	

25	11.440	3-Hydroxymandelic acid, methyl ester	$C_9H_{10}O_4$	182	1.29	
26	11.659	11-Bromoundecanoic acid	$C_{11}H_{21}BrO_2$	265	2.19	
27	11.894	2-Amino-4,6-dimethoxypyrimidine	$C_6H_9N_3O_2$	155	1.75	
28	12.159	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)	$C_{11}H_{16}O_2$	180	1.22	
29	12.834	Benzene, (1-methylenepropyl)	$C_{10}H_{12}$	132	0.45	
30	15.704	Methyleugenol	$C_{11}H_{14}O_2$	178	1.37	

31	16.634	Neophytadiene	$C_{20}H_{38}$	278	4.04	
32	16.748	Cyclohexane, 1,2,3-trimethyl	C_9H_{18}	126	0.88	
33	17.120	Phytol	$C_{20}H_{40}O$	296	0.99	
34	17.506	Phytol, acetate	$C_{22}H_{42}O_2$	338	1.48	
35	19.247	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	3.61	
36	19.769	Benzene, (cyclopropylidenemethyl)	$C_{10}H_{10}$	130	0.56	

37	19.982	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	0.99	
38	22.728	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	10.78	
39	23.458	9,12,15-Octadecatrienoic acid, (Z,Z,Z)	$C_{18}H_{30}O_2$	278	6.12	
40	23.981	Caryophyllene oxide	$C_{15}H_{24}O$	220	0.46	
41`	24.116	7,10,13-Hexadecatrienoic acid, methyl ester	$C_{17}H_{28}O_2$	264	0.75	
42	32.107	Phenol, 2,4-bis(1-methyl-1-phenylethyl)	$C_{24}H_{26}O$	330	0.46	

43	37.204	Heptacosane	$C_{27}H_{56}$	380	1.60	
44	39.698	Squalene	$C_{30}H_{50}$	410	0.40	
45	41.734	Eicosane	$C_{20}H_{42}$	282	1.14	
46	43.715	1,1,1,3,5,5,5-Heptamethyltrisiloxane	$C_7H_{22}O_2Si_3$	222	0.98	
47	46.322	Nonadecane	$C_{19}H_{40}$	268	0.92	
48	46.767	Vitamin E	$C_{29}H_{50}O_2$	430	8.11	

49	47.730	Arsenous acid, tris(trimethylsilyl) ester	$C_9H_{27}AsO_3Si_3$	342	0.53	
50	49.263	Benzo[h]quinoline, 2,4-dimethyl	$C_{15}H_{13}N$	207	0.88	
51	50.106	1,4-Bis(trimethylsilyl)benzene	$C_{12}H_{22}Si_2$	222	1.22	
52	52.206	.beta.-Sitosterol	$C_{29}H_{50}O$	414	4.96	
53	52.761	4-tert-Octylphenol, TMS derivative	$C_{17}H_{30}OSi$	278	0.65	

4.28. Gas chromatography-Mass spectrometry (GC-MS) analysis of ethyl acetate leaves extract of *Melia azedarach*

The ethyl acetate leaves extract of *Melia azedarach* was selected for GC-MS analysis. Upon GC-MS analysis, the extract contains 51 chemical constituents eluted between 6.51 and 56.89 minutes as shown in Figure 4.83. These compounds belong to different chemical classes and most of them are reported to exhibit important biological activities. The identified compounds with their peak number, retention time (RT), peak area (%), molecular formula and weight and structure are presented in Table 4.28. The major compounds identified were 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (12.66%), dl-.alpha.-Tocopherol (10.72%), Octacosane (5.92%), n-Hexadecanoic acid (5.62%), Phytol (5.29%), Tritriacontane (4.67%), .gamma.-Sitosterol (4.27%), Tetracosane (2.66%), Eicosane (2.24%), Cholesta-3,5-diene (1.95%), Neophytadiene (1.94%), Stigmasterol (1.54%), Heptacosane (1.49%), 9,12,15-Octadecatrien-1-ol, (Z,Z,Z)- (1.21%), Stigmastan-3,5,22-trien (1.16%) and Triacontane (1.06%).

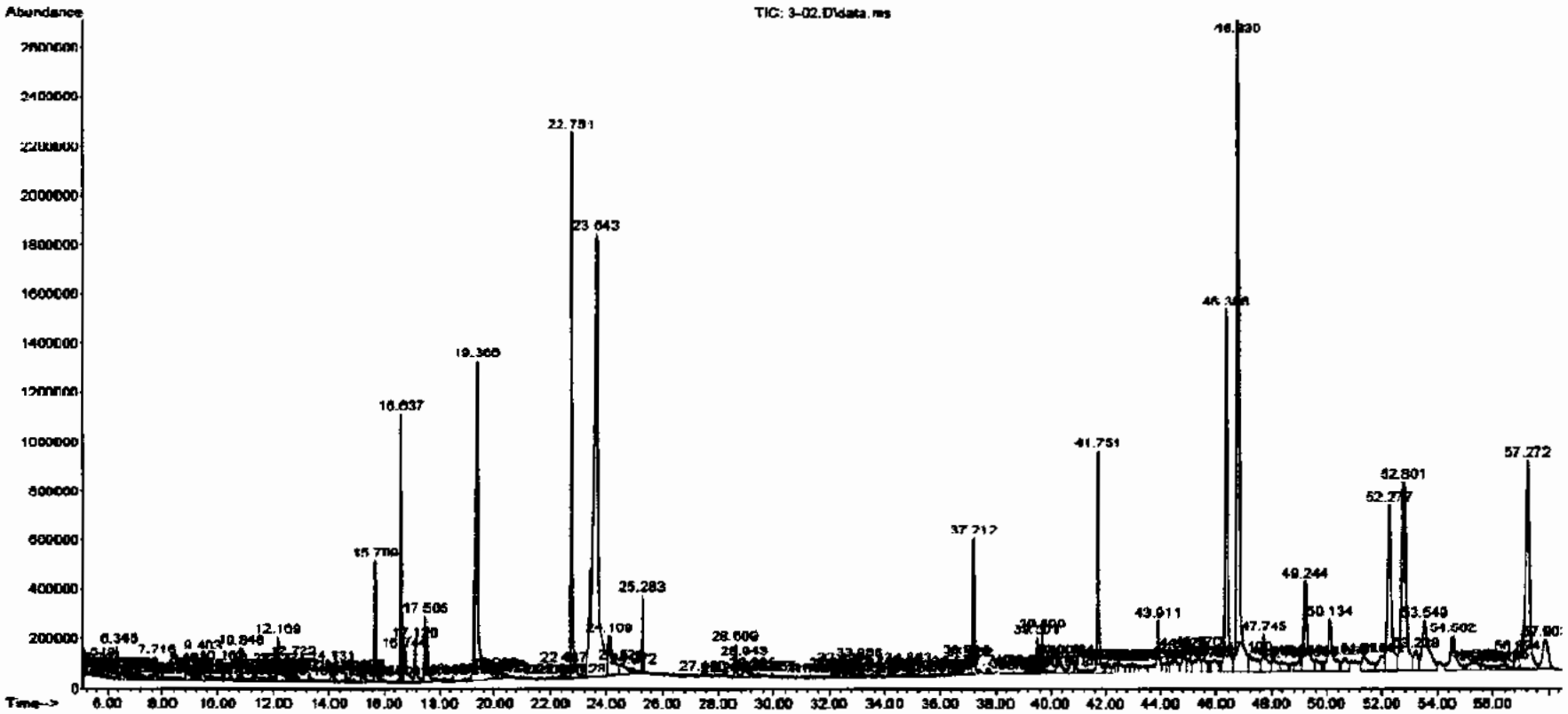
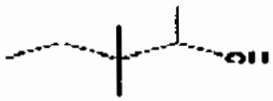
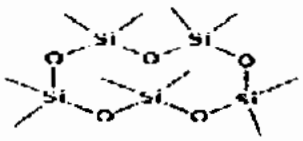
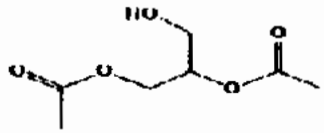
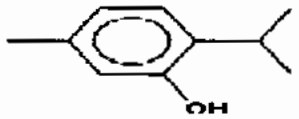
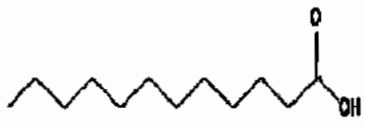
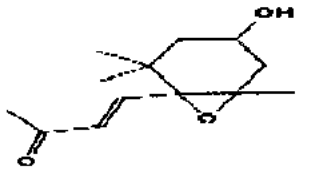
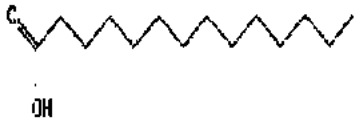
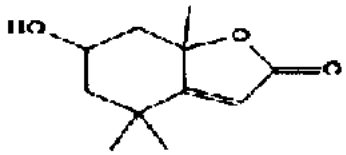
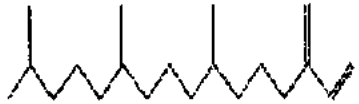
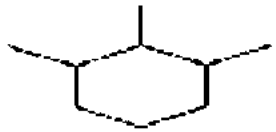
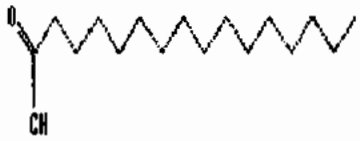
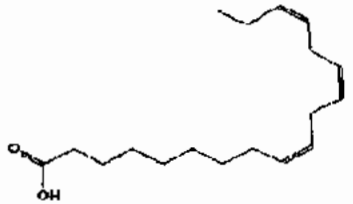
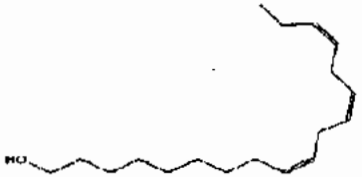


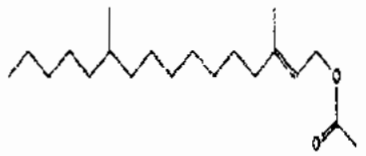
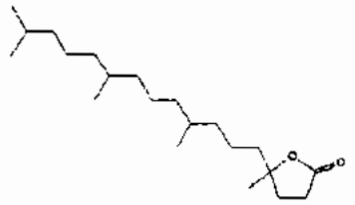
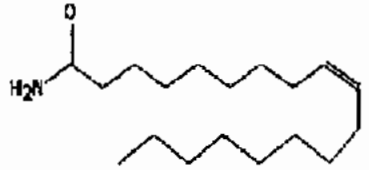
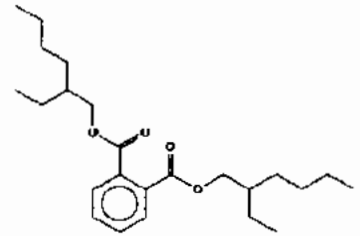
Figure 4.83: GC-MS chromatogram of ethyl acetate leaf extract of *Melia azedarach*

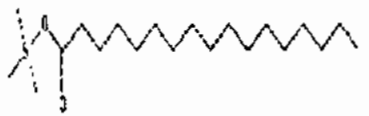
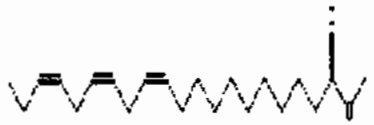
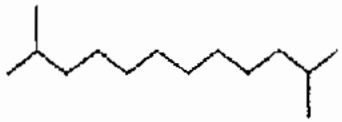
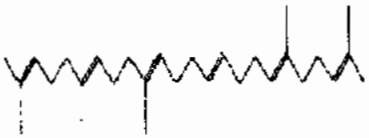
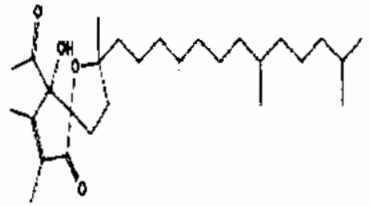
Table 4.28: Phytoconstituents identified in the ethyl acetate leaves extract of *Melia azedarach* by GC-MS

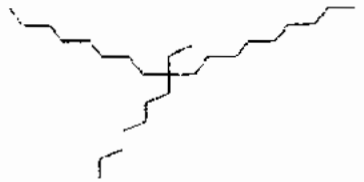
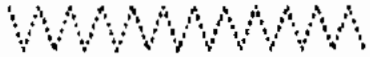
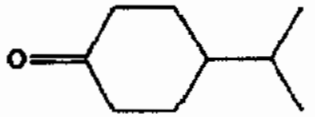
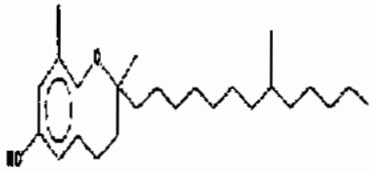
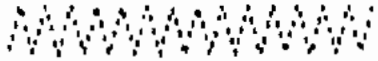
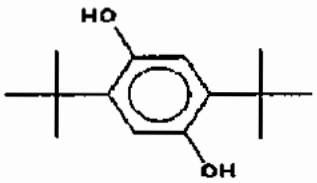
Sr. No.	RT	Compound	Molecular Formula	Molecular Weight	Peak Area %	Structure
1	6.513	3,3-Dimethyl-2-pentanol	$C_7H_{16}O$	116	0.26	
2	7.716	Cyclopentasiloxane, decamethyl-	$C_{10}H_{30}O_5Si_5$	370	0.21	
3	8.866	Glycerol 1,2-diacetate	$C_7H_{12}O_5$	176	0.28	
4	9.403	Thymol	$C_{10}H_{14}O$	150	0.29	
5	10.848	Caryophyllene	$C_{15}H_{24}$	204	0.22	
6	12.169	Dodecanoic acid	$C_{12}H_{24}O_2$	200	0.57	

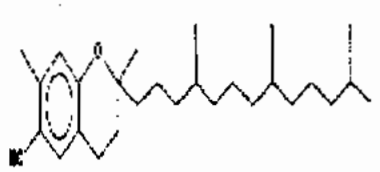
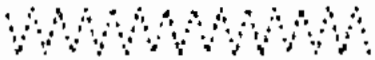
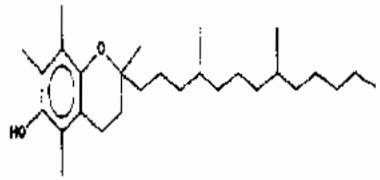
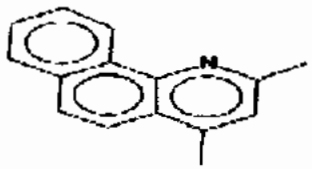
7	14.131	3-Buten-2-one, 4-(4-hydroxy-2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-	$C_{13}H_{20}O_3$	224	0.29	
8	15.186	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	0.20	
9	15.709	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	$C_{11}H_{16}O_3$	196	0.99	
10	16.637	Neophytadiene	$C_{20}H_{38}$	278	1.94	
11	16.744	Cyclohexane, 1,2,3-trimethyl-	C_9H_{18}	126	0.55	
12	17.120	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	0.52	

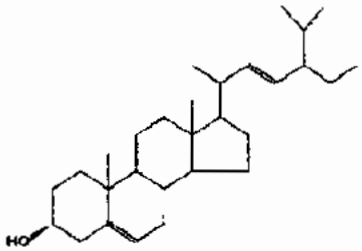
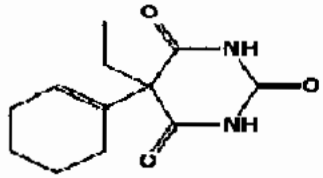
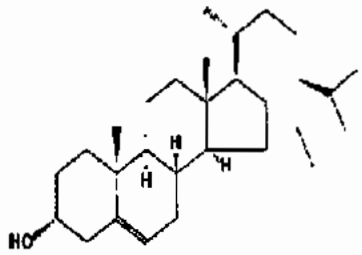
13	17.505	Bicyclo [3.1.1]heptane, 2,6,6-trimethyl-	$C_{10}H_{18}$	138	0.74	
14	19.365	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	5.62	
15	22.751	Phytol	$C_{20}H_{40}O$	296	5.29	
16	23.643	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	12.66	
17	24.109	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	$C_{18}H_{32}O$	264	1.21	
18	24.528	7,10,13-Hexadecatrienoic acid, methyl ester	$C_{17}H_{28}O_2$	264	0.56	

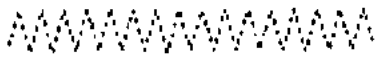
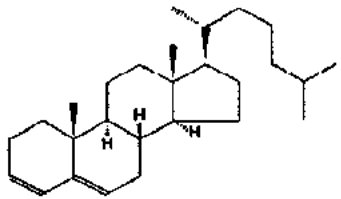
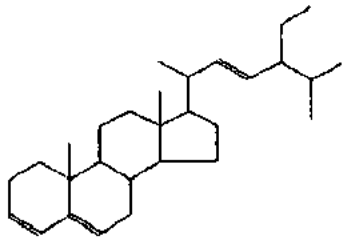
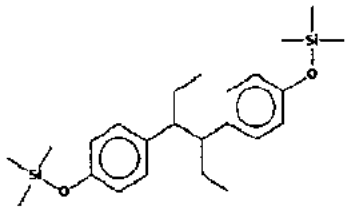
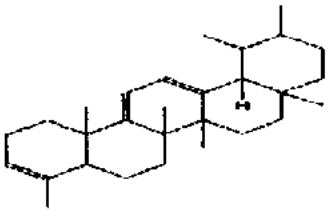
19	25.283	Phytol, acetate	$C_{22}H_{42}O_2$	338	0.70	
20	28.609	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	324	0.38	
21	28.943	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	281	0.33	
22	33.088	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	0.24	
23	36.548	Z,Z-11,13-Hexadecadien-1-ol acetate	$C_{18}H_{32}O_2$	280	0.22	

24	36.924	Heptadecanoic acid, TMS derivative	$C_{20}H_{42}O_2Si$	342	0.28	
25	37.053	(11Z,14Z,17Z)-Methyl icosa-11,14,17-trienoate	$C_{21}H_{36}O_2$	320	0.25	
26	37.212	Heptacosane	$C_{27}H_{56}$	380	1.49	
27	39.501	Dodecane, 2,6,11-trimethyl-	$C_{15}H_{32}$	212	0.41	
28	39.699	Squalene	$C_{30}H_{50}$	410	0.52	
29	40.308	.alpha.-Tocospiro A	$C_{29}H_{50}O_4$	462	0.46	

30	40.895	Octadecane, 9-ethyl-9-heptyl-	$C_{27}H_{56}$	380	0.21	
31	41.751	Tetracosane	$C_{24}H_{50}$	338	2.66	
32	42.049	4-Isopropylcyclohexanone	$C_9H_{16}O$	140	0.24	
33	42.497	.delta.-Tocopherol	$C_{27}H_{46}O_2$	402	0.34	
34	43.911	Triacontane	$C_{30}H_{62}$	422	1.06	
35	44.478	1,4-Benzenediol, 2,5-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O_2$	222	0.52	

36	44.819	.gamma.-Tocopherol	$C_{28}H_{48}O_2$	416	0.56	
37	45.370	Eicosyl isopropyl ether	$C_{23}H_{48}O$	340	0.63	
38	46.386	Octacosane	$C_{28}H_{58}$	394	5.92	
39	46.830	dl-.alpha.-Tocopherol	$C_{29}H_{50}O_2$	430	10.72	
40	47.745	Phytyl dodecanoate	$C_{32}H_{62}O_2$	478	0.89	
41	48.074	Benzo[h]quinoline, 2,4-dimethyl-	$C_{15}H_{13}N$	207	0.47	

42	49.244	Eicosane	$C_{20}H_{42}$	282	2.24	
43	50.134	Stigmasterol	$C_{29}H_{48}O$	412	1.54	
44	50.660	Cyclobarbital	$C_{12}H_{16}N_2O_3$	236	0.48	
45	51.348	Heneicosane	$C_{21}H_{44}$	296	0.76	
46	52.277	.gamma.-Sitosterol	$C_{29}H_{50}O$	414	4.27	

47	52.801	Tritriacontane	$C_{33}H_{68}$	465	4.67	
48	53.549	Cholesta-3,5-diene	$C_{27}H_{44}$	368	1.95	
49	54.562	Stigmastan-3,5,22-trien	$C_{29}H_{46}$	394	1.16	
50	55.348	Hexestrol, 2TMS derivative	$C_{24}H_{38}O_2Si_2$	414	0.41	
51	56.894	24-Norursa-3,9(11),12-triene	$C_{29}H_{44}$	392	0.40	

4.29. Gas chromatography-Mass spectrometry (GC-MS) analysis of methanolic leaves extract of *Justicia adhatoda*

The methanolic leaves extract of *Justicia adhatoda* was selected for GC-MS analysis. Upon GC-MS analysis, the extract contains 56 chemical constituents eluted between 5.20 and 55.79 minutes as shown in Figure 4.84. These compounds belong to different chemical classes and most of them are reported to exhibit important biological activities. The identified compounds with their peak number, retention time (RT), peak area (%), molecular formula and weight and structure are presented in Table 4.29. The major compounds identified in the methanolic leaves extract of *Justicia adhatoda* were Phytol, acetate (11.07%), 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one (6.72%), 5-Hydroxymethylfurfural (5.85%), n-Hexadecanoic acid (5.27%), Phenol, 4-(2-methylpropyl)- (5.24%), Hexanal, 2-methyl- (5.02%), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (4.49%), 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (4.22%), 1-Cyclohexyldimethylsilyloxy-3,5-difluorobenzene (3.91%), Carbamic acid, methyl-, ethyl ester (2.66%), 2-(Dimethylamino)ethanol, TMS derivative (2.37%), 2-Heptanone, 6-methyl- (2.07%), 2,3,5,6-Tetrafluoroanisole (1.94%), 1,3-Propanediamine,N-(3-aminopropyl)-N-methyl- (1.82%), Benzeneethanamine, N,N-dimethyl- (1.82%), 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one (1.76%), Thymol (1.66%), Mexiletine (1.53%), Betaine (1.52%), 2-Methoxy-4-vinylphenol (1.44%), Benzofuran, 2,3-dihydro- (1.36%), Phytol (1.32%), 1,3-Propanediamine, N,N-dimethyl- (1.24%), 7-Octen-2-one (1.11%), N,N'-Diethyl-1,6-hexanediamine (1.06%), 1,4-Butanediamine, N,N,N',N'-tetramethyl- (1.05%), 2,6-Difluorobenzoic acid, 2-methyloct-5-yn-4-yl ester (1.04%), 9,12-Octadecadienoic acid (Z,Z)- (1.04%) and .beta.-Sitosterol (1.00%).

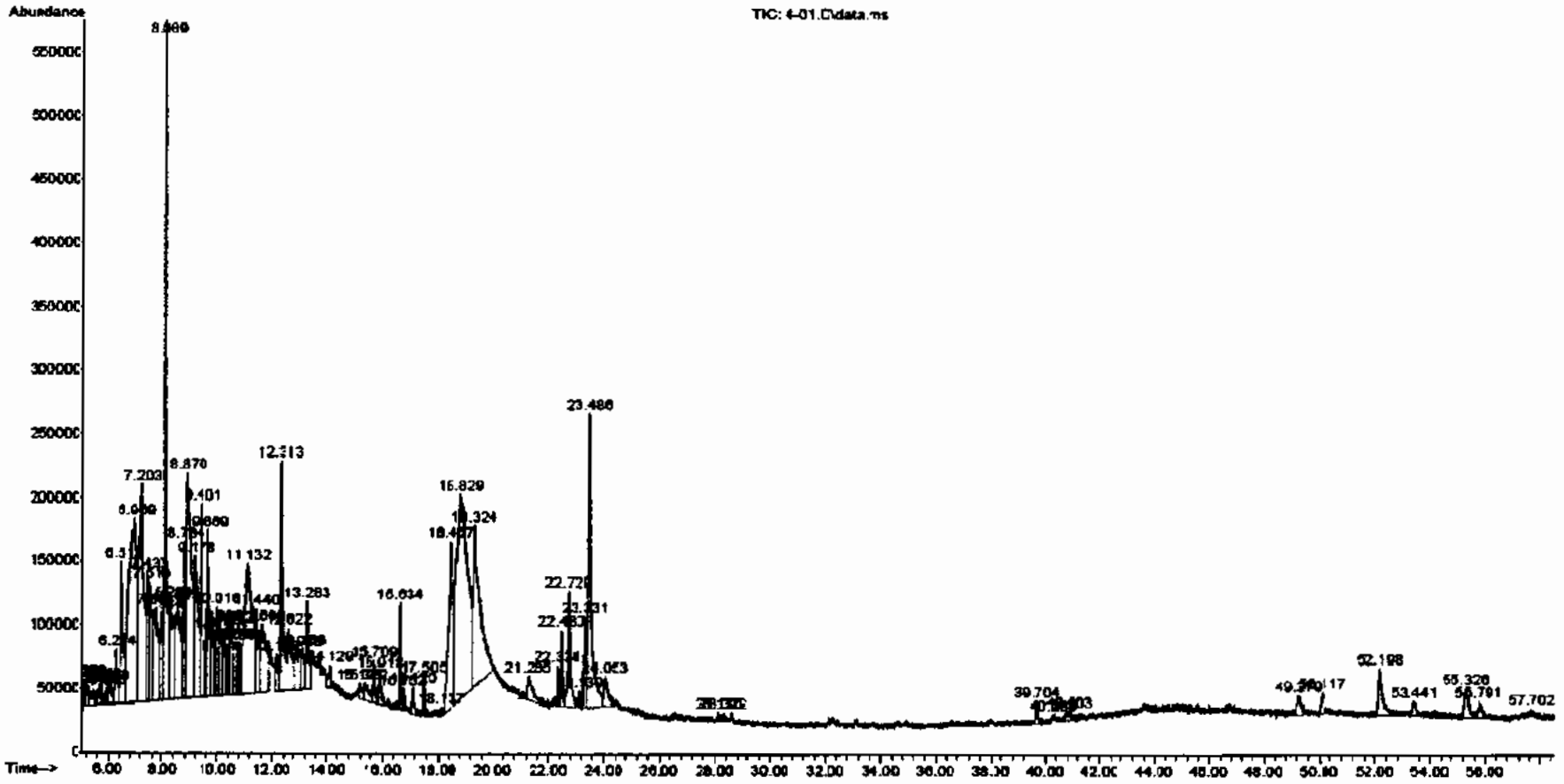
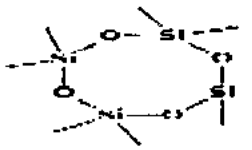
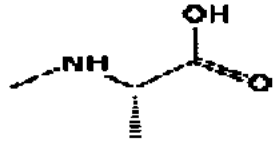
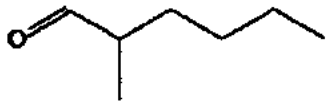
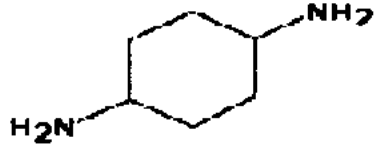
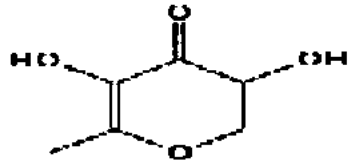
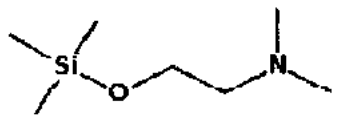
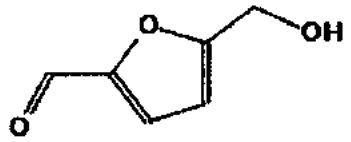
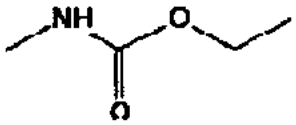
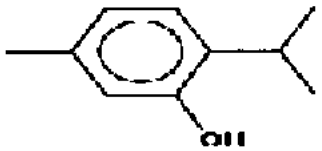
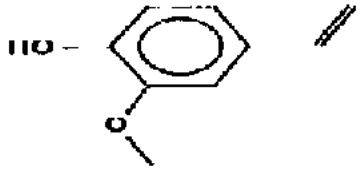


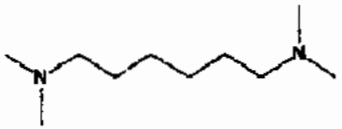
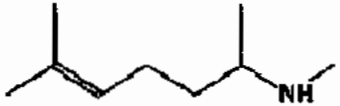
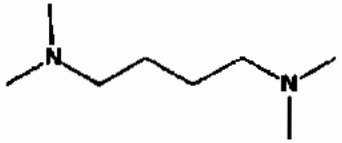
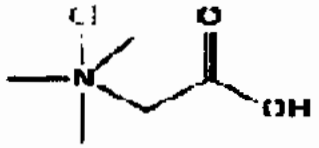
Figure 4.84: GC-MS chromatogram of methanolic leaf extract of *Justicia adhatoda*

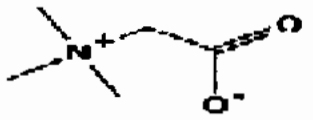
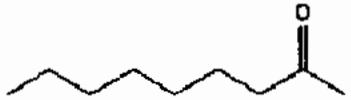
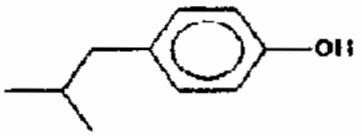
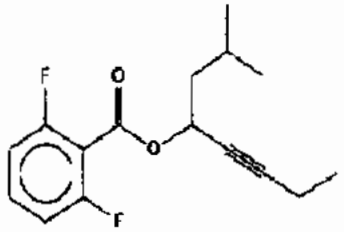
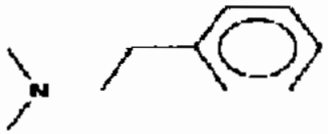
Table 4.29: Phytoconstituents identified in the methanol leaves extract of *Justicia adhatoda* by GC-MS

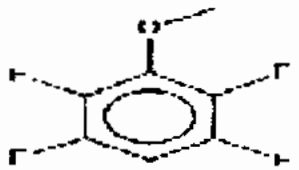
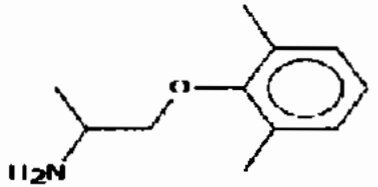
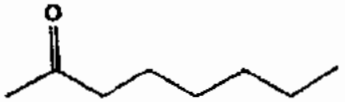
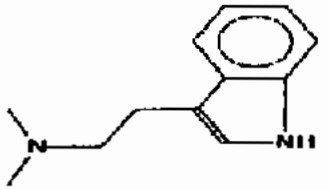
Sr. No.	RT	Compound	Molecular Formula	Molecular Weight	Peak Area %	Structure
1	5.207	2-Decanone	$C_{10}H_{20}O$	156	0.31	
2	6.046	1,4-Cyclohexanediol	$C_6H_{12}O_2$	116	0.31	
3	6.274	Cyclotetrasiloxane, octamethyl-	$C_8H_{24}O_4Si_4$	296	0.66	
4	6.513	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	144	1.76	
5	6.969	L-Alanine, N-methyl-	$C_4H_9NO_2$	103	6.72	
6	7.203	Hexanal, 2-methyl-	$C_7H_{14}O$	114	5.02	

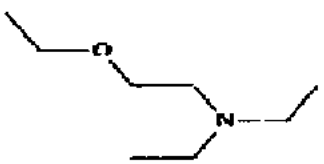
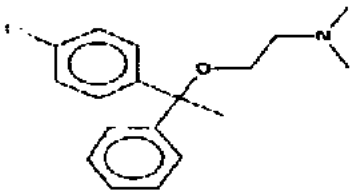
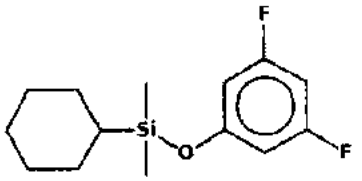
7	7.433	1,3-Propanediamine, N,N-dimethyl-	$C_5H_{14}N_2$	102	1.24	
8	7.510	Betaine	$C_5H_{11}NO_2$	117	1.52	
9	7.648	2-Heptanone, 6-methyl-	$C_8H_{16}O$	128	2.07	
10	7.984	trans-1,4-Cyclohexanediamine	$C_6H_{14}N_2$	114	0.98	
11	8.089	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	4.49	
12	8.298	1,3-Propanediamine,N-(3-aminopropyl)-N-methyl-	$C_7H_{19}N_3$	145	1.82	

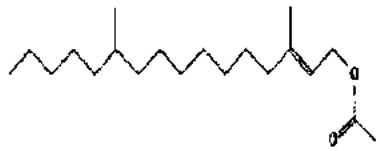
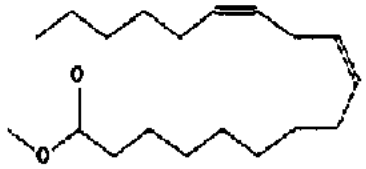
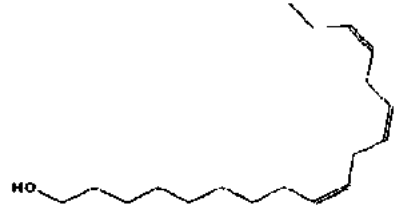
13	8.563	2-(Dimethylamino)ethanol, TMS derivative	$C_7H_{19}NOSi$	161	2.37	
14	8.784	Benzofuran, 2,3-dihydro-	C_8H_8O	120	1.36	
15	8.870	5-Hydroxymethylfurfural	$C_6H_6O_3$	126	5.85	
16	9.178	Carbamic acid, methyl-, ethyl ester	$C_4H_9NO_2$	103	2.66	
17	9.401	Thymol	$C_{10}H_{14}O$	150	1.66	
18	9.669	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	1.44	

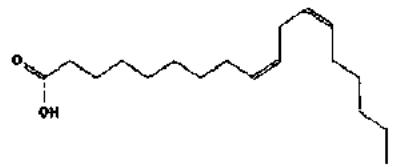
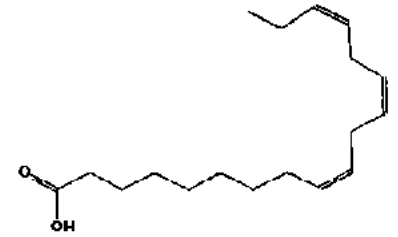
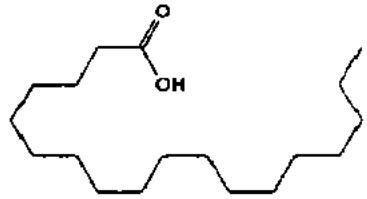
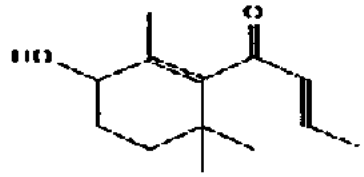
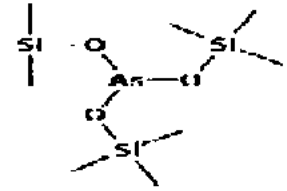
19	9.816	1,4-Cyclohexanediamine, cis-	$C_6H_{14}N_2$	114	0.97	
20	10.016	7-Octen-2-one	$C_8H_{14}O$	126	1.11	
21	10.090	1,6-Hexanediamine, N,N,N',N'-tetramethyl-	$C_{10}H_{24}N_2$	172	0.95	
22	10.304	5-Hepten-2-amine, N,6-dimethyl-	$C_9H_{19}N$	141	0.80	
23	10.500	1,4-Butanediamine, N,N,N',N'-tetramethyl-	$C_8H_{20}N_2$	144	1.05	
24	10.670	Betaine Hydrochloride	$C_5H_{12}NO_2 \cdot Cl$	153	0.82	

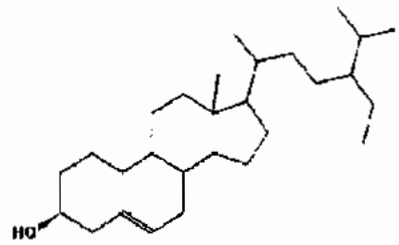
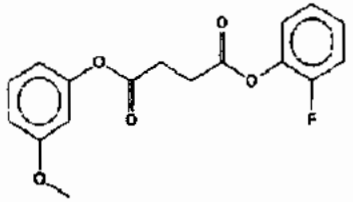
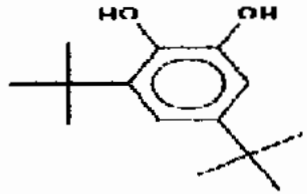
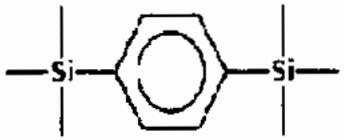
25	10.758	Betaine	$C_5H_{11}NO_2$	117	0.35	
26	10.840	2-Nonanone	$C_9H_{18}O$	142	0.37	
27	11.132	Phenol, 4-(2-methylpropyl)-	$C_{10}H_{14}O$	150	5.24	
28	11.440	2,6-Difluorobenzoic acid, 2-methyloct-5-yn-4-yl ester	$C_{16}H_{18}F_2O_2$	280	1.04	
29	11.636	Benzeneethanamine, N,N-dimethyl-	$C_{10}H_{15}N$	149	1.82	
30	12.159	Ethanamine, 2-chloro-N,N-dimethyl-	$C_4H_{10}ClN$	107	0.51	

31	12.313	2,3,5,6-Tetrafluoroanisole	$C_7H_4F_4O$	180	1.94	
32	12.622	Mexiletine	$C_{11}H_{17}NO$	179	1.53	
33	12.979	2-Octanone	$C_8H_{16}O$	128	0.94	
34	13.106	N,N-Dimethyltryptamine	$C_{12}H_{16}N_2$	188	0.59	
35	13.283	N,N'-Diethyl-1,6-hexanediamine	$C_{10}H_{24}N_2$	172	1.06	
36	15.372	1-Heptanamine, N,N-dimethyl-	$C_9H_{21}N$	143	0.42	

37	15.709	2-Ethoxyethyl,diethylamine	$C_8H_{19}NO$	145	0.38	
38	15.918	1,4-Benzenediol, 2-methyl-	$C_7H_8O_2$	124	0.37	
39	16.634	Neophytadiene	$C_{20}H_{38}$	278	0.62	
40	17.120	Benzeneethanamine, N,N-dimethyl-	$C_{10}H_{15}N$	149	0.28	
41	17.505	Ethylamine, 2-[1-(p-chlorophenyl)-1-phenylethoxy]-N,N-dimethyl-	$C_{18}H_{22}ClNO$	303	0.26	
42	18.467	1-Cyclohexyldimethylsilyloxy-3,5-difluorobenzene	$C_{14}H_{20}F_2OSi$	270	3.91	

43	18.829	Phytol, acetate	$C_{22}H_{42}O_2$	338	11.07	
44	19.324	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	5.27	
45	21.285	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294	0.78	
46	22.480	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	$C_{18}H_{32}O$	264	0.53	
47	22.729	Phytol	$C_{20}H_{40}O$	296	1.32	

48	23.331	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	1.04	
49	23.486	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	4.22	
50	24.053	Octadecanoic acid	$C_{18}H_{36}O_2$	284	0.56	
51	39.704	3-Hydroxy-.beta.-damascone	$C_{13}H_{20}O_2$	208	0.23	
52	49.279	Arsenous acid, tris(trimethylsilyl) ester	$C_9H_{27}AsO_3Si_3$	342	0.51	

53	52.198	.beta.-Sitosterol	$C_{29}H_{50}O$	414	1.00	
54	53.441	Succinic acid, 2-fluorophenyl 3-methoxyphenyl ester	$C_{17}H_{15}FO_5$	318	0.22	
55	55.326	1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O_2$	222	0.63	
56	55.791	1,4-Bis(trimethylsilyl)benzene	$C_{12}H_{22}Si_2$	222	0.29	

4.30. Gas chromatography-Mass spectrometry (GC-MS) analysis of ethanolic leaves extract of *Justicia adhatoda*

The ethanolic leaves extract of *Justicia adhatoda* was selected for GC-MS analysis. Upon GC-MS analysis, the extract contains 56 chemical constituents eluted between 5.36 and 57.71 minutes as shown in Figure 4.85. These compounds belong to different chemical classes and most of them are reported to exhibit important biological activities. The identified compounds with their peak number, retention time (RT), peak area (%), molecular formula and weight and structure are presented in Table 4.30. The major compounds identified were 9,12,15-Octadecatrienoic acid, (Z,Z,Z) (11.92%), Phytol, acetate (9.47%), n-Hexadecanoic acid (6.40%), .beta.-Sitosterol (3.87%), 9,12-Octadecadienoic acid (Z,Z) (3.86%), Phytol (3.73%), Squalene (3.50%), 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z) (3.45%), 5-Hydroxymethylfurfural (2.41%), Nonadecane (2.25%), Lethane (2.10%), Lupeol (1.92%), Phenol, 2-methyl-5-(1-methylethyl) (1.85%), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (1.71%), Hexadecanoic acid, ethyl ester (1.50%), Stearyltrimethylammonium chloride (1.48%), Neophytadiene (1.40%), Stigmasterol (1.40%), Linoleic acid ethyl ester (1.37%), Undecanoic acid, ethyl ester (1.36%), Thiirane, (methoxymethyl)- (1.34%), 1,6-Naphthyridine (1.33%), Tricosane, 2-methyl- (1.32%), 4-Hydroxy-3-methylacetophenone (1.24%), 1,3-Propanediamine, N,N-dimethyl- (1.19%) and Octacosane (1.13%).

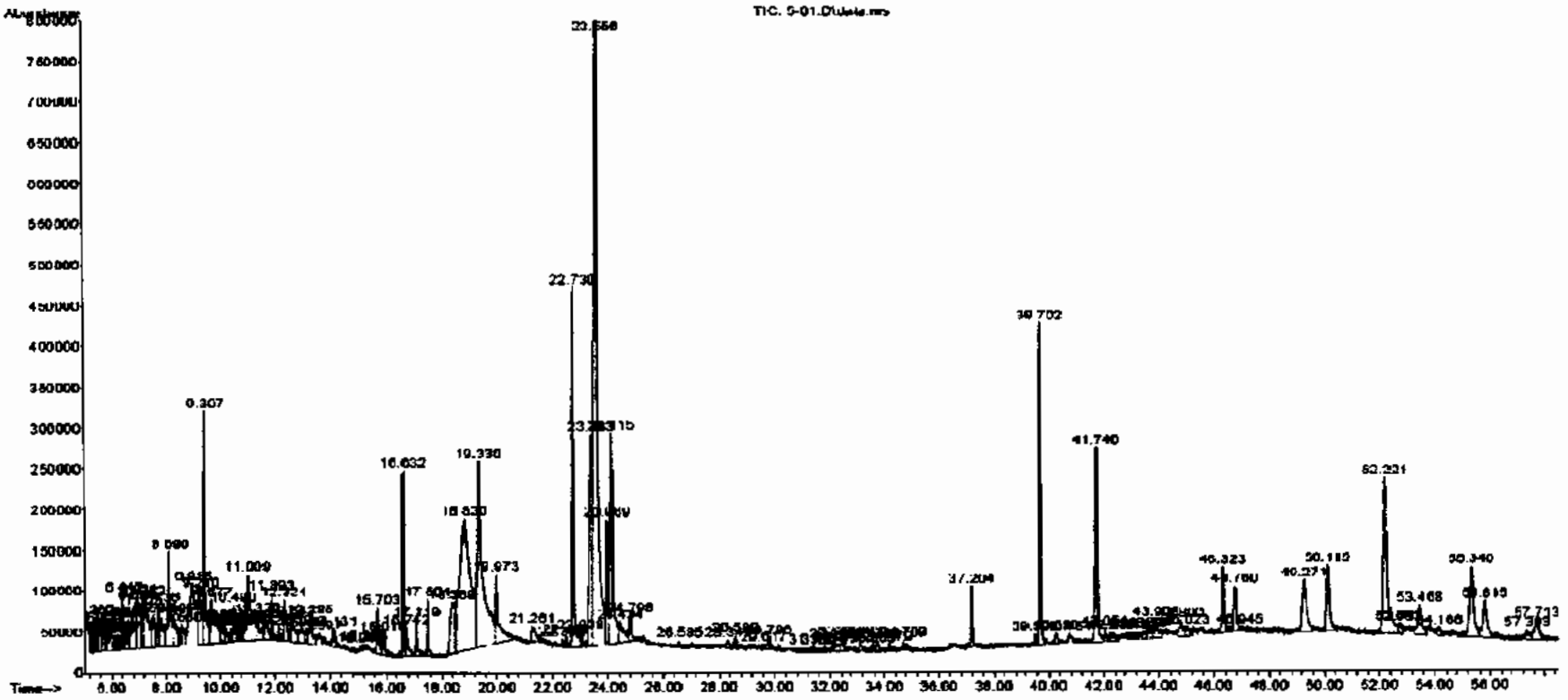
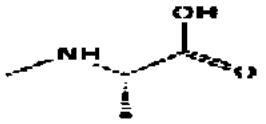
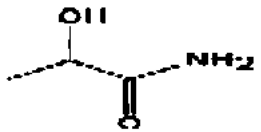
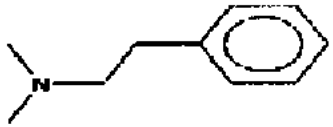
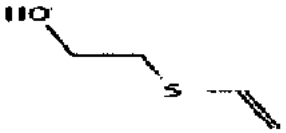
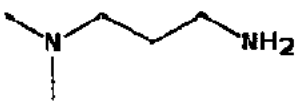
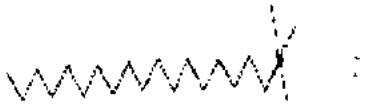
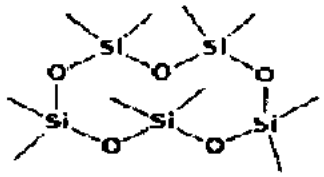
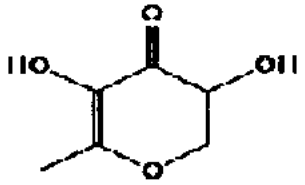
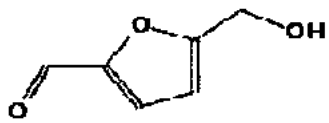
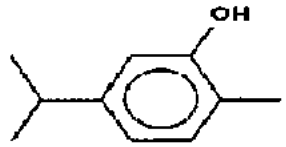
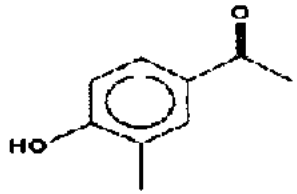
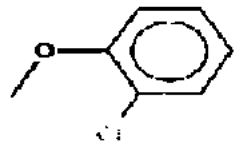
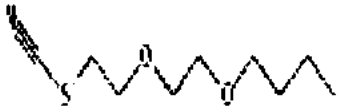


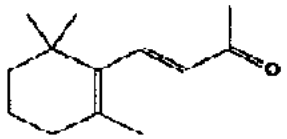
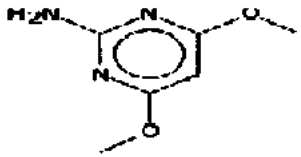
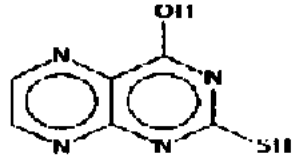
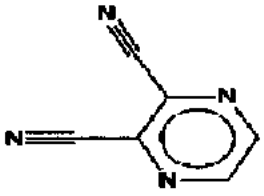
Figure 4.85: GC-MS chromatogram of ethanolic leaf extract of *Justicia adhatoda*

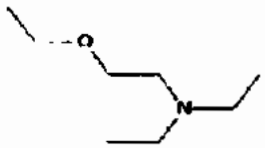
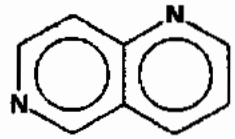
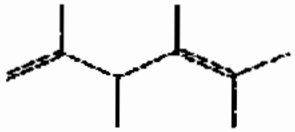
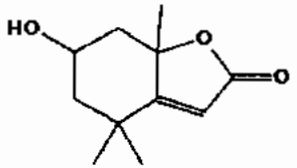
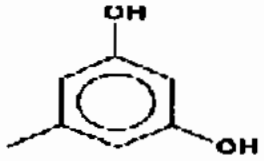
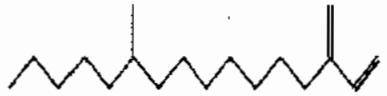
Table 4.30: Phytoconstituents identified in the ethanol leaves extract of *Justicia adhatoda* by GC-MS

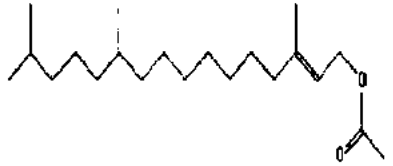
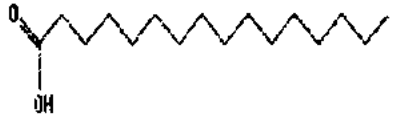
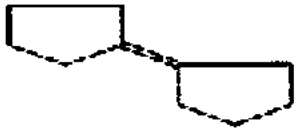
Sr. No.	RT	Compound	Molecular Formula	Molecular Weight	Peak Area %	Structure
1	5.364	L-Alanine, N-methyl	$C_4H_9NO_2$	103	0.31	
2	5.629	Propanamide, 2-hydroxy-	$C_3H_7NO_2$	89	0.38	
3	5.724	Phenelzine	$C_8H_{12}N_2$	136	0.33	
4	5.939	Benzeneethanamine, N,N-dimethyl	$C_{10}H_{15}N$	149	0.49	
5	6.595	2-Hydroxyethyl vinyl sulfide	C_4H_8OS	104	0.49	
6	6.879	Thiirane, (methoxymethyl)-	C_4H_8OS	104	1.34	

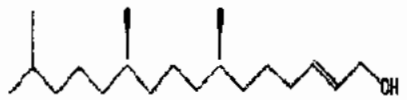
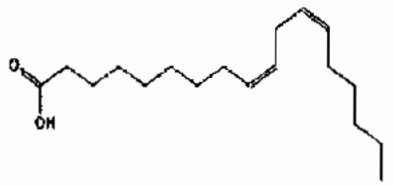
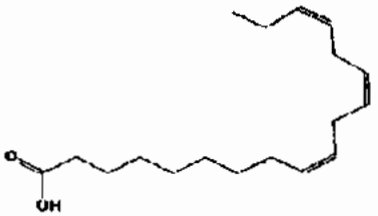
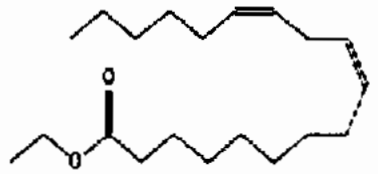
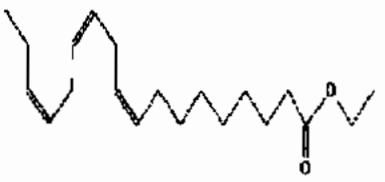
7	6.936	1,3-Propanediamine, N,N-dimethyl-	$C_5H_{14}N_2$	102	1.19	
8	7.125	Stearyltrimethylammonium chloride	$C_{21}H_{46}ClN$	348	1.48	
9	7.736	Cyclopentasiloxane, decamethyl	$C_{10}H_{30}O_5Si_5$	370	0.38	
10	7.800	2-Heptanone, 6-methyl-	$C_8H_{16}O$	128	0.52	
11	8.096	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	144	1.71	
12	8.560	1,3-Propanediamine, N-(3-aminopropyl)-N-methyl	$C_7H_{19}N_3$	145	0.41	

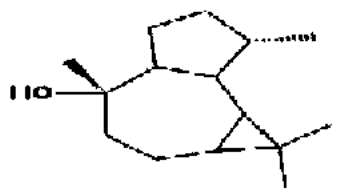
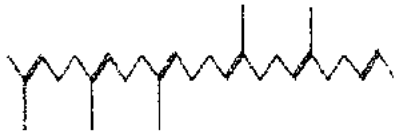
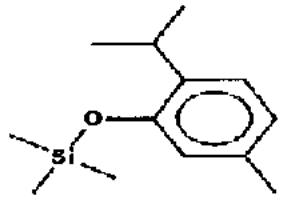
13	8.955	5-Hydroxymethylfurfural	$C_6H_6O_3$	126	2.41	
14	9.210	1,6-Naphthyridine	$C_8H_6N_2$	130	1.33	
15	9.397	Phenol, 2-methyl-5-(1-methylethyl)	$C_{10}H_{14}O$	150	1.85	
16	9.677	4-Hydroxy-3-methylacetophenone	$C_9H_{10}O_2$	150	1.24	
17	10.490	Benzene, 1-chloro-2-methoxy-	C_7H_7ClO	142	0.45	
18	11.009	Lethane	$C_9H_{17}NO_2S$	203	2.10	

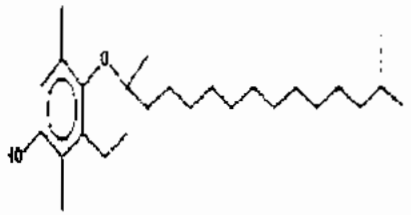
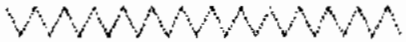
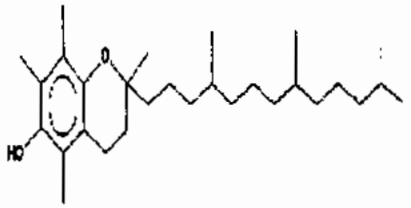
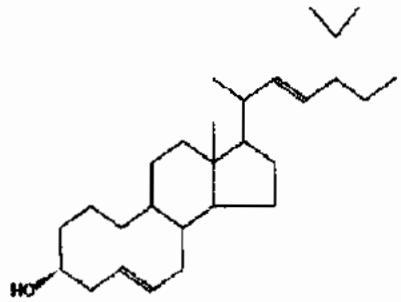
19	11.370	trans-.beta.-Ionone	$C_{13}H_{20}O$	192	0.36	
20	11.635	Ethanamine, 2-chloro-N,N-dimethyl-	$C_4H_{10}ClN$	107	0.49	
21	11.893	2-Amino-4,6-dimethoxypyrimidine	$C_6H_9N_3O_2$	155	0.29	
22	12.324	4-Hydroxy-2-mercapto pteridine	$C_6H_4N_4OS$	180	0.60	
23	12.613	8-Nonen-2-one	$C_9H_{16}O$	140	0.50	
24	12.947	2,3-Pyrazinedicarbonitrile	$C_6H_2N_4$	130	0.40	

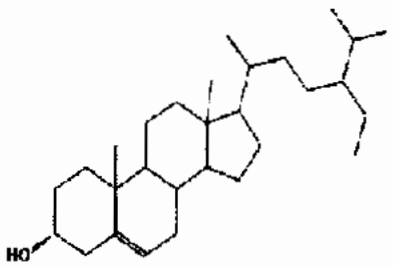
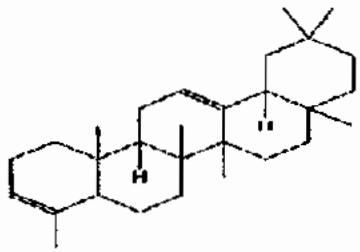
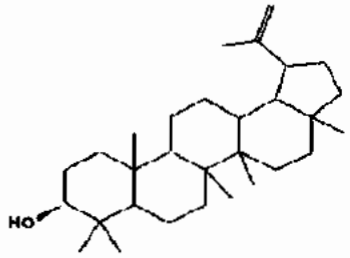
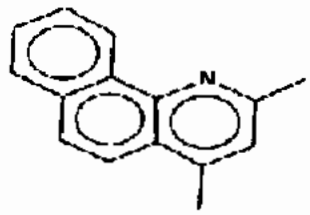
25	13.285	2-Ethoxyethyl,diethylamine	$C_8H_{19}NO$	145	0.58	
26	13.597	1,6-Naphthyridine	$C_8H_6N_2$	130	0.28	
27	14.131	1,4-Hexadiene, 2,3,4,5-tetramethyl	$C_{10}H_{18}$	138	0.29	
28	15.703	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	$C_{11}H_{16}O_3$	196	0.53	
29	15.915	Orcinol	$C_7H_8O_2$	124	0.38	
30	16.632	Neophytadiene	$C_{20}H_{38}$	278	1.40	

31	16.742	2-Hexadecene, 2,6,10,14-tetramethyl	$C_{20}H_{40}$	280	0.35	
32	18.389	Undecanoic acid, ethyl ester	$C_{13}H_{26}O_2$	214	1.36	
33	18.820	Phytol, acetate	$C_{22}H_{42}O_2$	338	9.47	
34	19.330	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	6.40	
35	19.973	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	1.50	
36	21.261	Bicyclopentylidene	$C_{10}H_{16}$	136	0.43	

37	22.730	Phytol	$C_{20}H_{40}O$	296	3.73	
38	23.383	9,12-Octadecadienoic acid (Z,Z)	$C_{18}H_{32}O_2$	280	3.86	
39	23.556	9,12,15-Octadecatrienoic acid, (Z,Z,Z)	$C_{18}H_{30}O_2$	278	11.92	
40	23.969	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308	1.37	
41`	24.115	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)	$C_{20}H_{34}O_2$	306	3.45	

42	24.434	Ledol	$C_{15}H_{26}O$	222	0.59	
43	24.798	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	0.35	
44	37.204	Eicosane	$C_{20}H_{42}$	282	0.69	
45	39.702	Squalene	$C_{30}H_{50}$	410	3.50	
46	41.740	Nonadecane	$C_{19}H_{40}$	268	2.25	
47	43.908	Thymol, TMS derivative	$C_{13}H_{22}OSi$	222	0.43	

48	44.800	.beta.-Tocopherol	$C_{28}H_{48}O_2$	416	0.35	
49	46.323	Octacosane	$C_{28}H_{58}$	394	1.13	
50	46.760	dl-.alpha.-Tocopherol	$C_{29}H_{50}O_2$	430	0.68	
51	49.271	Tricosane, 2-methyl-	$C_{24}H_{50}$	338	1.32	
52	50.115	Stigmasterol	$C_{29}H_{48}O$	412	1.40	

53	52.221	.beta.-Sitosterol	$C_{29}H_{50}O$	414	3.87	
54	53.468	24-Noroleana-3,12-diene	$C_{29}H_{46}$	394	0.86	
55	55.340	Lupeol	$C_{30}H_{50}O$	426	1.92	
56	57.713	Benzo[h]quinoline, 2,4-dimethyl	$C_{15}H_{13}N$	207	0.57	

4.31. Gas chromatography-Mass spectrometry (GC-MS) analysis of ethyl acetate leaves extract of *Justicia adhatoda*

The ethyl acetate leaves extract of *Justicia adhatoda* was selected for GC-MS analysis. Upon GC-MS analysis, the extract contains 47 chemical constituents eluted between 8.85 and 57.72 minutes as shown in Figure 4.86. These compounds belong to different chemical classes and most of them are reported to exhibit important biological activities. The identified compounds with their peak number, retention time (RT), peak area (%), molecular formula and weight and structure are presented in Table 4.31. The major compounds identified were 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (12.54%), 9,12-Octadecadienoic acid (Z,Z)- (9.41%), Hexacosane (8.17%), Octacosane (6.10%), Phytol (5.61%), Stigmasta-3,5-diene (5.19%), n-Hexadecanoic acid (4.95%), Cholesta-3,5-diene (3.48%), Squalene (2.79%), .gamma.-Sitosterol (2.63%), Octadecanoic acid (2.42%), Lupeol (2.30%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (1.64%), Stigmasterol (1.44%), dl-.alpha.-Tocopherol (1.42%), Stigmastan-3,5,22-trien (1.39%), 9,19-Cyclolanostan-3-ol, 24-methylene-, (3.beta.)- (1.28%), Benzo[h]quinoline, 2,4-dimethyl- (1.27%), Octadecane (1.26%), Cholest-5-ene, 3-methoxy-, (3.beta.)- (1.10%), Thymol (1.06%) and 9,12,15-Octadecatrien-1-ol, (Z,Z,Z)- (1.03%).

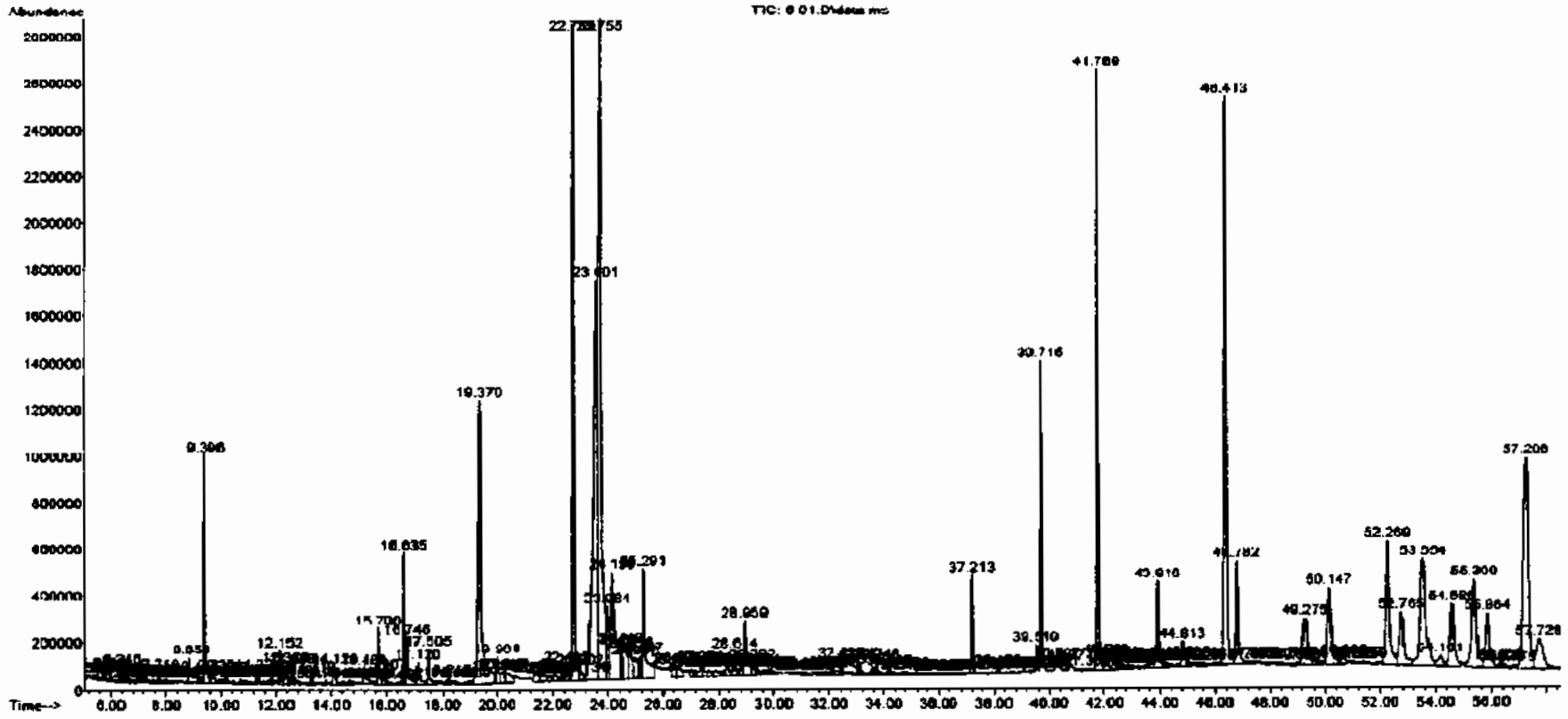
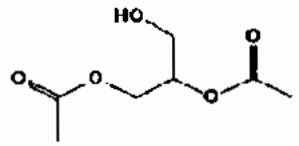
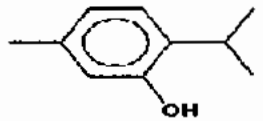
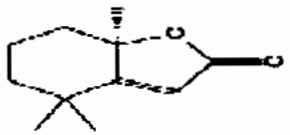
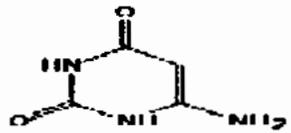
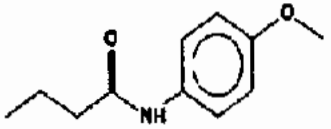
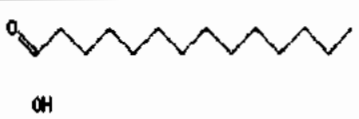
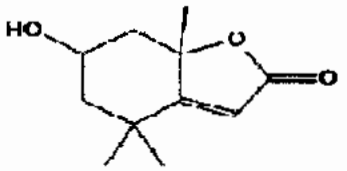
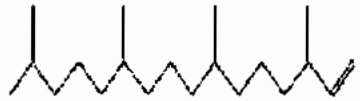
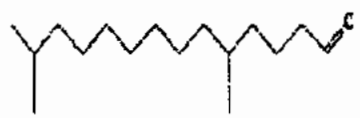
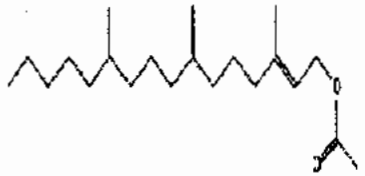
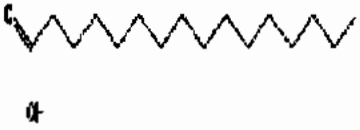
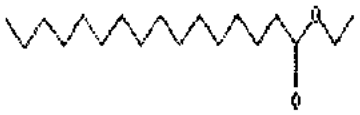
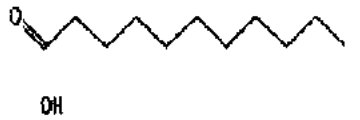
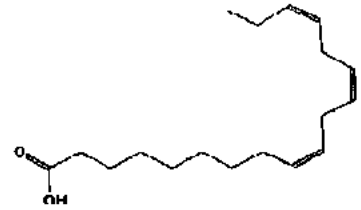


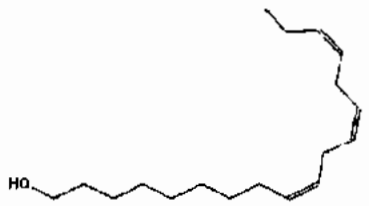
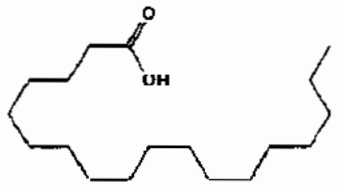
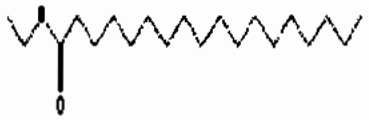
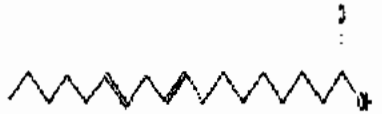
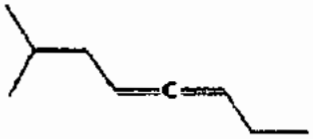
Figure 4.86: GC-MS chromatogram of ethyl acetate leaf extract of *Justicia adhatoda*

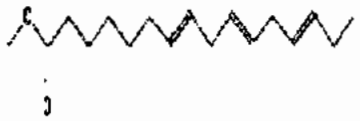
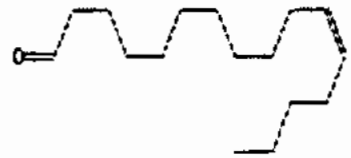
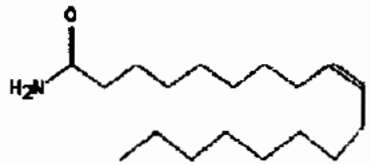
Table 4.31: Phytoconstituents identified in the ethyl acetate leaves extract of *Justicia adhatoda* by GC-MS

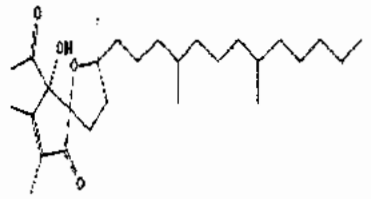
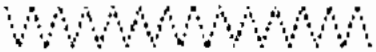
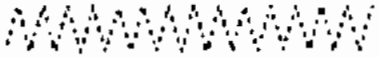
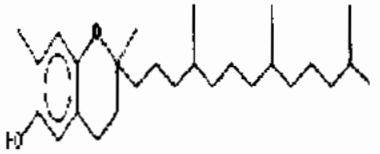
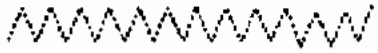
Sr. No.	RT	Compound	Molecular Formula	Molecular Weight	Peak Area %	Structure
1	8.859	Glycerol 1,2-diacetate	$C_7H_{12}O_5$	176	0.35	
2	9.396	Thymol	$C_{10}H_{14}O$	150	1.06	
3	12.152	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	$C_{11}H_{16}O_2$	180	0.21	
4	12.373	4-Amino-2,6-dihydroxypyrimidine	$C_4H_5N_3O_2$	127	0.20	
5	14.129	Butanamide, N-(4-methoxyphenyl)-	$C_{11}H_{15}NO_2$	193	0.24	
6	15.188	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	0.22	

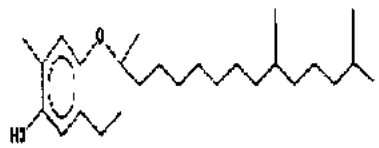
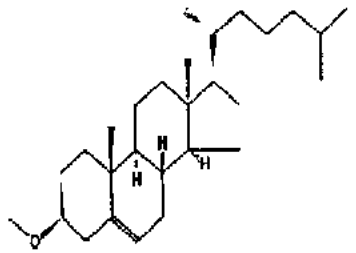
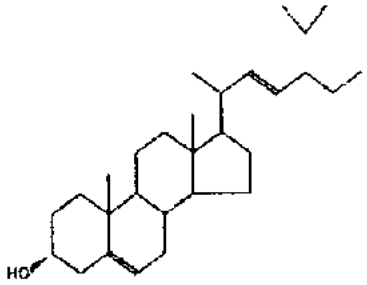
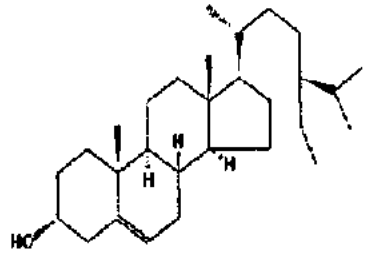
7	15.700	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	$C_{11}H_{16}O_3$	196	0.44	
8	16.635	Neophytadiene	$C_{20}H_{38}$	278	0.81	
9	16.746	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268	0.46	
10	17.120	Phytol, acetate	$C_{22}H_{42}O_2$	338	0.29	
11	17.505	9-Octadecyne	$C_{18}H_{34}$	250	0.36	
12	19.370	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	4.95	

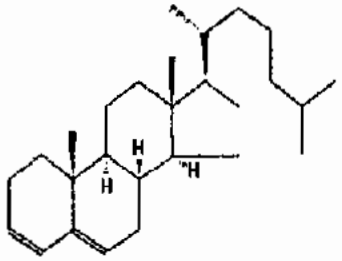
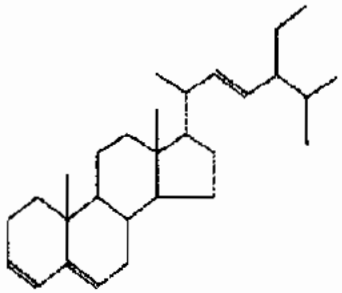
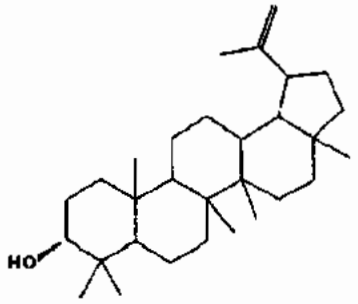
13	19.981	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	0.33	
14	20.146	Eicosane	$C_{20}H_{42}$	282	0.23	
15	20.310	Undecanoic acid	$C_{11}H_{22}O_2$	186	0.35	
16	22.759	Phytol	$C_{20}H_{40}O$	296	5.61	
17	23.601	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	9.41	
18	23.755	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	12.54	

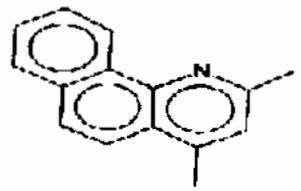
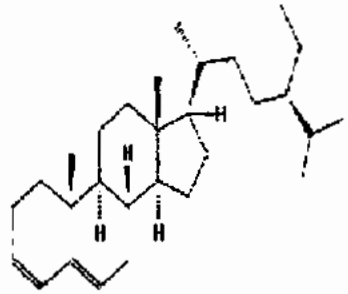
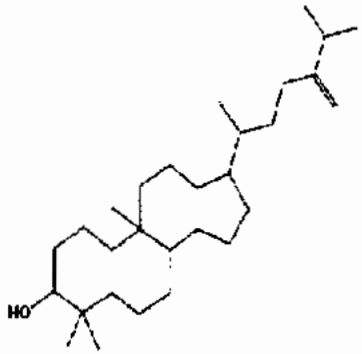
19	23.984	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	$C_{18}H_{32}O$	264	1.03	
20	24.159	Octadecanoic acid	$C_{18}H_{36}O_2$	284	2.42	
21	24.549	Cyclododecyne	$C_{12}H_{20}$	164	0.89	
22	24.814	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	0.53	
23	24.984	Linoelaidic acid	$C_{18}H_{32}O_2$	280	0.43	
24	25.186	3,4-Octadiene, 7-methyl-	C_9H_{16}	124	0.32	

25	25.291	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	1.64	
26	26.530	7,10,13-Hexadecatrienoic acid, methyl ester	$C_{17}H_{28}O_2$	264	0.34	
27	27.375	cis-11-Hexadecenal	$C_{16}H_{30}O$	238	0.26	
28	28.614	2(3H)-Furanone, dihydro-4-methyl-5-pentyl-	$C_{10}H_{18}O_2$	170	0.32	
29	28.959	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	281	0.79	
30	37.213	Tetracosane	$C_{24}H_{50}$	338	0.97	

31	39.715	Squalene	$C_{30}H_{50}$	410	2.79	
32	40.295	.alpha.-Tocospiro B	$C_{29}H_{50}O_4$	462	0.24	
33	41.789	Octacosane	$C_{28}H_{58}$	394	6.10	
34	43.918	Triacontane	$C_{30}H_{62}$	422	0.93	
35	44.813	.gamma.-Tocopherol	$C_{28}H_{48}O_2$	416	0.33	
36	46.413	Hexacosane	$C_{26}H_{54}$	366	8.17	

37	46.782	dl-.alpha.-Tocopherol	$C_{29}H_{50}O_2$	430	1.42	
38	49.275	Cholest-5-ene, 3-methoxy-, (3.beta.)-	$C_{28}H_{48}O$	400	1.10	
39	50.147	Stigmasterol	$C_{29}H_{48}O$	412	1.44	
40	52.269	.gamma.-Sitosterol	$C_{29}H_{50}O$	414	2.63	

41	52.765	Octadecane	$C_{18}H_{38}$	254	1.26	
42	53.554	Cholesta-3,5-diene	$C_{27}H_{44}$	368	3.48	
43	54.586	Stigmastan-3,5,22-trien	$C_{29}H_{46}$	394	1.39	
44	55.399	Lupeol	$C_{30}H_{50}O$	426	2.30	

45	55.864	Benzo[h]quinoline, 2,4-dimethyl-	$C_{15}H_{13}N$	207	1.27	
46	57.286	Stigmasta-3,5-diene	$C_{29}H_{48}$	396	5.19	
47	57.728	9,19-Cyclolanostan-3-ol, 24-methylene-, (3.beta.)-	$C_{31}H_{52}O$	440	1.28	

4.32. Gas chromatography-Mass spectrometry (GC-MS) analysis of methanolic leaves extract of *Ricinus communis*

The methanolic leaves extract of *Ricinus communis* was selected for GC-MS analysis. Upon GC-MS analysis, the extract contains 51 chemical constituents eluted between 5.37 and 55.38 minutes as shown in Figure 4.87. These compounds belong to different chemical classes and most of them are reported to exhibit important biological activities. The identified compounds with their peak number, retention time (RT), peak area (%), molecular formula and weight and structure are presented in Table 4.32. The major compounds identified were Lupeol (14.90%), 9,12,15-Octadecatrienoic acid, (Z,Z,Z) (12.55%), 1,4-Benzenediamine, N,N,N',N'-tetramethyl (12.21%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (4.03%), n-Hexadecanoic acid (3.82%), Nonane, 5-butyl- (3.43%), 1,2,3-Benzenetriol (2.95%), 5-Hydroxymethylfurfural (2.20%), cis-1-Ethyl-3-methyl-cyclohexane (2.16%), .beta.-Sitosterol (1.96%), N-(3-Chlorophenyl)-bis(2,2,3,3,3-pentafluoropropan)amide (1.88%), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (1.78%), 1,3,5-Benzenetriol (1.60%), 2-Methoxy-4-vinylphenol (1.46%), 1-Ethyl-3-methylcyclohexane (c,t) (1.36%), n-Decanoic acid (1.33%), 1,2,4-Benzenetriol (1.20%), 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one (1.18%), .alpha.-Tocospiro B (1.18%), Quinazoline, 4-methyl- (1.17%), Nonanoic acid (1.15%) and Benzenemethanol, 3-fluoro (0.99%).

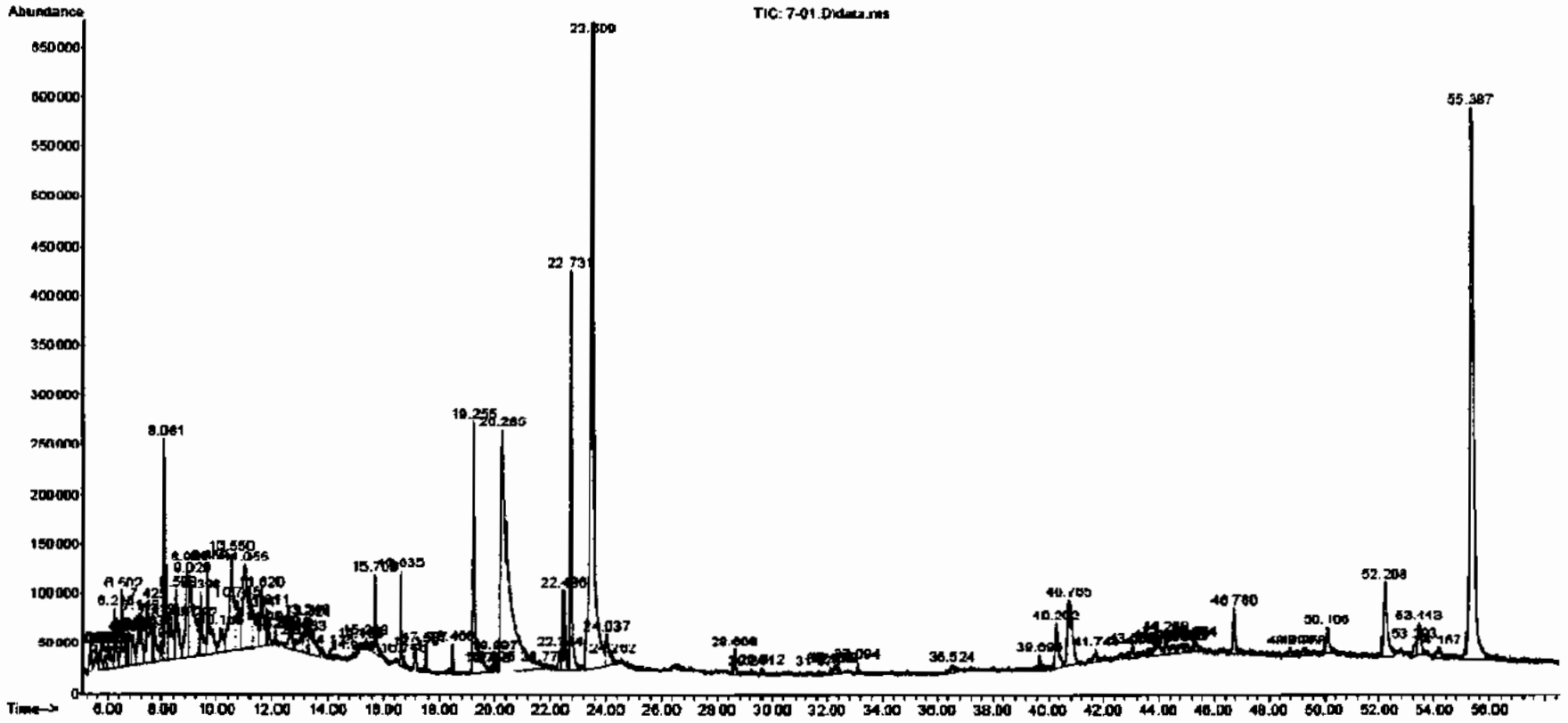
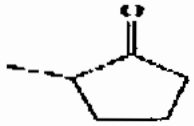
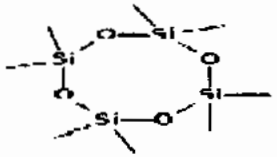
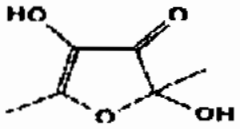
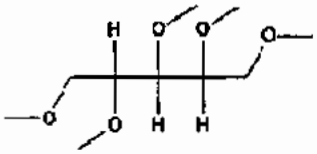
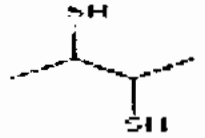
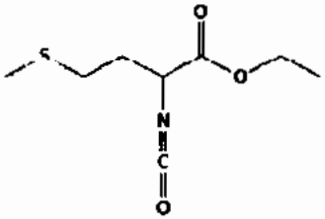
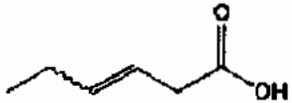
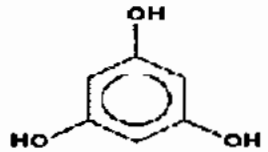
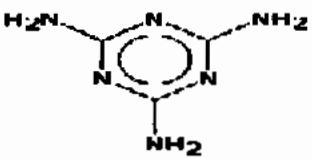
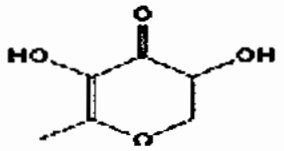
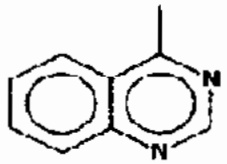
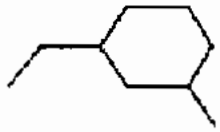
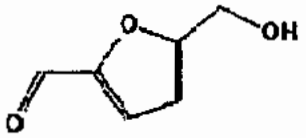
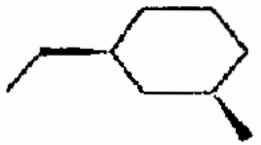
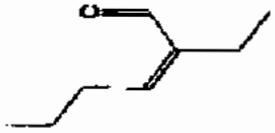


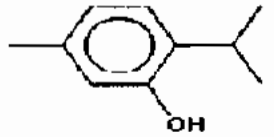
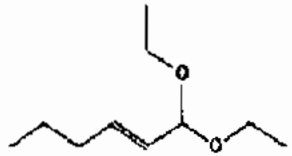
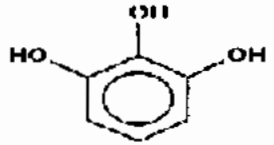
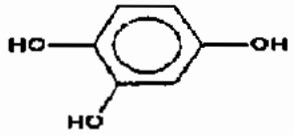
Figure 4.87: GC-MS chromatogram of methanolic leaf extract of *Ricinus communis*

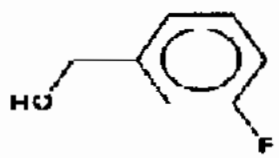
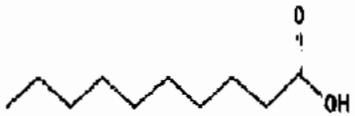
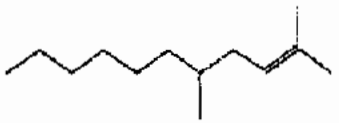
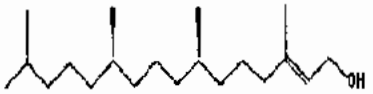
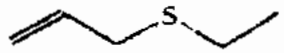
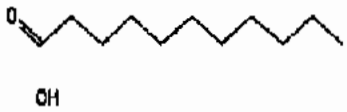
Table 4.32: Phytoconstituents identified in the methanol leaves extract of *Ricinus communis* by GC-MS

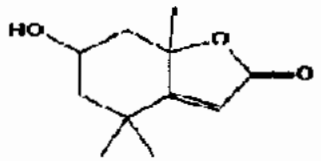
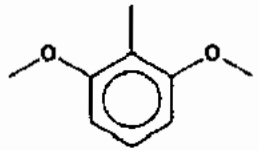
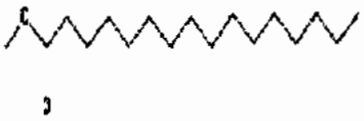
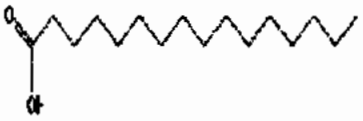
Sr. No.	RT	Compound	Molecular Formula	Molecular Weight	Peak Area %	Structure
1	5.371	Pyrazole, 1,4-dimethyl	$C_5H_8N_2$	96	0.35	
2	5.749	Bicyclo[4.2.0]octa-1,3,5-triene	C_8H_8	104	0.33	
3	6.027	Cyclopentanone, 2-methyl-	$C_6H_{10}O$	98	0.35	
4	6.216	Cyclotetrasiloxane, octamethyl-	$C_8H_{24}O_4Si_4$	296	0.76	
5	6.502	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	144	1.18	
6	6.658	Thiazole, 4,5-dihydro-2-methyl-	C_4H_7NS	101	0.29	

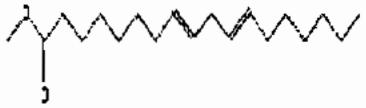
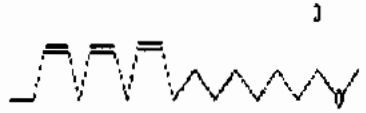
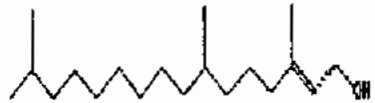
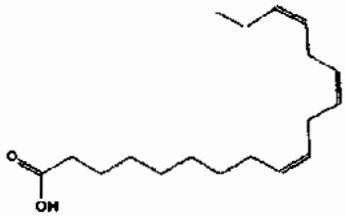
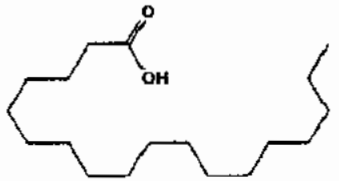
7	6.740	L-(-)-Arabitol, pentamethyl ether	$C_{10}H_{22}O_5$	222	0.31	
8	6.790	2,3-Butanedithiol	$C_4H_{10}S_2$	122	0.51	
9	6.942	Ethyl 2-isocyanato-4-methylthiobutyrate	$C_8H_{13}NO_3S$	203	0.52	
10	7.144	3-Hexenoic acid	$C_6H_{10}O_2$	114	0.88	
11	7.425	1,3,5-Benzenetriol	$C_6H_6O_3$	126	1.60	
12	7.711	1,3,5-Triazine-2,4,6-triamine	$C_3H_6N_6$	126	0.73	

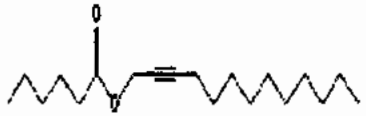
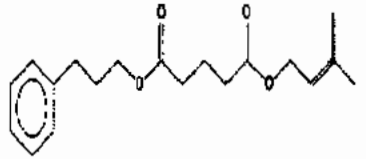
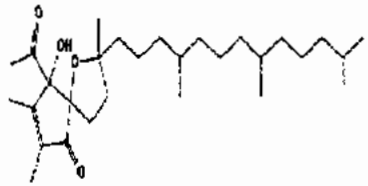
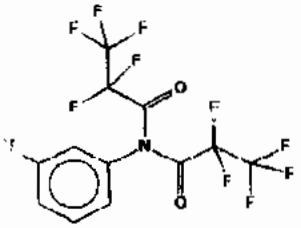
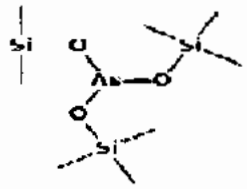
13	8.081	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	144	1.78	
14	8.203	Quinazoline, 4-methyl-	$C_9H_8N_2$	144	1.17	
15	8.506	1-Ethyl-3-methylcyclohexane (c,t)	C_9H_{18}	126	1.36	
16	8.923	5-Hydroxymethylfurfural	$C_6H_6O_3$	126	2.20	
17	9.029	cis-1-Ethyl-3-methyl-cyclohexane	C_9H_{18}	126	2.16	
18	9.292	2-Hexenal, 2-ethyl-	$C_8H_{14}O$	126	0.42	

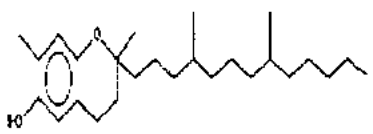
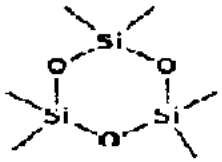
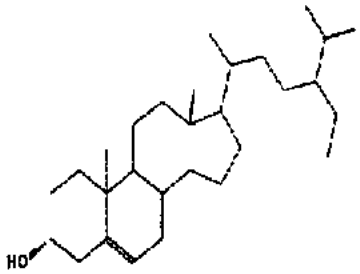
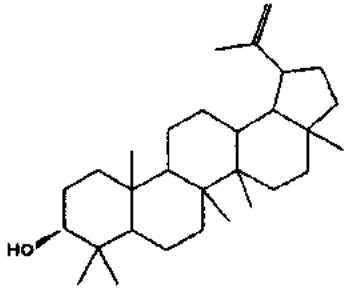
19	9.398	Thymol	$C_{10}H_{14}O$	150	0.92	
20	9.669	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	1.46	
21	10.165	2-Hexene, 1,1-diethoxy-	$C_{10}H_{20}O_2$	172	0.51	
22	10.550	1,2,3-Benzenetriol	$C_6H_6O_3$	126	2.95	
23	10.745	1,2,4-Benzenetriol	$C_6H_6O_3$	126	1.20	
24	11.055	Nonane, 5-butyl-	$C_{13}H_{28}$	184	3.43	

25	11.314	Benzenemethanol, 3-fluoro	C_7H_7FO	126	0.99	
26	11.620	n-Decanoic acid	$C_{10}H_{20}O_2$	172	1.33	
27	11.881	2-Undecene, 2,5-dimethyl-	$C_{13}H_{26}$	182	0.28	
28	12.613	Phytol	$C_{20}H_{40}O$	296	0.54	
29	13.130	3-Ethylthio-1-propene	$C_5H_{10}S$	102	0.61	
30	13.250	Undecanoic acid	$C_{11}H_{22}O_2$	186	0.55	

31	13.320	Nonanoic acid	$C_9H_{18}O_2$	158	1.15	
32	15.709	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	$C_{11}H_{16}O_3$	196	0.91	
33	16.635	Neophytadiene	$C_{20}H_{38}$	278	0.67	
34	17.181	2,6-Dimethoxytoluene	$C_9H_{12}O_2$	152	0.46	
35	18.466	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.31	
36	19.255	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	3.82	

37	20.285	1,4-Benzenediamine, N,N,N',N'-tetramethyl	$C_{10}H_{16}N_2$	164	12.21	
38	22.344	8,11-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	0.27	
39	22.486	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	$C_{19}H_{32}O_2$	292	0.92	
40	22.731	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	4.03	
41	23.509	9,12,15-Octadecatrienoic acid, (Z,Z,Z)	$C_{18}H_{30}O_2$	278	12.55	
42	24.037	Octadecanoic acid	$C_{18}H_{36}O_2$	284	0.71	

43	28.608	Hexanoic acid, tridec-2-ynyl ester	$C_{19}H_{34}O_2$	294	0.40	
44	32.277	Glutaric acid, 3-methylbut-2-en-1-yl 3-phenylpropyl ester	$C_{19}H_{26}O_4$	318	0.28	
45	40.292	.alpha.-Tocospiro B	$C_{29}H_{50}O_4$	462	1.18	
46	40.765	N-(3-Chlorophenyl)-bis(2,2,3,3,3-pentafluoropropan)amide	$C_{12}H_4ClF_{10}NO_2$	419	1.88	
47	44.268	Arsenous acid, tris(trimethylsilyl) ester	$C_9H_{27}AsO_3Si_3$	342	0.33	

48	46.760	dl-.alpha.-Tocopherol	$C_{29}H_{50}O_2$	430	0.46	
49	50.106	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si_3$	222	0.52	
50	52.208	.beta.-Sitosterol	$C_{29}H_{50}O$	414	1.96	
51	55.387	Lupeol	$C_{30}H_{50}O$	426	14.90	

4.33. Gas chromatography-Mass spectrometry (GC-MS) analysis of ethanolic leaves extract of *Ricinus communis*

The ethanolic leaves extract of *Ricinus communis* was selected for GC-MS analysis. Upon GC-MS analysis, the extract contains 39 chemical constituents eluted between 5.69 and 55.39 minutes as shown in Figure 4.88. These compounds belong to different chemical classes and most of them are reported to exhibit important biological activities. The identified compounds with their peak number, retention time (RT), peak area (%), molecular formula and weight and structure are presented in Table 4.33. The major compounds identified were Lupeol (19.64%), Phytol (15.69%), 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (15.40%), dl-.alpha.-Tocopherol (9.08%), Hexadecanoic acid, ethyl ester (7.97%), n-Hexadecanoic acid (3.91%), .beta.-Sitosterol (2.63%), Hexacosane (2.32%), 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)- (1.81%), 2-Diisopropylsilyloxybut-3-yne (1.33%), 6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a tetrahydrobenzofuran-2(4H)-one (1.12%) and 4-Methylheptane-3,5-dione (1.02%).

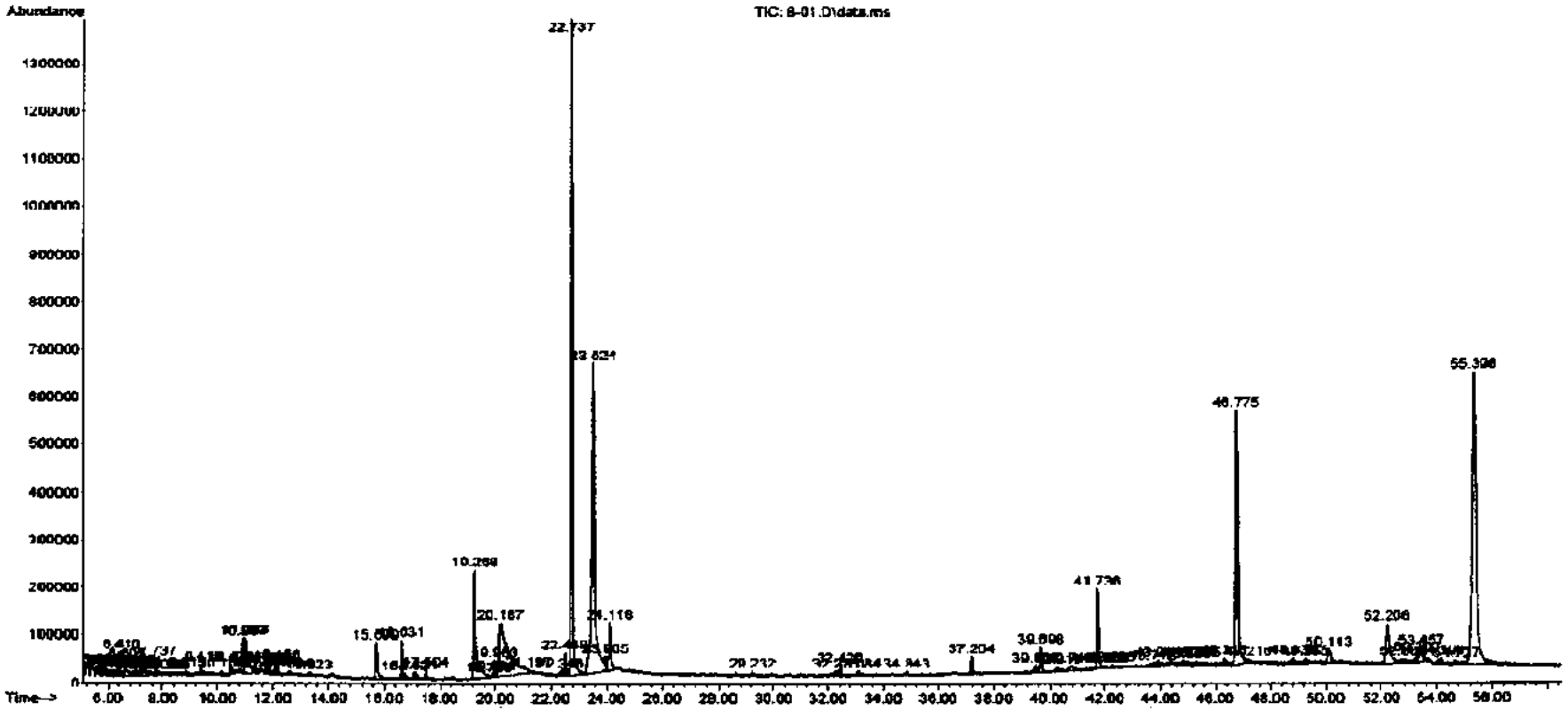
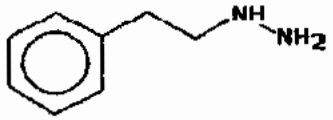
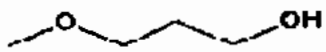
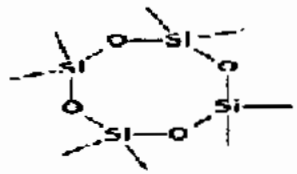
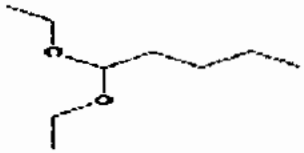
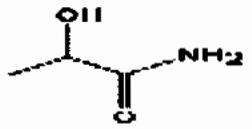
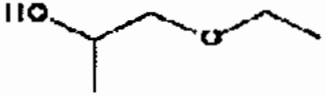
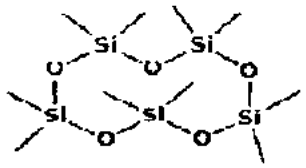
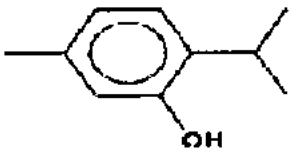
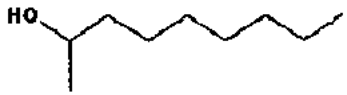
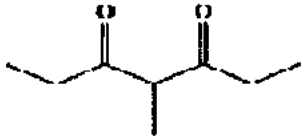
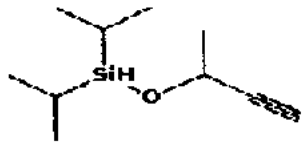
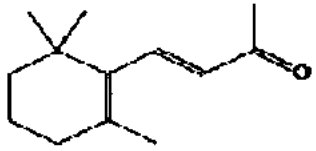
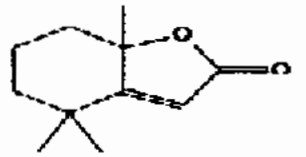
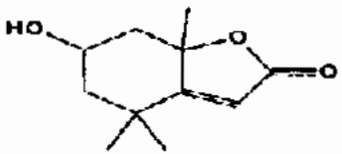
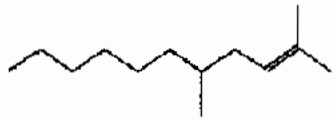
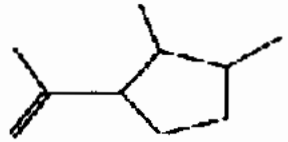
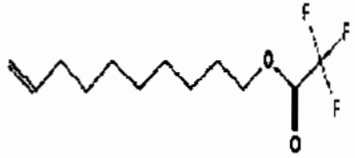


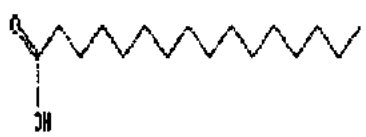
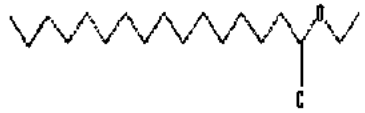
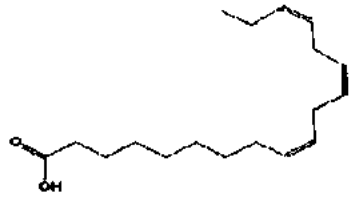
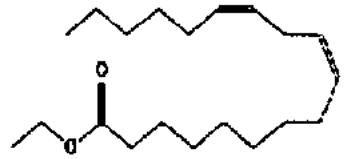
Figure 4.88: GC-MS chromatogram of ethanolic leaf extract of *Ricinus communis*

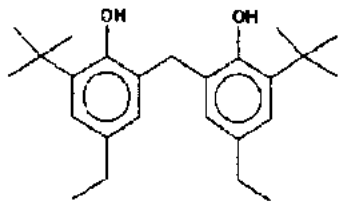
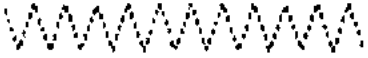
Table 4.33: Phytoconstituents identified in the ethanol leaves extract of *Ricinus communis* by GC-MS

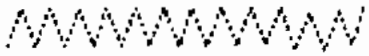
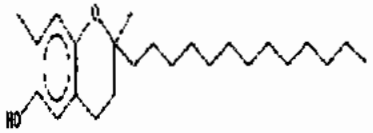
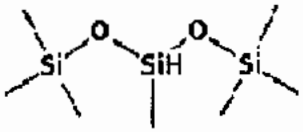
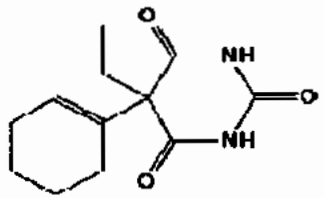
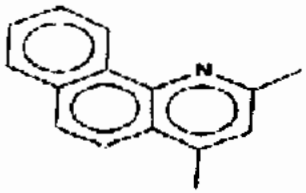
Sr. No.	RT	Compound	Molecular Formula	Molecular Weight	Peak Area %	Structure
1	5.692	Phenelzine	$C_8H_{12}N_2$	136	0.50	
2	5.920	Trimethylene glycol monomethyl ether	$C_4H_{10}O_2$	90	0.35	
3	6.410	Cyclotetrasiloxane, octamethyl-	$C_8H_{24}O_4Si_4$	296	0.33	
4	6.605	Pentane, 1,1-diethoxy-	$C_9H_{20}O_2$	160	0.56	
5	7.030	Propanamide, 2-hydroxy-	$C_3H_7NO_2$	89	0.40	
6	7.251	2-Propanol, 1-ethoxy-	$C_5H_{12}O_2$	104	0.41	

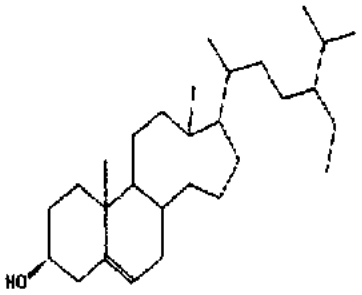
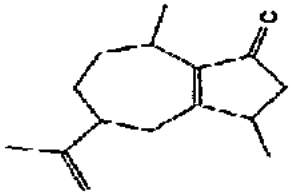
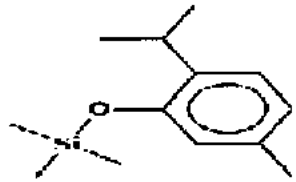
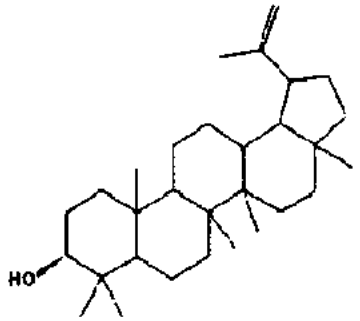
7	7.737	Cyclopentasiloxane, decamethyl-	$C_{10}H_{30}O_5Si_5$	370	0.29	
8	9.415	Thymol	$C_{10}H_{14}O$	150	0.34	
9	10.481	2-Nonanol	$C_9H_{20}O$	144	0.30	
10	10.937	4-Methylheptane-3,5-dione	$C_8H_{14}O_2$	142	1.02	
11	11.003	2-Diisopropylsilyloxybut-3-yne	$C_{10}H_{20}OSi$	184	1.33	
12	11.370	3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	$C_{13}H_{20}O$	192	0.29	

13	12.156	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	$C_{11}H_{16}O_2$	180	0.30	
14	15.699	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	$C_{11}H_{16}O_3$	196	1.12	
15	16.631	Neophytadiene	$C_{20}H_{38}$	278	0.73	
16	16.742	2-Undecene, 2,5-dimethyl-	$C_{13}H_{26}$	182	0.23	
17	17.124	Cyclopentane, 1,2-dimethyl-3-(1-methylethenyl)-	$C_{10}H_{18}$	138	0.28	
18	17.504	9-Decen-1-ol, trifluoroacetate	$C_{12}H_{19}F_3O_2$	252	0.27	

19	19.268	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	3.91	
20	20.187	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	7.97	
21	22.488	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	$C_{18}H_{32}O$	264	0.64	
22	22.737	Phytol	$C_{20}H_{40}O$	296	15.69	
23	23.524	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	15.40	
24	23.965	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308	0.40	

25	24.118	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306	1.81	
26	32.277	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-ethyl-	$C_{25}H_{36}O_2$	368	0.27	
27	32.426	Eicosane	$C_{20}H_{42}$	282	0.30	
28	37.204	Heptacosane	$C_{27}H_{56}$	380	0.67	
29	39.500	Tetracosane	$C_{24}H_{50}$	338	0.40	
30	39.698	Squalene	$C_{30}H_{50}$	410	0.80	

31	41.736	Hexacosane	$C_{26}H_{54}$	366	2.32	
32	46.775	dl-.alpha.-Tocopherol	$C_{29}H_{50}O_2$	430	9.08	
33	48.837	1,1,1,3,5,5,5-Heptamethyltrisiloxane	$C_7H_{22}O_2Si_3$	222	0.33	
34	49.266	Cyclobarbital	$C_{12}H_{16}N_2O_3$	236	0.37	
35	50.113	Benzo[h]quinoline, 2,4-dimethyl-	$C_{15}H_{13}N$	207	0.82	

36	52.208	.beta.-Sitosterol	$C_{29}H_{50}O$	414	2.63	
37	53.457	(3S,5R,8S)-3,8-Dimethyl-5-(prop-1-en-2-yl)-2,3,5,6,7,8-hexahydroazulen-1(4H)-one	$C_{15}H_{22}O$	218	0.78	
38	54.190	Thymol, TMS derivative	$C_{13}H_{22}OSi$	222	0.36	
39	55.396	Lupeol	$C_{30}H_{50}O$	426	19.64	

4.34. Gas chromatography-Mass spectrometry (GC-MS) analysis of ethyl acetate leaves extract of *Ricinus communis*

The ethyl acetate leaves extract of *Ricinus communis* was selected for GC-MS analysis. Upon GC-MS analysis, the extract contains 39 chemical constituents eluted between 9.40 and 57.19 minutes as shown in Figure 4.89. These compounds belong to different chemical classes and most of them are reported to exhibit important biological activities. The identified compounds with their peak number, retention time (RT), peak area (%), molecular formula and weight and structure are presented in Table 4.34. The major compounds identified were 9,12-Octadecadienoic acid, methyl ester (17.19%), Lupeol (13.85%), 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (11.92%), dl-.alpha.-Tocopherol (7.64%), Phytol (7.21%), n-Hexadecanoic acid (4.39%), Heptacosane (3.14%), .beta.-Sitosterol (2.83%), 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)- (1.24%), Stigmasterol (1.10%), Octacosane (1.09%), Lup-20(29)-en-3-one (1.06%) and Phytol, acetate (1.03%).

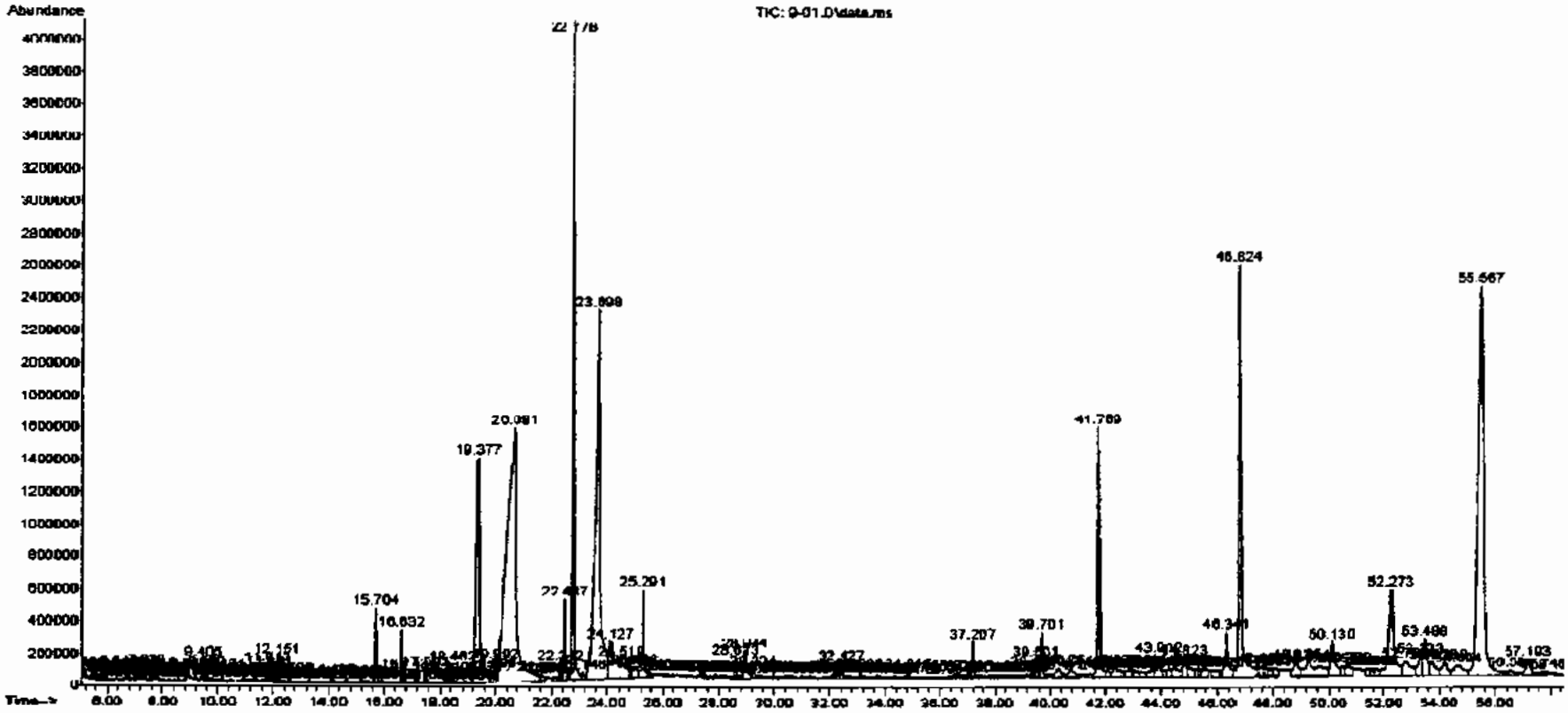
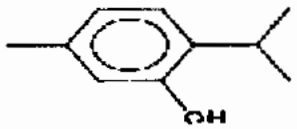
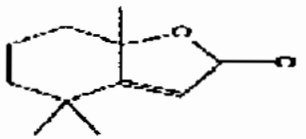
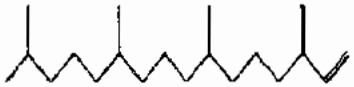
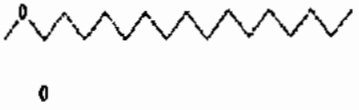
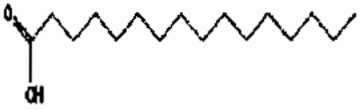
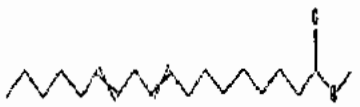
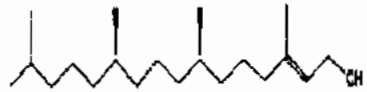
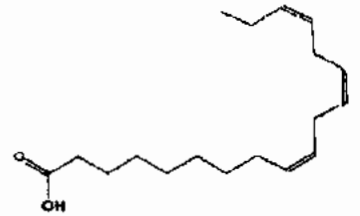
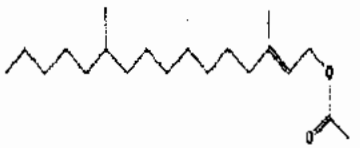
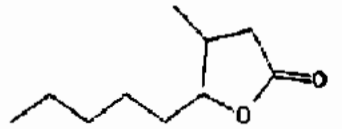
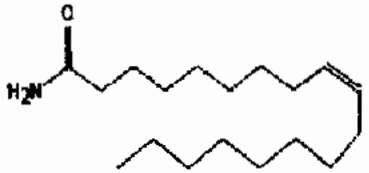
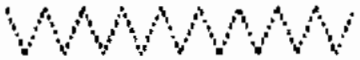
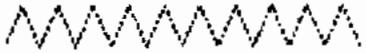


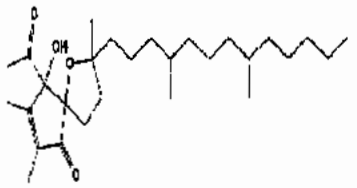
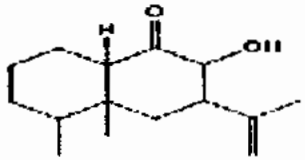
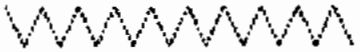
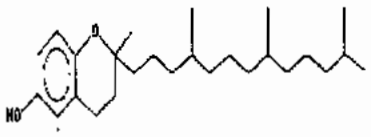
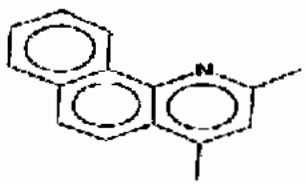
Figure 4.89: GC-MS chromatogram of ethyl acetate leaf extract of *Ricinus communis*

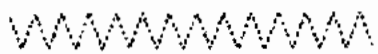
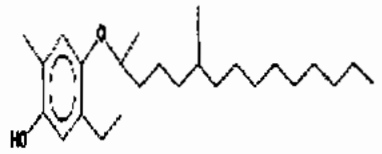
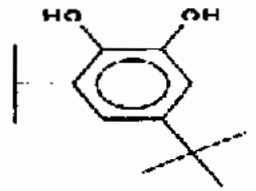
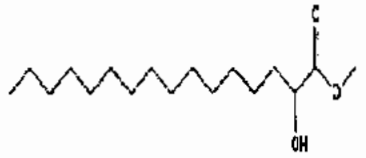
Table 4.34: Phytoconstituents identified in the ethyl acetate leaves extract of *Ricinus communis* by GC-MS

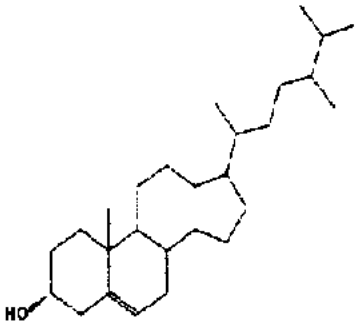
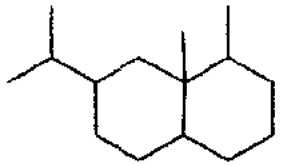
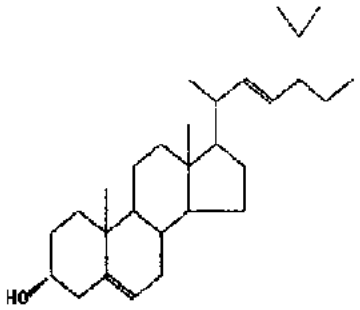
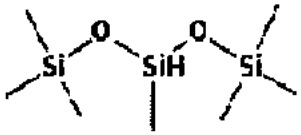
Sr. No.	RT	Compound	Molecular Formula	Molecular Weight	Peak Area %	Structure
1	9.402	Thymol	$C_{10}H_{14}O$	150	0.19	
2	12.152	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	$C_{11}H_{16}O_2$	180	0.23	
3	15.704	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	$C_{11}H_{16}O_3$	196	0.71	
4	16.632	Neophytadiene	$C_{20}H_{38}$	278	0.41	
5	18.461	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.19	
6	19.376	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	4.39	

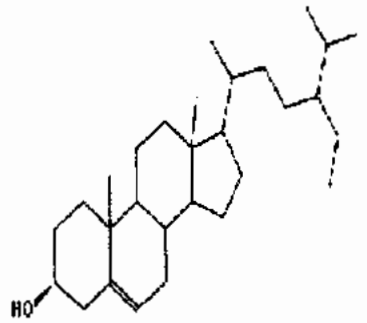
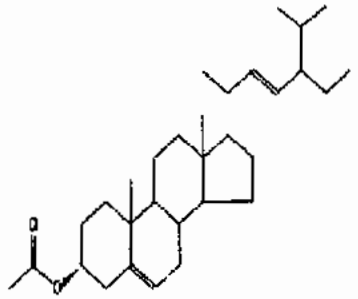
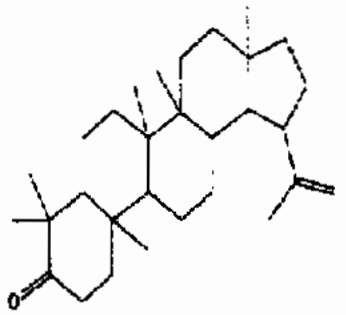
7	20.688	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	17.19	
8	22.486	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292	0.88	
9	22.776	Phytol	$C_{20}H_{40}O$	296	7.21	
10	23.697	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	11.92	
11	24.126	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306	1.24	
12	24.517	1,6-Cyclodecadiene	$C_{10}H_{16}$	136	0.61	

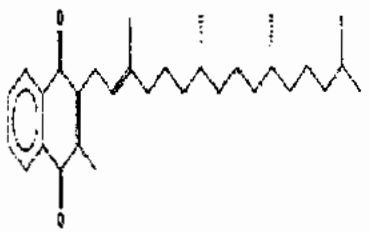
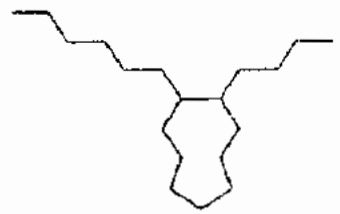
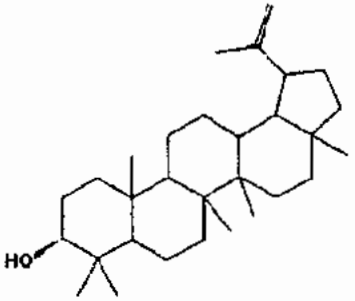
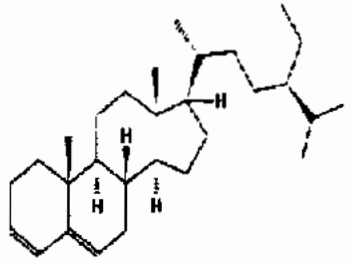
13	25.293	Phytol, acetate	$C_{22}H_{42}O_2$	338	1.03	
14	28.612	2(3H)-Furanone, dihydro-4-methyl-5-pentyl-	$C_{10}H_{18}O_2$	170	0.22	
15	28.946	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	281	0.42	
16	32.428	Nonadecane	$C_{19}H_{40}$	268	0.20	
17	39.500	Heneicosane	$C_{21}H_{44}$	296	0.24	
18	39.702	Squalene	$C_{30}H_{50}$	410	0.58	

19	40.750	.alpha.-Tocospiro A	$C_{29}H_{50}O_4$	462	0.40	
20	41.722	Heptacosane	$C_{27}H_{56}$	380	3.14	
21	42.377	(2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl)octahydronaphthalen-1(2H)-one	$C_{15}H_{24}O_2$	236	0.38	
22	43.910	Eicosane	$C_{20}H_{42}$	282	0.49	
23	44.825	.beta.-Tocopherol	$C_{28}H_{48}O_2$	416	0.44	
24	45.973	Benzo[h]quinoline, 2,4-dimethyl-	$C_{15}H_{13}N$	207	0.27	

25	46.339	Octacosane	$C_{28}H_{58}$	394	1.09	
26	46.825	dl-.alpha.-Tocopherol	$C_{29}H_{50}O_2$	430	7.64	
27	47.834	1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O_2$	222	0.22	
28	48.831	Hexadecanoic acid, 2-hydroxy-, methyl ester	$C_{17}H_{34}O_3$	286	0.57	

29	49.254	Campesterol	$C_{28}H_{48}O$	400	0.77	
30	49.664	Naphthalene, decahydro-1,8a-dimethyl-7-(1-methylethyl)-, [1R-(1.alpha.,4a.beta.,7.beta.,8a.alpha.)]-	$C_{15}H_{28}$	208	0.62	
31	50.130	Stigmasterol	$C_{29}H_{48}O$	412	1.10	
32	50.711	1,1,1,3,5,5,5-Heptamethyltrisiloxane	$C_7H_{22}O_2Si_3$	222	0.36	

33	52.275	.beta.-Sitosterol	$C_{29}H_{50}O$	414	2.83	
34	52.793	Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)-	$C_{31}H_{50}O_2$	454	0.80	
35	53.487	Lup-20(29)-en-3-one	$C_{30}H_{48}O$	424	1.06	

36	53.752	Phytonadione	$C_{31}H_{46}O_2$	450	0.49	
37	54.692	1H-Indene, 5-butyl-6-hexyloctahydro-	$C_{19}H_{36}$	264	0.64	
38	55.569	Lupeol	$C_{30}H_{50}O$	426	13.85	
39	57.190	Stigmasta-3,5-diene	$C_{29}H_{48}$	396	0.50	

CHAPTER FIVE

DISCUSSION

Natural products including plants, microorganisms, marine products and animals have served as an incessant reservoir of therapeutic moieties. Enhanced pathogenic resistance, advent of complex anomalies, irrational treatment approaches and emergence of adverse effects of synthetic drugs has caused a dire requirement to explore new resources to rationalize therapeutic mainstream. Natural products are potential candidates for exploring their pharmacological potential. Undoubtedly, these have paved the path for the future treatment approaches. The bioactive molecules derived from the natural products especially plants have served as the basis of new drug discovery. Plants have fulfilled both the nutritive and curative requirements of earthlings. Plants are a source of abundant molecules possessing biological activities (Cragg *et al.*, 2012). Plants have provided a foundation for the traditional systems of medicines which came into existence several years ago and are still used as remedies. Conferring to World Health Organization survey report, about 80% of the population of world still trusts on non-conventional medicines, particularly medicinal floras for their fitness issues (Bora and Sharma, 2011).

The extended past of the herbal medications is still present in many civilizations including China, India, Arab, North Africa, Southeast Asia and America (Central, South and North) (Zhang, 2000; Tapsell *et al.*, 2006). Recently, exploration on natural products have recaptured attention due to advances in screening programs along with growing understanding of their natural implication, identification of their sources and structural assortments (Conforti *et al.*, 2012). About 50-60% of all drugs at present available for the remedial purpose in clinical world are either natural products, natural products-derived compounds, contain active natural products-derived pharmacophores or modified natural products attached to targeting systems due to their extraordinary effectiveness and minor side effects. These natural products are desirable replacements for chemical therapeutics which have various severe adverse effects (Patwardhan *et al.*, 2004).

5.1. Phytochemical analysis

To identify the bioactive and novel compounds in herbal medicine research, phytochemical screening is the first step (Starmans and Nijhois, 1996; Jones and Kinghorn, 2012). Phytochemical screening is a qualitative chemical evaluation of the plant drug which indicates the presence of chemical constituents. The chemical evaluation of the plant drug leads to proper identification of biologically active groups (Gokhale and Kokate, 1997).

Ours study demonstrates that, different crude extracts of *Melia azedarach* contain the alkaloids, saponins, tannins, terpenoids, phenols, flavonoids, steroids and anthraquinone. Our results were in accordance with the previously published data. *Melia azedarach* extract contains terpenoids, flavonoids, phenols, glycosides, alkaloids, tannins, saponins and steroids (Ahmed *et al.*, 2012a). *Melia azedarach* extract contains tannins, saponins, alkaloids, Phenols, terpenoids, flavonoids glycosides and steroids (Rao *et al.*, 2012). Variety of compounds detected in *Melia azedarach* including phytosterols, diterpene, triterpenes, Terpene alcohol, flavonoid, alkane hydrocarbon, nalkanoic acid and vitamin E (Sen and Batra, 2012b). *Melia azedarach* extract contains saponins, phenols, alkaloids, tannins and flavonoids (Sultana *et al.*, 2013). *Melia azedarach* extract contains tannins, saponins, flavonoids, alkaloids and glycosides (Hossain *et al.*, 2013).

Melia azedarach extract contains alkaloids, saponins, tannins, flavonoids, terpins and steroids (Suad and Majeed, 2013). *Melia azedarach* extract contains saponins, tannins, alkaloids, flavonoids and glycosides (Asadujjaman *et al.*, 2013). Aqueous and ethanolic extracts of *Melia azedarach* contain the flavanoids, reducing sugars, alkaloids and carbohydrates (Krishnaiah and Prashanth, 2014). *Melia azedarach* extract contains tannins, phylobatannins, saponins, polyphenols, flavonoids, steroids, alkaloids, carbohydrates, glycosides, terpenoids, triterpenoids and proteins (Leela *et al.*, 2016). *Melia azedarach* extract contains phenols, alkaloids, tannins, saponins, cardioactive glycosides, phenolic glycosides, anthraquninone glycosides, cynogenic glycosides and flavonoids (Abbas *et al.*, 2017). Phytoconstituents like saponins and tannins, carbohydrates, flavonoids, proteins etc were present in *Melia azedarach* (Babu *et al.*, 2018).

Ours study demonstrates that, phytochemicals detected in the different crude extracts of *Justicia adhatoda* were alkaloids, saponins, tannins, terpenoids, phenols, flavonoids, steroids, anthraquinone and coumarins. Our results were following the same line as revealed by other researchers. Leaves of *Adhatoda vasica* possess tannins, oils, fats, phytosterol, carbohydrate, alkaloids, flavanoids, saponins, phenolic compounds and proteins (Vankata *et al.*, 2013). Leaves of *Adhatoda vasica* possess saponins, flavanoids, amino acids, phenols, tannins, alkaloids, anthraquninone and reducing sugars (Karthikeyan *et al.*, 2014).

Justicia adhatoda extract contains alkaloids, glycosides, steroidal/triterpenes, resin and saponins (Abhishek *et al.*, 2014). *Justicia adhatoda* ethanolic extract contains saponins, phenols,

steroids alkaloids, flavonoids and terpenoids (Bajpaia *et al.*, 2015). *Justicia adhatoda* extract contains saponins, phytosterols, triterpenoids, alkaloids, anthraquninone, flavonoids and polyphenols (Jayapriya and Shoba, 2015). *Justicia adhatoda* extract contains anthraquninone, saponins, flavonoids, phenols, tannins, alkaloids, amino acids and reducing sugars (Desai and Patel, 2015).

Ours study demonstrates that, phytochemicals detected in different crude extracts of *Ricinus communis* were alkaloids, saponins, tannins, terpenoids, phenols, flavonoids, steroids and anthraquinone. Our results were in accordance with the previously published data. The *Ricinus communis* extract contains the proteins, carbohydrates, tannins, phenols, flavonoids and saponins (Yadav and Agarwala, 2011). *Ricinus communis* extract contains proteins, tannins, carbohydrates, alkaloids and phenols (Khursheed *et al.*, 2012; Shabir *et al.*, 2011). *Ricinus communis* extract contains phytate, oxalate, saponins, cynogenic glycoside, tannin, phenol, alkaloid and flavonoid (Momoh *et al.*, 2012). *Ricinus communis* contains a variety of phytochemicals like terpenoids, glycosides, reducing sugars, saponins, tannins, flavonoids and phenols (Minakshi *et al.*, 2016). *Ricinus communis* seeds contain phytochemicals like phytate, tannins, saponins, flavonoids, alkaloid, phenol and steroids (Ezenobi *et al.*, 2016).

Alkaloids possess antibacterial and anticancer activities (Wirasathien *et al.*, 2006). Saponins have fungicidal and antibiotic activities (Sparg *et al.*, 2004). Anthraquinones possess antibacterial and antifungal activities. Terpenoids have antibacterial properties (Kanokmedhakul *et al.*, 2005). Flavonoids possess antibacterial and antioxidant activities (Miller, 1996). Phenolics possess diverse biological activities (Silva and Junior, 2010), antibacterial (Kubo *et al.*, 2004), antitumor (You *et al.*, 2010), antiviral (Uozaki *et al.*, 2007) and antioxidant (Kumar *et al.*, 2013a).

Phenols and flavonoids possess anti-carcinogenic and radical scavenging properties. Tannins possess antiviral activity (Lin *et al.*, 2004). Tannins have antibacterial properties (Akiyama *et al.*, 2001). Tannins possess antioxidant activity (Yokozawa *et al.*, 1998). Terpenoids represent another important class of secondary metabolites with many biological characteristics e.g. anticancer, antioxidant and antimicrobial characteristics (Omoyeni *et al.*, 2014). Coumarins have antioxidant and anti-carcinogenic properties (Guevara, 2005).

5.2. Antioxidant activity

Regarding antioxidant profile determination, DPPH assay is considered one of the best ways to gauge the quenching strength of the plant that is mandatory for radical neutralization. DPPH is a stable radical but in the presence of antioxidant it can get reduced by antioxidant's electron donation (Brand-Williams *et al.*, 1995). A purple colored bleaching solution of DPPH serves as a significant source of radical donor. It is frequently used to govern the electron donating capabilities of the plant (Nunes *et al.*, 2012). Phosphomolybdenum assay is a quantitative method to evaluate the total antioxidant capacity of the plant extracts. This method was employed on the principal of formation Phosphate/Mo (V) complex (green color) when Mo (VI) reduced by then antioxidants present in extracts to Mo (V) (Kumar *et al.*, 2014b).

Reducing power assessment is also very important parameter to gauge the antioxidant prospective of the extracts. Plant antioxidants have tendency to convert the ferric cyanide complex to ferrous form by contributing their one electron with the turning of the yellow colored solution to green. Reducing is actually responsible for antioxidant activity by bestowing hydrogen atom that will halt the fee radical chain mechanism (Gordon, 1990).

Ours study demonstrates that, MA-EAE showed the maximum quantity of TPC. Flavonoids were found to be rich in MA-EAE. The MA-EE exhibited a maximum scavenging activity. MA-EAE has the maximum total antioxidant capacity. MA-EAE has the maximum total reducing power. Our results were in accordance with the previously published data. *Melia azedarach* ethanolic leaves extract exhibited the great antioxidant activity than the other extracts. The reason is that they have the highest amount of phenolic compounds. Due to the hydroxyl groups present in the phenolic compounds, they have high scavenging property (Ahmed *et al.*, 2012b). Using DPPH assay, extract showed the free radical scavenging properties. Extract displayed free radical scavenging activity in quantitative assay (Hossain *et al.*, 2013).

Ethanolic leaves extract have antioxidant and antibacterial activities (Asadujjaman *et al.*, 2013). *Melia azedarach* extract possess significant inhibition of radical scavenging assays. So during oxidative stress *Melia azedarach* extract developed effective antioxidant (Marimuthu *et al.*, 2013). Ethanolic extract of *Melia azedarach* flowers showed antioxidant activity (Abbas *et al.*, 2017). *Melia azedarach* is an excellent source of natural antioxidants. *Melia azedarach* could be used for pharmaceutical and the cosmeceutical applications (Rabbet *et al.*, 2017). *Melia azedarach* leaves extract have strong antioxidant potential (Babu *et al.*, 2018).

Ours study demonstrates that, JA-EE showed the maximum quantity of TPC. Flavonoids were found to be rich in JA-EE. The JA-ME exhibited a maximum scavenging activity. JA-EE has the maximum total antioxidant capacity. JA-EAE has the maximum total reducing power. Our results were following the same line as revealed by other researchers. From ethanolic extract of *Adhatoda vasica Nees*, Vasicine was isolated. Vasicine exhibited significant DPPH inhibition activity and also indicated in the FRAP assay (Shahwar *et al.*, 2012). Antioxidant activity of *Adhatoda vasica* showed high antioxidant activity in various antioxidant experiments. The presence of high levels of polyphenol compounds was the reason behind the antioxidant activity of the plant (Vankata *et al.*, 2013). *Justicia adhatoda* can use as potential the source of natural antioxidants (Bajpaia *et al.*, 2015).

Ours study demonstrates that, RC-ME showed the maximum quantity of TPC. Flavonoids were found to be rich in RC-ME. The RC-ME exhibited a maximum scavenging activity. RC-ME has the maximum total antioxidant capacity. RC-ME has the maximum total reducing power. Our results were in accordance with the previously published data. The *Ricinus communis* leaves extract showed the maximum antioxidant properties. The identified compound can serve as antioxidant compound to reduce the oxidative stress (Jolly and Shetye, 2017).The biological properties of phenolic compounds include antiatherosclerosis, anti-inflammation, endothelial function improvement, anticancer, anti-apoptosis, cardiovascular protection, cell proliferation and angiogenesis inhibition activities. The medicinal plants rich in phenolic compounds have antioxidant properties in many studies (Mandal *et al.*, 2013). The natural antioxidants derived from the plants are mostly in the form of phenolic compounds (Ali *et al.*, 2008).

Flavonoids produced by plants in reaction to microbial infection are hydroxylated phenolic compounds (Marjorie, 1996). Flavonoids show anticancer activities and are effective antioxidants (Akinmoladun *et al.*, 2007). Polyphenols or phenolics are secondary metabolites which are generally found in plant and their products. A great antioxidant potential has been revealed by most of the phenolics (Razali *et al.*, 2008). Different polarity solvents are used to extract antioxidant substances of different chemical structures. It has been found in several studies that high concentrations of phenolics are present in the extracts obtained using polar solvents (Canadanovic- Brunet *et al.*, 2008).

At present it is an accepted notion that reducing efficiency is affiliated with antioxidant potential and it is governed by phenolic residents in numerous natural sources. The reducing capability of antioxidants has been credited to countless cause's for instance radical scavenging, hindrance of chain reaction initiation, disintegration of peroxides (Brand-Williams *et al.*, 1995). The free radical scavenging activity has been reported in the plants having antioxidant potential (Das and Pereira, 1990). Free radicals mainly contribute to a number of clinical diseases for instance diabetes mellitus, renal failure, liver diseases, cancer and degenerative disorders due to poor defense mechanism by natural antioxidants (Parr and Bolwell, 2000).

5.3. Cytotoxicity assay

Toxicity is pharmacology at lower doses that is why medicinal plant extracts are tested for cytotoxicity. A simple, fast and economical bioassay to test bioactivity of plant extracts is brine shrimp lethality assay which mostly associates well with antitumor and cytotoxic properties (McLauglin *et al.*, 1993). Brine shrimps have been known to be utilized in different applications including the analyses of mycotoxins, stream pollutants, pesticidal residues, morphine-like compounds, dinoflagellate toxins, anesthetics, carcinogenicity of toxicants and phorbol esters in marine environment. This bioassay has led to the isolation of numerous pesticidal and antitumor compounds from natural products (Meyer *et al.*, 1982; McLaughlin *et al.*, 1991; Sam, 1993). The differences in quantity and type of cytotoxic substances present in the extracts including flavonoids, tannins and terpenoids cause variations in brine shrimp assay results.

The brine shrimp cytotoxicity assay was considered as a suitable probe for preliminary evaluation of toxicity, pesticides, heavy metals, detection of fungal toxins and cytotoxicity testing of dental materials (Meyer *et al.*, 1982). It can also be extrapolated for antitumor activity and cell-line toxicity (Selvin *et al.*, 2004). The natural products evaluation by using brine shrimp cytotoxicity assay not only describes cytotoxicity but also antiviral, anticancer, pesticidal and insecticidal potential (Sheikh *et al.*, 2004).

Ours study demonstrates that, the MA-ME showed good cytotoxicity (86.66% at 1000 µg/ml). The LD_{50} value calculated was 15.12 µg/ml. The MA-EE showed good cytotoxicity (83% at 1000 µg/ml). The LD_{50} value calculated was 51.11 µg/ml. The MA-EAE showed good cytotoxicity (73.33% at 1000 µg/ml). The LD_{50} value calculated was 122.78 µg/ml. Our results were in accordance with the previously published data. A study carried out on cytotoxicity of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* leaf extracts was reported against

brine shrimp and LD_{50} was estimated over 500 μg/ml which is partially correlated with the current study. However, brine shrimp lethality activity in methanolic extract of *Melia azedarach* stem bark with 3.27 μg/ml LD_{50} value is in agreement with our findings (Shalabi *et al.*, 2015). Green aqueous extract of the plant fruits showed significant activity when compared to the ripe fruits. The LC_{50} value for green and ripen fruits was 18.07 μg/ml and 530.2 μg/ml, respectively. The brine shrimp activity indicates cytotoxicity as well as leads to further pharmacological activities (Apu *et al.*, 2013). *Melia azedarach* revealed a moderate toxicity with 383.58 μg/ml (Sharma and Kharel, 2019). All the compounds exhibited significant activity in the BST, in particular, azedarachin B showed remarkable BST activity with an LC_{50} value of 0.0098 μM (Fukuyama *et al.*, 2006).

Ours study demonstrates that, the JA-ME showed good cytotoxicity (63.66% at 1000 μg/ml). The LD_{50} value calculated was 84.91 μg/ml. The JA-EE showed good cytotoxicity (76%, at 1000 μg/ml). The LD_{50} value calculated was 41.58 μg/ml. The JA-EAE showed good cytotoxicity (66.66% at 1000 μg/ml). The LD_{50} value calculated was 287.50 μg/ml. Our results were in accordance with the previously published data. Crude extracts (n-hexane, ethyl acetate and chloroform soluble fraction) of *Justicia adhatoda*, were screened for cytotoxic activity using brine shrimp lethality bioassay. From the results of the brine shrimp lethality bioassay, it can be well predicted that n-hexane, ethyl acetate and chloroform soluble fraction of methanolic crude extracts possess cytotoxic principles (with LC_{50} 1.129 μg/ml, LC_{50} 1.402 μg/ml and LC_{50} 2.130 μg/ml respectively) comparison with positive control vincristine sulphate (with LC_{50} 0.563 μg/ml) (Meskat and Hussain, 2012).

Ours study demonstrates that, the RC-ME showed good cytotoxicity (93.33% at 1000 μg/ml). The LD_{50} value calculated was 11.14 μg/ml. The RC-EE showed good cytotoxicity (73.33% at 1000 μg/ml). The LD_{50} value calculated was 37.67 μg/ml. The RC-EAE showed good cytotoxicity (56.66% at 1000 μg/ml). The LD_{50} value calculated was 391.76 μg/ml. Our results were in accordance with the previously published data. Cytotoxicity was evaluated by brine shrimp assay. *Ricinus communis* seeds, stem, leaves, fruit and root methanolic extracts showed mild to moderate cytotoxicity against red blood cells (RBCs) of human and bovine. Brine shrimp lethality also revealed the cytotoxic nature of extracts with LC_{50} in the range of 0.22-3.70 (μg/ml) (shaking), 1.59-60.92 (μg/ml) (sonication) and 0.72-33.60 (μg/ml) (Soxhlet), whereas LC_{90} values were in the range of 345.42-1695.81 μg/ml, 660.50-14,794.40 μg/ml and 641.62-

15,047.80 µg/ml for shaking, sonication and Soxhlet extraction methods, respectively (Abbas *et al.*, 2018).

The methanolic extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* showed good activity against brine shrimps. This significant activity of crude plant extracts against brine shrimps indicates the presence of potent cytotoxic compounds which necessitates further study. The results of this assay are useful to lead the researchers on which fraction to prefer for further fractionation and isolation of cytotoxic compounds. Later on, specific bioassays and other cytotoxic tests may be performed on the isolated bioactive compounds.

5.4. Antidiabetic activity

Diabetes mellitus is a disease of various factors. It has great effect on health, quality of life and probability of patients. 230 million people have diabetes globally (Arumugan *et al.*, 2008). Diabetes results from damage cells of Langerhan islets. This make the body to produce the pancreatic hypoglycemic hormone called insulin. Excessive urine, glucose presence in urine and high glucose level in blood are main signs of diabetes (Koffi *et al.*, 2009). Glucose and lipid metabolism is changed due to the diabetes (Rajasekaran *et al.*, 2006).

Ours study demonstrates that, after 8 h treatment, MA-ME and MA-EE decreases the levels of blood glucose in the diabetic mice. After 28 days treatment, MA-ME and MA-EE decreases the levels of blood glucose in mice. After 28 days treatment with all the extracts, the levels of cholesterol, triglycerides, LDL, VLDL, bilirubin, ALT, ALP, AST, urea, creatinine and uric acid are significantly reduced and significant increase in HDL level. Our results were in accordance with the previously published data. *Melia azedarach* extract possess good antidiabetic activity (Appalaraju *et al.*, 2014). *Melia azedarach* ethanolic flower extract possess antidiabetic activity (Asokan *et al.*, 2015). The *Melia azedarach* leaf extract decreases the levels of blood glucose and improved the peripheral glucose disposal (Seifu *et al.*, 2017).

Ours study demonstrates that, after 8 h treatment, JA-ME and JA-EE decreases the levels of blood glucose in the mice. After 28 days treatment, JA-ME and JA-EE at the 200 mg/kg and 400 mg/kg dose decrease the levels of blood glucose in the mice respectively. After 28 days treatment with all the extracts, the levels of cholesterol, triglycerides, LDL, VLDL, bilirubin, ALT, ALP, AST, urea, creatinine and uric acid are significantly reduced and significant increase in HDL level. Our results were in accordance with the previously published data. The leaves and roots extracts of *Justicia adhatoda* was studied. On the glucose tolerance, lipid profile and body

weight of the animals, significant effects were observed (Gulfraz *et al.*, 2011). The results of *Adhatoda vasica Nees* give a protective role against diabetes which sum of its glucose lowering action (Mohan *et al.*, 2014).

Ours study demonstrates that, after 8 h treatment, RC-ME and RC-EAE significant decreases the levels of blood glucose in the diabetic mice. After 28 days treatment, RC-ME and RC-EAE at 200 mg/kg and 400 mg/kg dose, decreases the levels of blood glucose in the mice respectively. After 28 days treatment with all the extracts, the levels of cholesterol, triglycerides, LDL, VLDL, bilirubin, ALT, ALP, AST, urea, creatinine and uric acid are significantly reduced and significant increase in HDL level. Our results were following the same line as revealed by other researchers. The *Ricinus communis* have a promising value for the development of a phytomedicine for diabetes (Shokeen *et al.*, 2008).

Higher contents of flavonoids (Oladele *et al.*, 1995; Rao and Rao, 2001; Sharma *et al.*, 2008), alkaloids, terpenoids (Reher *et al.*, 1991; Shane-McWhorter, 2001) and steroid glycosides (Ivorra *et al.*, 1989; Adallu and Radhika, 2000) have been usually reported in anti-diabetic and anti-hyperglycemic medicinal plants. Higher concentration of these phytoconstituents in the extract could explain its significant hypoglycemic effect, either separately or in synergy with one another. Alloxan prompts diabetes by destroying the insulin secreting cells of the pancreas accelerating hypoinsulinemia and hyperglycemia (Szuldelski, 2001). Alloxan prompts hyperglycemia by specific cytotoxic impact on the pancreatic beta cells (Yadav *et al.*, 2002). The unnecessary quantity of glucose in the blood impels secretion of insulin. The insulin secretion will excite utilization of marginal glucose and controls the processing of glucose through numerous pathways (Andrew, 2000).

In diabetes mellitus, the profile of serum lipids is increased and such an increase in lipids prompts coronary illness. The elevated serum lipids levels are because of the uninhibited activities of lipolytic hormones on the fat stores primarily because of the low activity of insulin. In diabetic state, lipoprotein lipase is not initiated because of the insulin inadequacy bringing about hyper triglyceridaemia (Pushparaj *et al.*, 2007). Additionally, insulin lack is connected with hypercholesterolaemia. Lack of insulin may be accounted for dyslipidaemia (Murali *et al.*, 2002).

Enzymes levels are mostly used for the evaluation of hepatic injury. Intracellular enzymes can be measured in the serum. Elevated levels of AST specify hepatic injury, which is

caused by the cardiac infarction, muscle damage and viral hepatitis. The alanine is converted to glutamate and pyruvate which is catalyzed. Hence, ALT is a better parameter to identify hepatic damage because it is more specific to liver. Enzymes in the serum have higher concentrations indicates the loss of hepatic membrane functional integrity and cell leakage (Drotman and Lawhorn, 1978). ALP and bilirubin are also linked to the liver cell function. The elevation in ALP in the presence of rising biliary pressure is because of the improved synthesis, (Muriel *et al.*, 1992).

Urinary examination may be knowledgeable about kidney function. In normal conditions, there is no urobilinogen excreted into the urine but it only excretes with urine in a high concentration when illness arises. Urobilinogen is the end product of bacterial reduction of conjugated bilirubin which passes through the bile ducts and metabolized in the intestine thus converted to urobilinogen (Ogeturk *et al.*, 2005; Simerville *et al.*, 2005).

5.5. Antibacterial activity

Currently antimicrobial resistance is on top among serious health intimidations due to infections resulting from resistant bacteria. Such types of infections are mostly prevalent in susceptible patients experiencing cancer chemotherapy, dialysis due to renal failure, and surgery; principally organ transplantation for which the recipient capability to combat subsidiary infections is very essential. The causes of antibiotic resistance are complex. Development of new and novel antibiotics is the need of the hour. Medicinal plants have been in use since century for treating different ailments. The universality and usefulness of traditional medicine/medicinal herbs are obvious from their persistent use by a substantial portion of the world's inhabitants (Gilani *et al.*, 2010; Caceres *et al.*, 1995; Shinwari and Qaisar, 2011).

Ours study demonstrates that, MA-ME has the highest antibacterial activity against *Staphylococcus aureus* followed by MA-EE against *Staphylococcus aureus* and MA-EAE against *Pseudomonas aeruginosa*. Our results were in accordance with the previously published data. Different extracts of the plant showed significant inhibition against bacteria tested (Khan *et al.*, 2008). To control the infections, extracts of *Melia azedarach* could be effective antibiotics (Khan *et al.*, 2011). Alcoholic extract of *Melia azedarach* have the maximum zone of inhibition against all the microorganisms, thus this extract is effective herbal medicine to treat infections (Sen and Batra, 2012a). *Melia azedarach* extracts showed significant antibacterial activity (Neycee *et al.*, 2012). By using the diffusion method, bioactive compounds of *Melia*

azedarach were effective against the majority of the bacterial strains (Marzoqi *et al.*, 2015). All the concentrations of *Melia azedarach* extract showed significant antibacterial activity (Akacha *et al.*, 2016).

Ours study demonstrates that, JA-ME has the highest antibacterial activity against *Escherichia coli* followed by JA-EE against *Escherichia coli* and JA-EAE against *Staphylococcus aureus*. Our results were following the same line as revealed by other researchers. *Justicia adhatoda* has broad spectrum of antibacterial activity and could be useful to control the infectious diseases (Pa and Mathew, 2012). Against all the bacteria *Justicia adhatoda* extracts showed antibacterial activity (Karthikeyan *et al.*, 2014). All extracts showed antibacterial activity against all the organisms except *Pseudomonas aeruginosa* and *Proteus vulgaris* (Jayapriya and Shoba, 2015). Anti-microbial assay was found that ethanolic extract was effective against all the bacterial strains (Desai and Patel, 2015). *Justicia adhatoda* leaves essential oil has a strong antibacterial activity (Shukla *et al.*, 2017).

Ours study demonstrates that, RC-ME has the highest antibacterial activity against *Pseudomonas aeruginosa* followed by RC-EE against *Pseudomonas aeruginosa* and RC-EE against *Bacillus subtilis*. Our results were following the same line as revealed by other researchers. Oil, leaf, stem and seed extract of *Ricinus communis* were screened against pathogenic bacteria. The methanol and ethanol extracts showed maximum zone of inhibition Whole parts of plant are effective in antibacterial activity assay (More *et al.*, 2014). The leaves, stem and roots extract of *Ricinus communis* shown antibacterial activity (Minakshi *et al.*, 2016).

The organisms used in the investigation were selected based on their association in varied pathologic human infections. For example; *E. coli* causes septicemia and lungs infection, gall bladder and skin lesions besides various forms of foodstuff borne ailments that commonly consequences in diarrhea (Molbak *et al.*, 2002). *Staphylococcus aureus* infection is a leading reason of skin, bone, joint, soft-tissue, respiratory and endovascular disorders (Lowy, 1998). Due to their ability to interact with soluble and extracellular protein, to formulate the bonding with cell wall of microbes and stimulating the bacterial growth inhibition, flavonoids are potential antimicrobial substances (Cowan, 1999).

Our results indicate that polar fractions showed higher potential might be due to combined influences of various compounds present in each fraction of the plant. This might act mutually with the conformation of cell structure of bacteria. Related antimicrobial findings of

methanol extract fractions accredited antimicrobial potential to bioactive phytochemicals were reported in earlier studies (Negi and Dave, 2010).

Antimicrobial potential of the plants is perhaps due to high percentage of phenolics as both are always linked together (Ravikumar *et al.*, 2009). Flavonoids and tannins present in the plant extracts have been associated with antimicrobial effects (Abo *et al.*, 1999). Flavonoids are known to be inhibitory to the *Staphylococcus aureus* and it has been used as a cure for inflamed tissues (Ali *et al.*, 1996). Plant derived alkaloids have antimicrobial potential (Omulokoli *et al.*, 1997). Alkaloids may be helpful against AIDS associated intestinal infections (McDevitt *et al.*, 1996) as well as HIV infection (Sethi, 1979).

5.6. Antifungal activity

The synthetic antimicrobials are frequently coupled with side effects. The antimicrobials agents present in plants have vast therapeutic potential (Fair and Tor, 2014). The fungi are destroyers of food materials and they are unfit for human consumption (Amrouche *et al.*, 2011). Therefore, there is a requirement to constantly explore plant derived antimicrobials. Detailed investigation is desired to identify and resolve the full scale of efficiency of the antimicrobial compounds from these plants. Antimicrobial drugs derived from plants have been included as mainstream antimicrobials owing to ineffectiveness of traditional antibiotics (Ncube *et al.*, 2008). Many natural-products chemists believe that the various potentially useful phytochemical structures can be synthesized chemically before they could be lost permanently due to species extinction (Das *et al.*, 2010).

Ours study demonstrates that, MA-ME has the highest antifungal activity against *Aspergillus flavus* followed by MA-EE against *Fusarium oxysporum* and MA-EAE against *Aspergillus flavus*. Our results were following the same line as revealed by other researchers. The methanolic extract of *Melia azedarach* possesses strong antifungal activity (Khan and Javaid, 2013). The extracts of *Melia azedarach* were found to be more effective against the fungal strains (Leela *et al.*, 2016). Methanolic extracts of this plant showed better zone of restriction against *Rhizopus oryzae* than *Aspergillus niger*. The result clearly indicated that bark of *Melia azedarach* L. contains phytochemicals which are responsible for inhibition of fungal growth and manifestation (Khatoon *et al.*, 2016). All tested concentrations of *Melia azedarach* extracts showed the significant antifungal activity. *Melia azedarach* could be

considered as a good medicinal agent (Akacha *et al.*, 2016). The leaf extracts of *Melia azedarach* extracts possess good antifungal activity (Abbas *et al.*, 2017).

Ours study demonstrates that, JA-ME has the highest antifungal activity against *Aspergillus niger* followed by JA-EE against *Aspergillus niger* and JA-EAE against *Aspergillus niger*. Our results were in accordance with the previously published data. The *Ricinus communis* leaves showed the antifungal activity against *Aspergillus flavus*, *Candida albicans* (Pa and Mathew, 2012). *Justicia adhatoda* extracts showed the significant antifungal activity (Ramachandran and Sankaranarayanan, 2013).

Ours study demonstrates that, RC-ME has the highest antifungal activity against *Aspergillus flavus* followed by RC-EE against *Aspergillus flavus* and RC-EAE against *Aspergillus flavus*. Our results were following the same line as revealed by other researchers. The *Ricinus communis* leaves showed the antifungal activity against *Aspergillus niger*. The moderate activity was found against the *Rhizopus syphilis*. The *Ricinus communis* leaves extract showed moderate activity against *Aspergillus flavus* (Khursheed *et al.*, 2012; Shabir *et al.*, 2011).

Persistent opportunistic fungal infections have turned out to be a main factor for mortality and morbidity in immunocompromised patients (Bodey and Anaissie, 1989). The plant secondary metabolites possess antifungal activity (Quiroga *et al.*, 2001). Due to the presence of phenolic compounds, many investigations accredited the preventive outcome of plant extracts against microorganisms (Baydar *et al.*, 2004; Rodriguez *et al.*, 2007). Saponins possess the antifungal properties (Mohanta *et al.*, 2007) and the polyphenolic compounds (Negri *et al.*, 2014).

5.7. Anticancer activity

In most countries of the world, cancer is the leading cause of death. It is influenced by a number of factors including diet, life style and carcinogens (Key *et al.*, 2004). Cancer is considered as one of the most prevalent ailments in the world. According to WHO, more than 10 million cases of cancer new cases are reported per year worldwide (Center *et al.*, 2011). The underlying etiology of cancer, a debilitating and multipronged disorder, involves processes like epigenetic mechanisms, mutations and carcinogens etc. Prolonged inflammatory responses in the body may also initiate cancer due to production of certain chemotaxins responsible for genetic alterations. This disease has three stages: initiation, promotion, and progression (Kandouz and Batist, 2010).

Ours study demonstrates that, MA-ME demonstrated effective results on HepG2 cell line with percent viability of 12.33%, 6.55% at 48 h and 19.72% at 72 h. MA-EE demonstrated effective results on HepG2 cell line with percent viability of 16.79% at 48 h and 15.53% at 72 h. MA-EAE demonstrated effective results on HepG2 cell line with percent viability of 20.01% at 48 h and 24.70% at 72 h. The MA-ME demonstrated effective results on HCCLM3 cell line with percent viability of 22.83% at 48 h and 10.84% at 72 h. The MA-EE demonstrated effective results on HCCLM3 cell line with percent viability of 20.31% at 48 h and 19.69% at 72 h. The MA-EAE demonstrated effective results on HCCLM3 cell line with percent viability of 26.03% at 48 h and 20.57% at 72 h. MA-ME showed significant accumulation of cells in sub-G1 phase, dose dependently with 11.24%, 64.81% and 87.21% cell cycle arrest, followed by MA-EE (26.73%, 66.14% and 81.12%) cell cycle at 100 µg/ml, 500 µg/ml and 1000 µg/ml respectively. Our findings were following the same line as revealed by other researchers. *Melia azedarach* ethanolic extract have a potent anticancer activity (Kim and Kang, 2009). The hexane extract of *Melia azedarach* have an anticancer activity (Kim and Kang, 2012).

Ours study demonstrates that, JA-ME demonstrated effective results on HepG2 cell line with percent viability of 20.31% and 16.52% at 48 h and 31.06% at 72 h. The JA-EE demonstrated effective results on HepG2 cell line with percent viability of 20.13% at 48 h. The JA-EAE demonstrated effective results on HepG2 cell line with percent viability of 18.13%, 9.84% and 6.14% at 48 h and 17.42% and 10.89% at 72 h. The JA-ME demonstrated effective results on HCCLM3 cell line with percent viability of 32.03% at 48 h. The JA-EE demonstrated effective results on HCCLM3 cell line with percent viability of 23.99% and 19.43% at 48 h. The JA-EAE demonstrated effective results on HCCLM3 cell line with percent viability of 15.86%, 13.30% and 11.73% at 48 h and 20.48%, 14.33% and 9.91% at 72 h. JA-EAE showed significant accumulation of cells in sub-G1 phase, dose dependently with (13.39%, 55.63% and 92.29%) cell cycle arrest at 100 µg/ml, 500 µg/ml and 1000 µg/ml respectively. Our findings were following the same line as revealed by other researchers. Methanol extract of *Justicia adhatoda* also showed considerable inhibition of cancer cells (Batool *et al.*, 2017).

Ours study demonstrates that, RC-EE demonstrated effective results on HepG2 cell line with percent viability of 21.14% at 48 h. The RC-EAE demonstrated effective results on HepG2 cell line with percent viability of 12.81%, 9.17% and 8.33% at 48 hours. The RC-EAE demonstrated effective results on HepG2 cell line with percent viability of 17.71% and 13.87%

at 72 h. The RC-EE demonstrated effective results on HCCLM3 cell line with percent viability of 36.20% and 9.75% at 48 h and 34.18% at 72 h. The RC-EAE demonstrated effective results on HCCLM3 cell line with percent viability of 9.42%, 6.15% and 5.97% at 48 h and 21.62%, 18.33% and 13.15% at 72 h. RC-EAE showed significant accumulation of cells in sub-G1 phase, dose dependently with (17.38%, 70.95% and 98.86%) cell cycle arrest at 100 µg/ml, 500 µg/ml and 1000 µg/ml respectively. Our findings were following the same line as revealed by other researchers. This study further provides the information about the use of terpenoids. Terpenoids are inducers of apoptosis in the cancer cells (Darmanin *et al.*, 2009). The *Ricinus* communis has the strong anticancer activity (Al-Mamun *et al.*, 2016).

The phenolics of plant extracts possess various biological activities such as antitumor (You *et al.*, 2010), antioxidant (Kumar *et al.*, 2013a), antibacterial (Kubo *et al.*, 2004) and antiviral (Uozaki *et al.*, 2007). Phenolics and flavonoids possess anti-carcinogenic and radical scavenging properties (Miliauskas *et al.*, 2004). Terpenoids represent another important class of secondary metabolites with many biological characteristics e.g. anticancer, antioxidant and antimicrobial characteristics (Omoyeni *et al.*, 2014). Coumarins possess antioxidant, anti-inflammatory, antiviral, anti-carcinogenic and Hepatoprotective activities (Guevara, 2005).

The chief plant originated constituents implicited to be responsible for protection against cancer are flavonoids and dietary fibers (Le-Marchand, 2002; Surh, 2003; Cohen *et al.*, 2000). Flavonoids have been validated to exert a multiplicity of therapeutic utilities via inhibiting the cell cycle, retreating oxidative trauma, augmenting the efficiency of enzymes detoxification, persuading apoptosis and exciting the immune functions. These intrinsic possessions of flavonoids sort them as a class of valuable compounds which own health-promoting and ailment-preventing nutritional efficacy, together with usefulness in cancer prevention (Thiery-Vuillemin *et al.*, 2005; Yang *et al.*, 2001).

5.8. Elemental analysis

Medicinal plants are effective against various diseases because of their pharmacological efficacy which depends on their elemental concentrations. Phytochemicals like primary and secondary metabolites are formed in various combinations of major, minor and trace elements which play curative and preventive role in most of the dangerous diseases. Hence elemental profile and concentrations of medicinal plants are directly or indirectly related with the curative ability of medicinal plants, so the quantitative analysis of various elemental concentrations is

essential for the determination of effectiveness and functioning of drugs prepared from medicinal plants which play an important role in curing the various diseases. Elements are known to possess profound influence in the regulation of glucose-tolerance, maintenance of the cardiac rhythms, functions of nerves and muscles, hormone regulation, blood clotting and cellular mortality (Jimoh and Oladiji, 2005).

In order to maintain the good health, the human body needs a number of minerals (Ajasa *et al.*, 2004). Both microelements and macro elements influence the biochemical processes in the human body (Kolasani *et al.*, 2011). Medicinal values of some plant species used in homoepathic system (Vartika *et al.*, 2001). Since the quality of foods and medicines depends upon the content and type of minerals, so determination of mineral elements in plants is very important (Bahadur *et al.*, 2011). For many tropical developing countries, malnutrition is of the major concern. Deficiency of elements cause number of disorders e.g. one third of the world population effects Iron deficiency anemia (Leterme *et al.*, 2006).

To determine the levels of elements in all the three plants, this study is the first attempt. In our study, the levels of elements were determined using ICP-OES, and thus the plants contain significant levels of elements including Fe, Zn, Cu, Cr, Mn, Co, Mg, Al, Ca, Na, K, Ba, B, and P in *Melia azedarach* leaves. The Chloride, Fluoride, Nitrate, Phosphate, Sulfate, Carbon, Hydrogen and Nitrogen have been found in *Melia azedarach* leaves. *Melia azedarach* leaves contain rich source of mineral such as calcium, magnesium, potassium, iron, manganese and zinc. The leaves of *Melia azedarach* could be a potential source of nutrition and minerals (Leela *et al.*, 2016).

Ours study demonstrates that, using ICP-OES, the levels of elements were determined and thus the plants contain significant levels of elements including Fe, Zn, Cu, Cr, Mn, Co, Mg, Al, Ca, Na, K, Ba, B, and P in *Justicia adhatoda* leaves. The Chloride, Fluoride, Nitrate, Phosphate, Sulfate, Carbon, Hydrogen and Nitrogen have been found *Justicia adhatoda* leaves. By atomic absorption spectrophotometer, *Justicia adhatoda* contain significant levels of elements including K, Na and Mg, Cu, Ni, Zn, Mn, Cr, Cd, Co and Fe (Ghani *et al.*, 2016). The Calcium was maximum in *Justicia adhatoda.* Potassium content in leaves of Justicia adhatoda is effective in the blood pressure (Dastagir *et al.*, 2017).

Ours study demonstrates that, levels of elements were determined using ICP-OES, and thus the plants contain significant levels of elements including Fe, Zn, Cu, Cr, Mn, Co, Mg, Al,

Ca, Li, Na, K, Ba, B, P, Ni and Mo in *Ricinus communis* leaves. The Chloride, Fluoride, Nitrate, Phosphate, Sulfate Carbon, Hydrogen and Nitrogen in *Ricinus communis* leaves. Iron is an essential element. It is an essential component of hemoglobin. It helps to control the body weight. Zinc is the component of many enzymes and it is responsible for sperm manufacture, fetus development and proper function of the immune response (Serfor-Armah *et al.*, 2002). Copper is an essential nutrient and plays an important role in the production of hemoglobin (Cobanoglu *et al.*, 2010). Chromium acts as an activator in most of the enzymes and helpful in lipoproteins, carbohydrate and nucleic acid metabolism. Higher concentration of chromium causes damage of kidney, liver and lungs whereas deficiency causes decreasing insulin activity which is responsible for increasing cholesterol and sugar level in the animal body (Zayed and Terry, 2003).

Manganese is second most important minor element present in plant and animal body, required in various biochemical reactions. In animal body Manganese is stored in kidney and liver and it is essential for normal functioning of reproductive and central nervous system (Guenther and Konieczynski, 2003). Manganese deficiency causes the reproduction failure in male and female (Lokhande *et al.*, 2010). Cobalt is main part of vitamin B-12 and help to make DNA and blood cells. Its deficiency causes serious problem in biological processes (Sullivan, 2002). Nickel plays an important role in the production of insulin. The deficiency of nickel causes liver disorder (Pendias and Pendias, 1992).

Calcium and Phosphorus are most important elements in animal as well as plant metabolism. Phosphorus plays key role in maintenance and development of skeletal tissues, acid-base balance and osmotic pressure maintenance in animal body. Specific phosphate like ATP is essential in energy utilization and transfer. Phosphorus is also involved in (a) protein synthesis (b) fatty acid transport (c) amino acid exchange (d) growth and cell differentiation (e) appetite control (f) efficiency of feed utilization and (g) fertility. Phosphorus is one of the major parts of phosphoproteins, nucleic acids, phospholipids, sugar phosphate, enzymes etc. For increasing the plant growth, phosphorus is one of the important constituents. Calcium is main constituents of bones, teeth, heart functions and muscular system. It is mostly necessary in blood coagulation activity. Calcium, Potassium and Magnesium are together essential for red blood cell production and maintaining body mechanism (WHO, 1996).

For many essential processes, the high concentration of potassium in plants is needed (Martin *et al.*, 1985). Boron and Molybdenum are essential for the growth as well as health of the animals and plants (Linder and Hazeg-Azam, 1996). Boron deficiency is responsible for alteration in brainwave activity Boron plays role in preserving neuronal function and stabilize neuronal membrane (Penland, 1994). Lithium is a trace mineral most effective in mental health due to its neuroprotective potential, its deficiency influences common metal illness and social ills (Chuang, 2004).

5.9. GC-MS analysis

The active compounds in the plants are studied using various analytical and extraction methods (Iordache *et al.*, 2009). GC-MS is an ideal technique for analysis of volatile and semi-volatile compounds. It is made by the combination of ideal separation technique (Gas Chromatography) with the best identification technique (Mass Spectrometry). Gas Chromatography-Mass Spectrometry is one of the best methods to identify the bioactive constituents of ester, acids, alcohols, long chain and branched chain hydrocarbons etc (Palawat and Lodha, 2014).

Ours study demonstrates that, major compounds identified in MA-ME were Chloroacetic acid allyl ester (19.92%), D-Galactose (6.82%), 1H-Indene, 2,3-dihydro-4-methyl- (5.52%), 3,4-Dimethylthiophene-2-thiol (4.99%) and 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (3.79%). The major compounds identified in MA-EE were 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (10.78%), Vitamin E (8.11%), 9,12,15-Octadecatrienoic acid, (Z,Z,Z) (6.12%), 4-Methylheptane-3,5-dione (5.54%) and Beta.-Sitosterol (4.96%). The major compounds identified in MA-EAE were 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (12.66%), Dl-.alpha.-Tocopherol (10.72%), Octacosane (5.92%), n-Hexadecanoic acid (5.62%) and Phytol (5.29%). Our findings were following the same line as revealed by other researchers. Varieties of compounds have been detected in *Melia azedarach* including flavonoids, phytosterols, vitamin E, nalkanoic acid, tri-terpene etc (Sen and Batra, 2012b). In the methanolic extract of *Melia azedarach*, 13 bioactive phytochemical compounds were identified (Marzoqi *et al.*, 2015).

Ours study demonstrates that, major compounds identified in JA-ME were Phytol, acetate (11.07%), 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one (6.72%), 5-Hydroxymethylfurfural (5.85%), N-Hexadecanoic acid (5.27%) and Phenol, 4-(2-methylpropyl)- (5.24%). The major compounds identified in JA-EE were 9,12,15-Octadecatrienoic acid, (Z,Z,Z) (11.92%), Phytol,

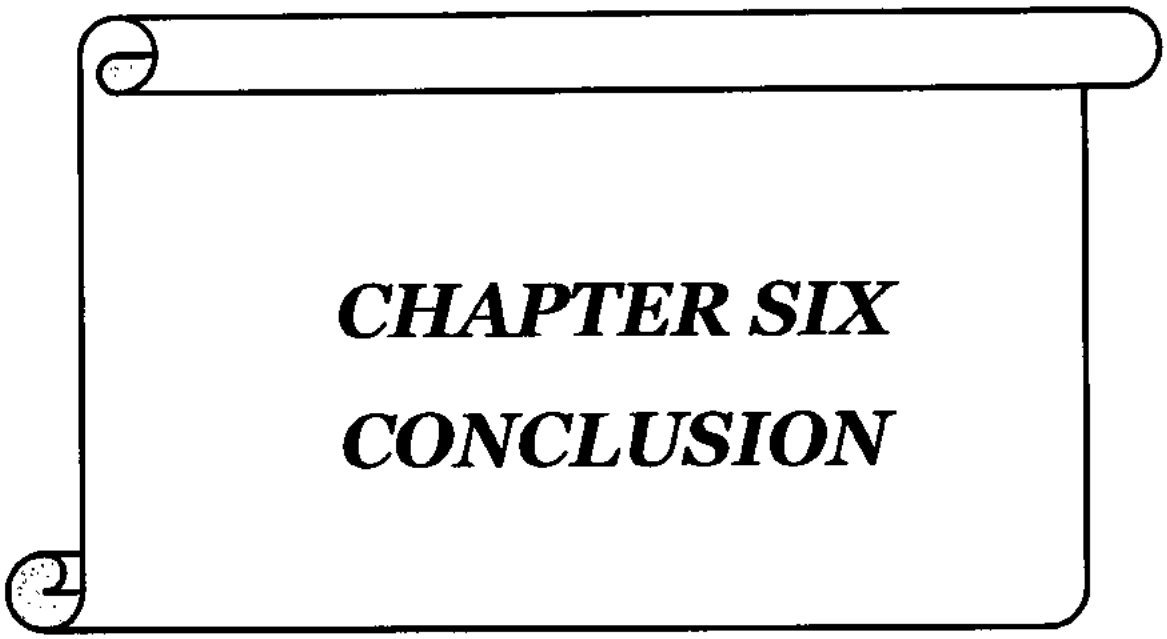
acetate (9.47%), N-Hexadecanoic acid (6.40%), Beta.-Sitosterol (3.87%) and 9,12-Octadecadienoic acid (Z,Z) (3.86%). The major compounds identified in JA-EAE were 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (12.54%), 9,12-Octadecadienoic acid (Z,Z)- (9.41%), Hexacosane (8.17%), Octacosane (6.10%) and Phytol (5.61%). Our findings were following the same line as revealed by other researchers. From the hydro distillation of *Adhatoda vasica (Nees.)* leaves, essential oil was obtained by GC-MS method. Eleven compounds from the oil of *Justicia adhatoda* were identified (Sarkera *et al.*, 2011). In the Petroleum ether extract of *Justicia adhatoda*, nine major bioactive components were identified (Jayapriya and Shoba, 2015). GC-MS analysis of *Justicia adhatoda* methanolic leaf extracts contains the major compounds (Shukla *et al.*, 2017).

Ours study demonstrates that, major compounds identified in RC-ME were Lupeol (14.90%), 9,12,15-Octadecatrienoic acid, (Z,Z,Z) (12.55%), 1,4-Benzenediamine, N,N,N',N'-tetramethyl (12.21%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (4.03%) and N-Hexadecanoic acid (3.82%). The major compounds identified in RC-EE were Lupeol (19.64%), Phytol (15.69%), 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (15.40%), Dl-.alpha.-Tocopherol (9.08%) and Hexadecanoic acid, ethyl ester (7.97%). The major compounds identified in RC-EAE were 9,12-Octadecadienoic acid, methyl ester (17.19%), Lupeol (13.85%), 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (11.92%), Dl-.alpha.-Tocopherol (7.64%) and Phytol (7.21%). Our findings were following the same line as revealed by other researchers. The major compounds of *Ricinus communis* essential oil are α-thujone (31.71%) and 1, 8-cineole (30.98%), α pinene (16.88%), camphor (12.92%) and camphene (7.48%). From the extract of *Ricinus communis*, Lupeol and 30-Norlupan3β-ol-20-one are obtained (Malcolm *et al.*, 1968). *Ricinus communis* extract contains two alkaloids, ricinine and N-demethylricinine and six flavones (Kang *et al.*, 1985). The flavonoids rutin, isoquercetin, and nicotiflorin, as well as the toxic alkaloid ricinine were detected (Mamoucha *et al.*, 2016). *Ricinus communis* revealed the existence of major bioactive components in the extract of *Justicia adhatoda* (Hussein *et al.*, 2016).

Vitamin E is effective in various hematological disorders and malagnancies (Bieri *et al.*, 1983). Vitamin E possesses anti-oxidant, hypocholesterolemic, cancer preventive and anticoronary properties. Vitamin E is a blood thinner which is another significant health benefit. In other words, it prevents the clumping of blood platelets. The risk of sunstroke is reduced by high levels of vitamin E (Basu *et al.*, 2014). Squalene, another major constituent identified is

structurally identical to β-carotene, function as a potent scavenger of singlet oxygen in skin and protects the skin from UV and ionizing radiations; boost immune functions, reduces triglyceride and cholesterol levels in animal models, its supplementation in human with cholesterol lowering agents potentiates their efficacy. It also possesses anticancer properties (Kelly, 1999).

Phytol compounds present in all the extracts. This indicates the relevance of anticancer, antimicrobial and anti-inflammatory prospective of the plant (Kalaisezhiyen and Sasikumar, 2012). Hexadecanoic acid has antioxidant, anti-inflammatory and antimicrobial potentials (Bodoprost and Rosemeyer, 2007). The presence of Hexadecanoic acid may be used as potential antifeedant agents against insects (Kumar *et al.*, 2010). N-Hexadecanoic acid exhibited Antioxidant activity. 9,12-Octadecadienoic acid (Z,Z) possess antiinflammatory, anticancer activities. Phytosterols documented as cancer preventative agents. It is called cancer preventive agent with other secondary plant products such as carotenoids, flavonoids and phytoestrogens. Stigmasterol exhibit anti-inflammatory, anti-pyretic and immunomodulating activity (Careri *et al.*, 2001). Beta.-Sitosterol prevents cancer. It also prevents angiogenesis. These sterols induced the apoptosis (Rathee *et al.*, 2012). Lupeol possess anti-inflammatory and anticancer activities (Saleem, 2009). Lupeol blocks tumorigenesis which is involved in the cell proliferation and cell death (Saleem *et al.*, 2008). Lupeol revealed potent anti-mutagenic property (Lira *et al.*, 2008).



CHAPTER SIX

CONCLUSION

- These diverse types of biological activities performed on polarity based extracts of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* proved the medicinal potentials of the plants in different fields.
- Qualitative analysis confirmed the presence of alkaloids, saponins, tannins, terpenoids, phenols, flavonoids, steroids, anthraquinone and coumarins in different plant extracts which is indication for its diverse biological activities. Phenolic compounds have been found to be beneficial in controlling diabetes. All the plant extracts showed the presence of phenolic compounds and flavonoids.
- *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* extracts possess potent antioxidant potential which was related to high contents of phenolics and flavonoids. Thus offering effective protection from free radicals and are a promising source of natural antioxidants of high significance. Such remarkable properties can be used as efficient food/ feed additives for the betterment of human health.
- Extracts of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* have shown profound cytotoxic activities. Hence they might be potential producers of cytotoxic secondary metabolites and can be utilized for the development of novel anticancer drug leads.
- All the extracts of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* exert significant hypoglycemic effect in diabetic mice which supports the traditional use of these plants for controlling hyperglycemia in diabetics. Administration of all the extracts to alloxan monohydrate induced diabetic mice reduces the blood glucose level and restored the lipid profile and body weight after 28 days treatment. Thus the extracts possess an antidiabetic effect. Uric acid levels were considerably decreased in mice treatment with plant extracts thus having potential in treating diabetes. Liver enzymes revealed that mice administered with plants extracts had little effect on liver function thus having little or no toxicity.
- Most of the medicinal plant extracts showed potential antimicrobial activities against the tested bacterial and fungal strains. Antifungal activities were found moderate in crude extract of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis*. The antimicrobial activities may be due to strong occurrence of active compounds i.e. saponins, tannins, alkaloids, steroids, phenols and flavonoids.

- Ethyl acetate extracts of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis* have shown profound anticancer activity. Hence they might be potential producers of secondary metabolites and can be utilized for the development of novel anticancer drug leads.
- All the plants contain significant levels of elements including Fe, Zn, Cu, Cr, Mn, Co, Ni, Mg, Al, Ca, Li, Na, K, Ba, B, P and Mo. Ni. These elements are known to possess profound influence in the regulation of glucose-tolerance, maintenance of the cardiac rhythms, functions of nerves and muscles, hormone regulation, blood clotting and cellular mortality.
- GC-MS was undertaken to determine the chemical constituents of the leaves extracts of *Melia azedarach*, *Justicia adhatoda* and *Ricinus communis*. It was demonstrated that plants were richly supplied with important chemical components which could serve the purpose of therapeutic and medicinal approaches.
- The presence of various bioactive compounds in different plant extracts justifies the use of plant for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents and subjecting it to the biological activity will definitely give fruitful results.
- Therefore, further studies are needed to isolate and characterize the bioactive compounds responsible for the observed antidiabetic, antibacterial, antifungal and anticancer properties of all the extracts.

CHAPTER SEVEN

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