Cytotoxicity of Green Synthesized Silver Nanoparticles against Cancer Cell lines

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Nanoparticles

Silver nanoparticles

Thin layer chromatography

بسم الله الرحمن الرحيم

In the name of Allah,

The most Gracious, The most merciful.

"And say: My Lord increase me in Knowledge"

(Qur'an, Ta-Ha 20:114)

"My Lord, put my heart at peace for me, makes my task easy for me, and loose a knot from my tongue so they may understand my speech."

(Qur'an, 20:25-2)

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FINAL APPROVAL

It is certified that we have read the thesis entitled "Cytotoxicity of Green Synthesized Silver Nanoparticles against Cancer Cell Lines" submitted by Ms. Noreen Akhtar and it is our judgment that this project is of sufficient standard to warrant its acceptance by the International Islamic University, Islamabad for the MS degree in Biotechnology.

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A thesis submitted to Department of Biological Sciences, International Islamic University, Islamabad as a partial Fulfillment of requirement of the award of the

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(MSBT)

This humble effort is

Dedicated

To

My beloved respected

Parents,

Brothers and

Sisters

Who inspired me for higher ideals of Life

DECLARATION

I hereby solemnly declare that the work **"Cytotoxicity of Green Synthesized Silver Nanoparticles against Cancer Cell lines"** presented in the following thesis is my own effort, except where otherwise acknowledged and that the thesis is my own composition. No part of the thesis has been previously presented for any other degree.

Dated: 12-02-2020

Noreen

Noreen Akhtar

345 -FBAS/MSBT/F17

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(Noreen Akhtar)

ABBREVIATIONS

Abbreviations	Definitions
%	Percentage
µg	Microgram
µl	Micro liter
B.w	By weight
Conc.	Concentrated
AgNPs	Silver Nanoparticles
HCl	Hydrochloride Acid
HER	Human Estrogen Receptor
gm	Gram
mg	Mili gram
Hr	Hour
ml	Mili liter

mm	Mili meter
nm	Nano meter
°C	Degree Centigrade
pH	Power of Hydrogen Ions
Rpm	Revolution per Minute
DNA	Deoxyribonucleic acid
PMA	Poly(methacrylic acid)
RB	RB Retino Blastoma
RES	Reticulo Endothelial System
ROS	Reactive oxygen species
RPMI-1630	Roswell park memorial institute-130
RT	Reverse transcriptase
Av	Average
HCC	Hepatocellular Carcinoma
ROS	Reactive Oxygen Species

SRB	Sulforhodamine B
IC_{50}	Inhibitory Concentration 50
TCA	Tricarboxylic Acid
$AgNO_3$	Silver Nitrate
UV-Visible	Ultraviolet Visible
XRD	X-Ray Diffraction
SEM	Scanning Electron Microscopy
FTIR	Furiour Transformed Infrared
IST	Institute of Space Technology
NCP	National Centre for physics

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ABSTRACT

Fagonia cretica is a known medicinal plant containing important biomolecules .The present study was designed to evaluate extracts of *Fagonia cretica* and then synthesized silver nanoparticles to test against bacteria and Human liver carcinoma cells (HepG2). Qualitative analysis of the Ethanolic, Methanolic and aqueous extracts revealed that it contains Flavonoids, Alkaloids, Tannins, Steroids, Cardiac glycoside, Terpenoids, Saponins and Anthraquinones glycosides. Thin layer chromatography confirmed the presence of bioactive molecules.

The essential component of the biosynthesized silver nanoparticles (AgNPs) formation by the extracts of *Fagonia cretica* were determined by functional groups using FTIR (IRPRESTIGE-21) Spectroscopy (FTIR), the wave number range from 500 to 4000 cm^{-1} Using 21 scans. Different peaks i.e. 2θ of 38°, 45°, 65 and 77°that correlate with the reticulum position of 111, 200, 220 and 311, were observed by the XRD Spectrum of biologically formulated silver nanoparticles. Finer crystallinity of biologically formulated silver nanoparticles is assured due to the observance of more precise diffraction peaks.

In vitro antibacterial potential of green incorporate of silver nanoparticles was investigated on various strain of bacteria for instance *Enterobacter Klebsiella pneumoniae* and *Streptococcus pyogenes*. The Fagonia cretica extract and Biosynthesized (AgNPs) Silver nanoparticles showed maximum circle of inhibition against different bacteria.

Green synthesized silver nanoparticles anticancer activity experiments were followed to analysis the cytotoxicity of *Fagonia cretica* extract and green synthesized silver nanoparticles (AgNPs).

The cytotoxic activity of AgNPs against cancer lines suggested that the significant role of AgNPs in cell death. The current research suggested that AgNPs would play for development of an effective anticancer drug that treats the cancers.

CHAPTER 1

INTRODUCTION

1. INTRODUCTION

The size of nanoparticles is normally ranging from 1 to 100 nm. The different structure of nanoparticles display new superior physical and chemical properties in ratio the upper cover area per volume of particles (Cushing *et al*, 2004). Metallic nanoparticles' being easy to synthesize are the most widely studied nanoparticle. Additionally, these particles play an vital role in different application such as detectors, antibacterial and antimicrobial etc. According to Ajitha *et al,* 2015, the most studied metallic nanoparticles is silver nanoparticles.

Silver has strong antibacterial ability and have high toxicity to cell. Ag being capable of dismantling the cell wall of bacteria due to their unsettles its metabolic activities. The silver nanoparticles interact with macromolecules such as DNA, proteins to degrade that are present in the cell, also rupture the cell membrane ultimately to the death. The biosynthesis silver nanoparticles are more reactive then the synthetic nanoparticles. The AgNPs have more potential of antibacterial ability (Vazquez-Muñoz *et al,* 2017 & Rai *et al*, 2009). For these reasons AgNPs are considered to be most important especially is the field of health and medicine.

There are many methods to synthesize AgNPs but synthetic way are widely practiced to synthesize AgNPs because they are structurally fine (Zieli′nska *et al*, 2009). In this method sodium citrate and borohydride is a reducing material to reduce Ag Na (Sondik and Salopek-Sondi 2004). According to Singh *et al*, 2010) by using chemicals for synthesizing AgNPs may cause chemosorption of lethal chemicals such as organic chemical and reducing material.

These chemicals hinder its applications. Consequently, environmental friendly methods are desirable. To counter this problem, plants or microorganisms on natural products could be used for synthesizing AgNPs. Method is called as green synthesis method (Solomon et al, 2007). The biosynthesis of (AgNPs) by taken out secondary metabolites by plant (Jirovetz et al, 2003). The biological agent ability asstabilizers and reducing the procedure. (Chandran *et al,* 2006).

For obtaining AgNPs both conventional and unconventional techniques are in use today. Two different methods "top-down" and "bottom-up" are used.

Numerous conventional methods including solution microwave, electrochemical, radiation thermal decomposition of silver particles or compounds are used for obtaining nanoparticles. But they usually involve release of harmful chemicals, least conversions of compounds, more energy demanding and costly purifications (Mourato *et al,* 2011 & Haiza *et al,* 2013). In recent years green chemistry and biosynthetic methods have become more attractive ways for obtaining AgNPs. Modern days methods involves the use of whether associated microbes (e.g.: bacteria, fungi yeasts and so on) or numerous fermented or aquatic plant secretions. Green production offers diverse advantages relative to common routes because these are less costly, environmental viable and less demanding for pressure, temp and energy or harmful chemicals. (Kharissova *et al,* 2015). But plant-mediated synthesizing methods are even more advantageous especially because they are environmental friendly, pathogens free, require single step techniques, can easily be improved, and are less bio hazardous. These methods also eliminate the need of growing cell cultures.

Plant extracts contain numerous medicinally important biomolecules including vitamins, terpinoids, alkaloids, phenols, polysaccharides and tannins. Although they have complex structures but are environmentally not threating (Roy and Das *et al,* 2015). It is believed from some traces of knowledge that flavanone and perpenoids extracted from leaf can stabilize silver nanoparticles formation. It is also believed that polyol and some other heterocyclic components soluble in water are source of silver ions reductions. According to (Logeswari *et al,* 2015) AgNPs exhibits brownish appearance in water solution in accordance to surface vibration excitation. AgNPs are extracted from either aqueous plant extract or alcoholic plant extract. Aqueous plant extract is widely preferred because AgNPs are further used in different medical of biological applications.

The steps involved for obtaining aqueous plant extract are

- Collection of plant's important parts.
- Thorough wash with distilled or tap water
- Shade-drying (7-10 days or more if necessary).
- Grinding the dried portion into a powder.
- Boiling a certain amount of the dried powder with tap water.
- Filter the infusion till no raw material left
- One the aqueous plant extract is obtained, some milliliters of it are poured into Silver Nitrate compound. This reduces uncontaminated Silver(I) ions into Silver(0). The formation of Ag nanoparticles are possible to be checked by witnessing the alteration

of color into brown while measuring UV-Vis spectra at different time gaps (Chung *et al,* 2016).

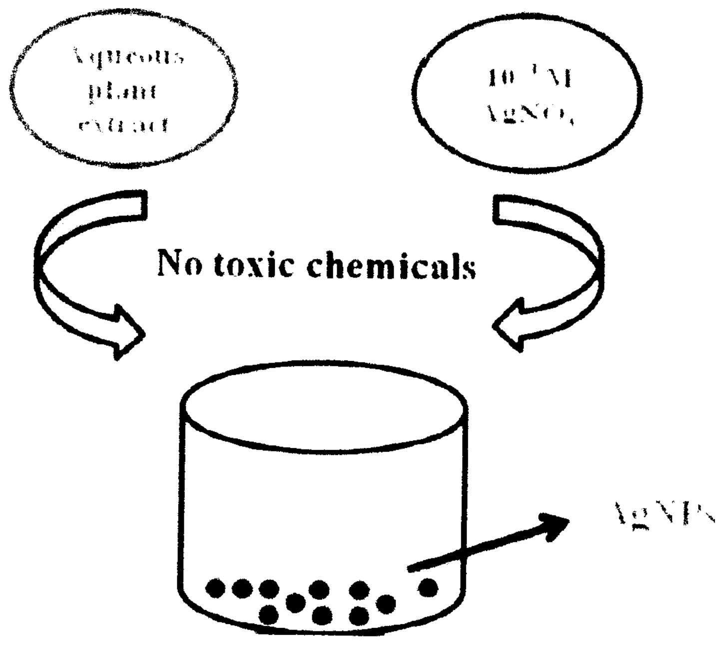

Fig: 1.1 Production of AgNPs by plant extracts (Alexandra Sorescu et al, 2016)

Fig: 1.2 *Fagonia cretica*

1.1 *Fagonia cretica*

Fagonia is a slight, spiny, and small sized shrubs with glabrous, branchless and stander, \type of shapes. Its leaves are opposite, 1-3 foliate; petioles are flexible lengthwise that range anywhere between 3 - 30 mm in length, slim and striate; a couple of sharp slight thorns of stipules, occasionally more than 12 milli meter in length. It has cannabis branch and woody from the base and erect.

A large quantity of species like *Fagonia cretica, F. Arabica, and many other species*. has been identified. These are popular conventional medicine in Afghanistan and Sind. This plant is used has booster and febrifuge, likewise children are being treated with it as prophylactic in Peshawar region to treat small pox. Its leaves are believed to have cooling affects. Scrofula and neck inflation is also being treated in Ormara hills with this. The suspension came from it is then applied on the bodies of children. A hot water mixute in Kharan is used while taking shower if you are suffering from temperature. So, this plant is recommended to treat Las Beta and itch in these areas. For Levy tracts, this plant is crushed, mixed with mill and then used on all over the body for three days. (Rathore *et al,* 2011)

Fagonia species also were studied because of its medicinal uses. Recent studies have shown that this plant exhibits numerous activities including analgesic, antitumor, anitoxident, astringent, febrifuge and prophylactic to beat all the small pox agents. Some of the *Fagonia* were used also to treat kidney infections, stomach issues, tooth pain, urine issues cancer isses and much more. Species of *Fagonia* are observed to have biomolecules like coumarins, amino acids and proteins, sterols alkaloids and other trace elements (Alqasoumi *et al,* 2011).

From centuries, herbal medicinal treatments are popular for treatment of human diseases because plants contain various unique compounds that are affective against number of diseases. Family of *Zygophyllaceae* has twenty five genera with two hundred and forty species limited to humid, sub region and hot condition, but mostly in dry parts of land. This family's all the species particularly genus *Fagonia* have great pharmacological importance because of their potential to treat number of diseases. Aqueous extract upper parts of *F. cretica* L. believed to be considerably effective for early stages cancer therapy (Anjum *et al.* 2007).

F. cretica L. is extensively termed as plant with importance in pharmacology, phytochemistry, and ethnobotany. Pharmacological researchers are emphasizing pharmacological activity of these herbal extracts should be examined thoroughly to identify the biomolecules that are affective against the diseases as mentioned above. Sound antimicrobial, anti-inflammatory and antioxidant activity evidence of this rate plant suggest that this plant very effective to be examined to be investigated for clinical trials. Phytochemicals of *F. cretica* including phenol, pectin, flavonoids, glycosides, and protein ingredients with important three multivitamins like niacin (B5), Vitamin (C) and riboflavin (B2) fried material of this plant were calibrated in terms of quantity in past (Hussain *et al*, 2017)

1.2 Silver Nanoparticles (AgNPs)

The Nanoparticles being three dimensional ingredients fluctuating range 1–100 nm became of great interest in recent years due to its unique characteristic, arrangement to form superstructures and applications superior to their bulk counterparts. The characteristics properties of nanoparticles with accordance to the rules of quantum mechanics instead of old version physics (Millinor *et al,* 2004).

The silver nanoparticles are attractive day by day due to its extra valuable characteristic properties. Now a days silver nanoparticles gets enough attention in the field of biological potential. It would be used in drug delivery, food preservation, bio-labeling, wound healings, and many other applications (Zhang *et al,* 2016).

Moreover, AgNPs are also of great importance in different industries for instance catalysis, textiles and electronics (Handoko *et al,* 2017). The plant-mediated green producing (Ag) nanoparticles has developed in the field of nanotechnology. Due to its unique potential it is cost effective and ecofriendly. Relative to chemical hazards it is less toxic. Green synthesis has number of benefits then physical or chemical nanoparticles synthesis.

(1) Non-Toxic properties,

(2) Rapid, easy to produce.

(3) Reduced energy Consumes and activate under working environment

(4) Silver nanoparticles and plant active ingredient Combines the potency.

Biosynthesis (AgNPs) more used instead of synthetic silver naoparticles (Choudhury *et al,* 2016).

1.3 Anticancer Activity of *Fagonia cretica* and Silver Nanoparticles

Hussain *et al,* (2007) examined that the *Fagonia cretica* plant has remedial activity specific against tumors and cancer, the analysis performed at lab stage by performing cytotoxic antitumor DNA damage assay and. The maximum cytotoxicity potential was observed in opposition to brine shrimp at lethal dose with 11.89 particles per meter loading. The Potato disc assay was used to inhibit the tumor. The Invitro-Antitumor activity was observed in opposition to all cancer inducing strains, the (MTI) was observed (77.04%) in opposition to At10. Therefore, the plant extract has not observed any lethal potential in opposition to bacterial strain, DNA breakage has not being detected.

Zulfiqar *et al,* (2019) studied that a powerful reducing agent extracted from *F. cretica* was used to synthesize AgNPs. The Ultraviolet-Visible confirms the production of nanoparticles. Morphological and structural analysis determined by XRD and the size of nanoparticles is 16 nm crystalline. In the Fagonia cretica extract Compositional analysis was performed for the availability of active bio reducing and stabilizing agents. By using the different concentrations of $AgNO_3$ and *F. cretica* extract were used to get higher potential with improved stability of silver nanoparticles. Silver nanoparticles showed maximum invitro antibacterial potential in opposition to different strains. The AgNPs showed maximum (ROS) in *Proteus vulgaris* as compared to bacterial strains to induced cell toxicity,

Ullah *et al,* (2017) stated that the tropical disease leishmaniosis is universally mistreated caused by parasite. The biosynthesis of (AgNPs) using plant leaves of Fagonia indica ethanolic extract. The viability of MTT cell assay was analyze to regulate the antileishmanial activity of the extract and non-toxicological concentration of the extract. The Silver nanoparticles AgNPs are non-toxic to macrophage cell that are above than 30 μg/ml. The IC50 value was calculated as 8.16±0.63 μg/ml and 4.8±0.819 μg/ml for extract against parasites. It was found that both the extract and Silver Nanoparticles produced by increasing the nitrogen oxide. Overall, the F. *indica* leaves extract and Silver Nanoparticles are showed good activity against antileishmanial agents.

Leela, & Vivekanandan (2008) mentioned that the expansion of biological procedures for synthesizing nanoparticles is emerging as an vital in the field of nanotechnology. Biosynthesis of silver nanoparticles by the extracts of various plants *Helianthus annus etc,* showed bio reduction behavior in the synthesis of silver nanoparticles. The strong potential for speedy reduction of silver ions determined by Ultraviolet-Visible XRD and SEM.

Safaepour *et al,* (2008) reported that the plant extract producing biogenesis of AgNPs using for instance of *P. graveolens* and *Pelargonium graveolens,* The formation of silver nanoparticles by using geraniol was investigated and successful synthesis of uniformly dispersed, uniform sized and shaped AgNPs in the range of 1 to 10 nm. The cytotoxicity of the biosynthesis silver nanoparticles was determined using a abnormal cell line. The sample showed a directly portion response; directly proportion values using nanoparticles. The strength of 1 µg/ml, of (AgNPs) was potential to kill the cell line's growth by minimum 30%. The administration of 5 µg/ml (Ag) nanoparticles remarkable inhibited the cell line's growth. It was identified that the dose needed to formation of Fifty percent cell death by using 2.6 µg/ml for this AgNPs prepared with geraniol.

Bibi *et al,* (2019) have found that the *F. cretica.* Is a well-important remedial plant consisting of numerous a vital biomolecules. The initial study was conducted to prepare the AgNPs from *F. cretica* L. AgNP were seen to be morphologically characterized and their antibacterial activity was estimated using different techniques. The Silver Nanoparticles distinguished were crystalline, having spherical shape, size ranging from (20-50) nm. There were various functional groups observed in Silver Nanoparticles by the FTIR. Some important antibacterial potential in opposition to *Klebsiella pneumoniae* and *Staphylococcus aureus* were investigated. Invitro Anti-bacterial potential of AgNPs fifty milli gram was collated with ciprofloxacin (10 mg) whereas −ve control produces an immense inhibitory circle in according to −ve & +ve control in a concentration conditional way. The Silver Nanoparticles developed from *F. cretica* extract and potential in opposition to various clinical isolates of strain.

Lam *et al,* (2012) declared that the medicinal plants have shown a vital origin of anti-cancer drugs. It was examined that the cytotoxic potential of water extract of *F. cretica,* was used commonly in opposition to cancer. It was reported as time & dose-dependent arrest in

G0/G1 phase of the cell cycle and apoptosis the treatment of extract in MCF-7 (WT-p53) and MDA-MB-231 (mutant-p53) as human abnormal cell lines with a considerable decrease effect on the primary mammary epithelial cells. Furthermore, FOXO3a was found to be needed for activity in the absence of p53. All these findings stipulated that *F. cretica* aqueous extract contains very important anti-cancerous agents which act either individually or in groups against breast cancer cell line.

Bhattarai *et al,* (2018) described the synthesis of nanoparticles (of Au and Ag) was evaluated by following green chemistry metrics, while emphasizing on process mass intensity (PMI).

Abdel-Fattah and Ali (2018) assessed the existing review of AgNPs because of their high-level physicochemical and biological properties being intensely dealt with. The appropriate knowledge of these attributes is vital to make most of their potential applications in multiple areas while decreasing their hazards to human and the surrounding environment. This document aims to review the synthesis of Silver Nanoparticles critically via different approaches, its usage in treatment of cancer and the future challenges.

Rajkumar *et al,* (2019) studied the Silver nanoparticles and found out that they possess numerous distinctive drugs characteristics and their applications as compared to other types of metallic nanoparticles .At present, Silver nanoparticle is biosynthesized by using an water extract of *Zea mays* L. The vital effects of concentration of ingredients, extract, condition and time of synthesis were also assessed beside the cytotoxic and radical rummage potential. Ultraviolet–vis spectra of the Silver nanoparticles provide at ~420 nm. The Silver nanoparticles having monodispersed and aggregated spherical shape were noticed by SEM image along with its identification via visual indications in the Ag region of EDX. The confirmation of crystallinity and cubic face-centered structure was done by XRD spectra. FT-IR spectra revealed the existence of phyto compounds in the synthesis of AgNPs. Moreover, the AgNPs exhibited powerful cytotoxic potential against the HepG2 cells along with its viable decline with an elevation in concentration of AgNPs to the control cells.

Song and Kim (2009) proved that the AgNPs are formed by the treatment of water suspension of $AgNO_3$ with extracts of plant as depletion of Ag+ to Ag^0 is much more stable. Ultraviolet V-visible spectroscopy examined the quantitative development of AgNPs. The synthesized AgNPs were identified with inductively coupled plasma spectrometry (ICP), XRD and SEM. This eco-friendly procedure of biological production of AgNPs provide rate of synthesis comparable or faster than chemical procedures can commonly use in various different human appearance region such as food, and medicinal solicitations.

Xiu *et al,* (2012) explained mechanism of poisonousness of AgNPs to bacteria and other organisms. *E. coli* endurance was acclaimed by low concentration of almost all the tested Silver Nitrate and Silver Nanoparticles, proposing a hermetic action that acts be unjust to antimicrobial utilization. In general, this suggested that the morphological properties of AgNPs are indirect effectors that basically influence the release of Ag+. Moreover, it was seen that the antibacterial potential would be managed by outer response the release of Ag+, possibly through particle size, shape, and varieties of outer sheeting.

Zulfiqar *et al,* (2019) exhibited the manufacturing of Silver Nanoparticles using a productive bio reducing agent derived from the extract of *Fagonia cretica* showing benefits of being eco-friendly in chemical and physical ways.

The presence of active stabilizing agents and bio reducing in *Fagonia cretica* extract was confirmed by using compositional analysis. Numerous concentrations of *Fagonia cretica* extract and Silver Nitrate were employed to get highest profit of Silver Nanoparticles with finest stability. The resulted Silver Nanoparticles indicated very effective antibacterial activity against *Escherichia coli, Proteus vulgaris* and *Klebsiella pneumoniae*. It was studied that the Nanoparticles induced maximum reactive oxygen species (ROS) in *Proteus vulgaris* as compared to *Escherichia coli* and *Klebsiella pneumoniae*. Whereas, the production of ROS in presence of AgNPs was 30% higher than the plant control and release in all of the three bacterial strains.

Remya *et al,* (2015) found that the method to yield bio-functionalized Silver Nanoparticles by the use of aqueous release of *Cassia fistula* flowers. The crystalline, face-centered cubic structure of Silver nanoparticles was revealed by the XRD pattern which showed round morphology as given by FE-SEM. Various functional groups were identified by FTIR studies including effective capping of Silver nanoparticles. The produced Silver nanoparticles showed very effective cytotoxic potential against the MCF7. During this, the inhibitory concentration (IC50) was recorded at 7.19 mg/mL. The AO/EB staining defined the apoptotic effects of the Silver nanoparticles.

Mfouo-Tynga *et al,* (2014) examined that the multifunctional effects of AgNPs have made nanostructures extremely potent compounds for biomedical applications. Cytotoxic effects on both A549 lung and MCF-7 breast cancer cell lines were assessed with the help of Trypan blue exclusion assay, inverted light microscopy, lactate dehydrogenase membrane integrity assays and ATP luminescence. Results revealed that the Silver Nanoparticles used during the existing research were found to be spherical in shape, with -0.0261 mV surface net charges, having average size of 27 nanometers and being positively identified in both cell lines.

The exposed Silver Nanoparticles encouraged the reduction in proliferation and aviability increased cytotoxicity with induced programmed cell death through the mechanism of apoptosis. Photodynamic activity in both cancer cell lines was showed by Silver Nanoparticles whereas MCF-7 cells showed enhanced cytotoxic effects as compared to A549 cells. The maximal absorbance of Silver Nanoparticles lies within the wavelength range of 370–450 nanometers. Silver Nanoparticles formed and used in this research had peak absorption at 631 nanometers. This working demonstrates that the Silver Nanoparticles which were activated at 635 nanometers contribute remarkably to the cytotoxic induction in cancer cells but more in breast cancer cells (MCF-7) as compared to lung cancer cells (A549).

Ahmed and Mustafa (2019) explained that AgNPs Plant-mediated fusion is preferred due to its accessibility and different metabolites. The current review has discovered the diversity and potentiality of AgNPs biological activities which originated from the combination of phyto constituents and Ag (mostly traditionally used Sudanese medicinal and aromatic plants). Additionally, some parameters affect the synthesis of plant-mediated AgNPs, their different biological activities and characterization techniques are summarized and discussed.

1.4 AIMS AND OBJECTIVES

- ✓ Bio-synthesis of Silver nanoparticles (AgNPs) from *Fagonia Cretica*
- ✓ Characterization the plant extract & green synthesized silver nanoparticles
- ✓ To explore the antibacterial activity of Silver nanoparticles
- ✓ Evaluation of cytotoxicity in HepG2 cell lines of silver nanoparticles.

CHAPTER 2

MATERIALS AND METHODS

2. MATERIALS AND METHODS

In this research study I have prepared the silver nanoparticles (AgNPs) by using green chemistry. *Fagonia cretica* was used for the biosynthesis of silver nanoparticles. Biosynthesis by various plant extracts is indefinite, it has been revealed that the biomolecules in plant extract such as protein, phenol and flavonoids play an important role in the reduction of metals ions and coating the biosynthesized nanoparticles (Saravanan *et al*, 2018). Green synthesis of nanoparticles has is cost effective, ecofriendly and does not require high pressure, energy, temperature or the use of toxic chemical reagents (Kharissova *et al,* 2015).

2.1 Collection of Plant Material

Mature plant samples of Fagonia cretica was collected from the local market of Islamabad Pakistan. After that Fagonia cretica was sent to Botanical department of Arid Agriculture University for the identification of Species.

2.1.1 Plant Extracts Preparation

Fagonia cretica aqueous extract was prepared. Plant Fagonia cretica was washed with purified distilled water until no adulteration remained. The fresh plant leaves were chopped into smaller lump Fig 2.1. 10 g sample of *Fagonia cretica* was mixed in 100 milli liter of distilled aqueous beaker. The blend was then heated at 60°C about 20 minutes while stirring occasionally. Allow the mixture to cool at normal temperature, then filtered the mixture using the whattman 42 filter paper and then centrifuged for 20minutes. The biosynthesis of Ag nanoparticles from AgNO3 forerunner solution, the extract was stored in the refrigerator.

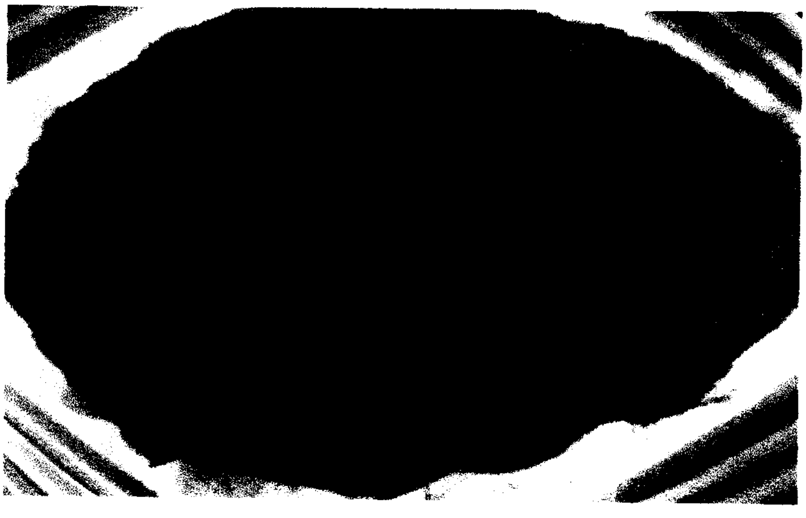

Fig: 2.1 Dry powder of *Fagonia cretica*

2.1.2 Separation by Thin Layer Chromatographic Technique

About 25g of dried *Fagonia cretica* was subjected to Soxhlet extraction for 2, 5 or 24 hr. Fig 2.2. Under vacuum, extracts were dried in a rotary evaporator and desiccated for 24 hours. The TLC technique was then used for the dissociation of compounds in a mixture. Under UV detection, the separated compounds were visualized. The plant extracts was used 500ug put in to TLC plate. The solvent benzene was used on TLC plate which was developed at 7.5 cm. (Sarker *et al,* 2006)

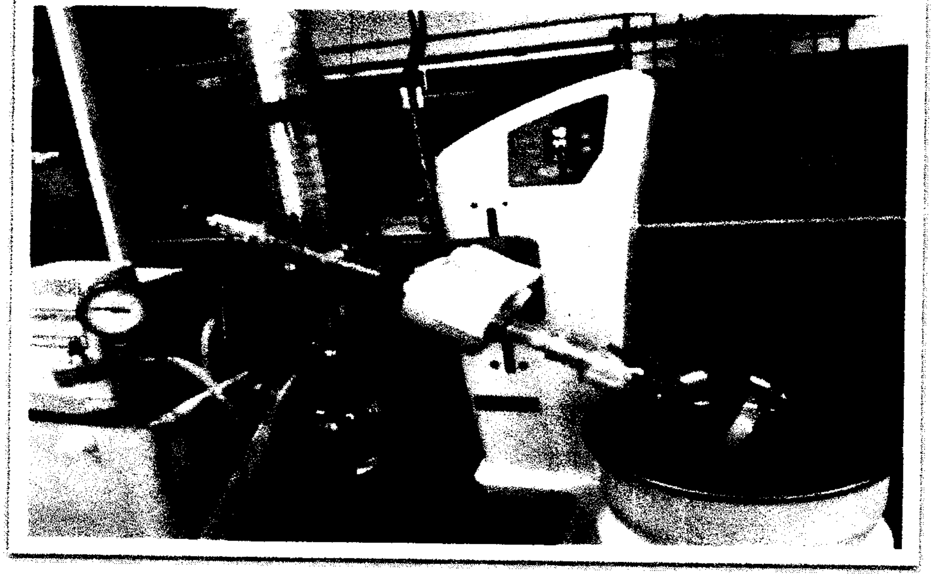

Fig: 2.2 Soxhlet extraction of Fagonia cretica for (TLC) analysis

2.1.3 Fourier Transform Infrared (FTIR) Analysis of *Fagonia cretica*

FTIR spectra of sample of *Fagonia cretica* was determined using Fourier Transform Infrared IRPRESTIGE-21 (Shemadzo) to recognize the Functional group of the bioactive molecules based on the different wavelength number. One mg dried samples of Fagonia cretica were placed on sample slot respectively and then recorded for FT-IR measurement in the wavelength range from 4000 to 500 cm^{-1} Using 33 scan (Ravi *et al,* 2011).

2.2 Silver Nanoparticles (AgNPs) Synthesis

The properties of Silver nanoparticles (AgNPs) are unique, which can be incorporated into Invitro-antimicrobial applications, Medical treatment, Pharmaceuticals and biosensor materials. Powder $AgNO_3$ was liquefying in distilled water to produce 10mM $AgNO_3$ ware solution. Then a series of 1 mM, 2 mM, and 5mM AgNO3 solutions were make-ready from the stock solution. The aqueous extract of *Fagonia cretica* plant was mixed with $AgNO_3$ solutions at a ratio of 1:1 volume of 50mL in a beaker (Anthony et al, 2014). The beaker was packed with an aluminum foil and then heated in an aqueous medium for 5 hours at 60°C. The blend was then stored in the refrigerator for further analysis.

2.2.1 Extraction of Nanoparticles

The medium containing $AgNO_3$ particles were separated through centrifuge machine at 12000 revolutions per minutes for 15 minutes to get pellet having synthesized Silver Nano particles. The pellet was washed 3 times and centrifugation was repeated. After having the final pellet it was subjected to be dried in a slightly open centrifuge tube.

2.2.2 Silver nanoparticles (AgNPs) Characterization

The characterization of Nanoparticles is mandatory for the analysis of quality and description of nanoparticls. The complete morphology of nanoparticles was determined by latest techniques. Moreover, by these techniques intercalation, dispersion and orientation of nanoparticles and nanotubes in Nano-composite materials could be determined. The biosynthesis of nanoparticls is mandatory for their study to control the size and morphology during synthesis. The synthesis of silver nanoparticles (AgNPs) by biological rout is discussed, which is simple, easy and convenient route for preparing nanometer range particles.

2.2.2.1 UV-Vis absorption Spectroscopy

The Ultraviolet visual spectrum was used to determine the reduction of pure silver ions. The chemical reaction chamber after dissolved a minute quantity of extract sample into distilled water. The chemically reaction mixture of metal ion solution & *Fagonia cretica* extract determined through Ultraviolet visual spectral analysis by the changing of color. By using UV-1800 (Shimadzu) at the wavelength of 180–820 nanometer .

2.2.2.2 FTIR of Silver nanoparticles (AgNPs)

The FTIR spectroscopy analysis was used to identify the molecules on the basis of functional groups. The FTIR spectra of dried pellets of (AgNPs) were determined using Fourier Transform Infrared IRPRESTIGE-21 (Shemadzo) to recognize the Functional group of the bioactive molecules construct on different wavelength number. The dried pelleted was carefully weighed on a sensitive electronic weigh machine. One mg of dried pellets of (AgNPs) were placed on sample slot respectively and then recorded for FT-IR measurement wavelength range from 4000 to 500 cm^{-1} Using 33 scan. (Ravi *et al,* 2011).

2.2.2.3 XRD of Silver Nanoparticles (AgNPs)

X-ray diffraction spectroscopy avail oneself of to identify the structure & composition of green (Ag) nanoparticls were determined at National Centre for Physics, Islamabad (NCPI). On the basis of peak at degree the normally the size of particles were investigated, The average solid crystal size of nanoparticles is D & k is the judgment of X-ray origin, the half maximum of different angular of full width is represent (Bibi *et al,* 2019)

2.2.2.4 SEM of silver nanoparticles

In SEM, the samples are focused with a beam of electrons which interact with atoms of the samples producing single which are detected and contains information about the sample SEM achieves resolution better than 1 mm. it was used to study the morphological characteristic of the nanostructure (Goldstein, 1992). The morphological and particles size of the nanostructure were studied by SEM. The nanostructures synthesized were focused with an electron beam of 1 kv and image was produced. The SEM was performed by using high performance in nanospace "MIRA3 TESCAN" technology system from "Institute of Space technology Islamabad.

2.3 Antibacterial Activity

For antimicrobial activity test three different infectious strains like, *Klebsiella pneumonia, Streptococcus pyogenes,* and *Enterobacter* were collected from Benazir Bhutto Hospital Rawalpindi. All the strains were placed separately in the prepared inoculum in LB broth media gL^{-1} each analysis tube having unique strain was then placed in a shaking rotatory incubator for 24 Hours at 100 revolutions per minutes at 37°C. The antimicrobial tests were done through agar well diffusion method in applied Microbiology and Biotechnology laboratory, IIUI.

Lysogeny broth (LB) agar was make-ready and autoclaved at 121°C for 20 minutes to perform the experiment then stream into culturing plats after cooling. Diluted 20µl of microbial strain were than seeding and using a sterile glass (L) rod made a lawn culture. Plates were left for dry. Five borehole of 6 mm were bored on each plate and 25µg, 50µg , 75µg and 100µg of each sample of AgNPs was poured in each borehole and plates were incubated at 40°C for 24 hr. In each plate one well in the center was of control named Clarithromycin. Clarithromycin was used as positive control. After 24 hr. circle of inhibition were determined in millimeter (mm) (Bibi *et al,* 2019)

2.4 Culture Conditions of HepG2 Cell Line

HepG2 cell line was cultured in 75 cm^2 (250ml) flasks. Cells were augmented with 10% heat deactivated FBS and 1 percent GPPS and were kept as monolayer in RPMI 1640. Humidified and 10% CO2 incubator was used to keep the flask at 37°C.

2.4.1 Passaging

Cultures having confluency in between 70-90% were harvested by trypsinization and microscopic study of flask was performed.

- Mature media was unloaded and removal of dead cells by using 5ml of 1% PBS
- Cohered single layer was separated by keeping in incubator for one minute at optimum condition and by inducing 1-1.5 ml of 0.5mM EDTA.
- Culture medium was appended 5 milli liter, after that trypsin was making inactive and cells were congregated in fifteen milli meter in rotary tube.
- Centrifugation of specimen was evaluated at 1000 revolution per minute for 5mins at optimum condition
- Floating material was then drained and resuscitative pellet in 5 milli liter of fresh culture medium the cultured cells were calculated using neubauer chamber.

2.4.2 Cell Counting

Cellular suspension and 0.4% trypan blue were assorted thoroughly in equal volumes and added over the neubauer chamber beneath the coverslip lying flat over the chamber. Living cells didn't get stained while dead cells turned blue by permeability of tryptan blue by permeable cell membrane. Large Central Square of haemocytometer was used to count the viable cells. Following formula was used to compute Cellular concentration per unit volume

Number of cells = Number of cells in large central square × dilution factor ×104

Where, Dilution factor = 2

Harvested cells were either subcultured in 75 cm^2 flask at cellular density of 3x104 to 3.5x104 cells/ml or used in experiments.

Cell Cultures having 80% confluency were treated, by trypsinizing with 1 ml of 0.5 mM trypsin/EDTA for 1 minute at 37° C. Cells were harvested and media was drained by centrifuging at 1000rpm for 5 minutes. Pellet was resuspended in appropriate volume of culture medium and cells were stained with tryptan blue and counted on neubauer chamber (strobe, 2001). Flasks were reseeded at a density of 3x104 to 3.5x104 cells/ml.

2.4.3 Frosty

Media was decanted by centrifuging at 1000 revolution per min for 5 min after the cultured cells were trypsinized. In a suitable volume of frosty part (10% DMSO + RPMI 1640 + 45% FBS), cell pellets were resuspended to capitulate about 525 viable cells /ml. In each cryovial, 1 milli liter volumes of cell solution were poured and these samples were maintained at -70°C for short period of time and further shifted to liquid nitrogen for longer durations.

2.4.4 Thawing

In the cryovial, culture medium was incorporated drop by drop and resuspended cells were harvested along with it. After harvesting all the cells from cryovials, these were centrifuged at 1000 revolution per minute for 5 minute. The cells were resuspended in 10ml of culture medium and set horizontally at particular culture stat. After one day, cells were amplified with make new medium and older medium was withdrawn. Before experimenting, cells were let to flourish for one week.

2.4.5 Doubling time of Cell Lines

Trypan blue method was used to trypsinize the cells. 105 cells/well, cells were implanted in 6 well plates. After every 24 hours, cells were collected for four days and counted.

2.5 Cytotoxicity Screening

2.5.1 Cytotoxicity Screening of Everolimus and Lorlatinib

Everolimus and Lorlatinib were used as positive control to determine the cytotoxicity analysis. Everolimus and Lorlatinib were tested against the cell lines (HepG2)., healthy cells were poured in 96 borehole plates at a density of (1x105) cells per ml. After that plates were incubated for 24 hr. for culture environment to allow the cells to bind with the outer surface of culturing plate and recover from the trypsnization stress. After one day culture medium was revitalize and cell were unprotected to serial dilutions of Everolimus (0.1, 0.5, 1, 5, 10, 50 and 100μM) and Lorlatinib (3, 30, 100 and 300μM). After that plates containing cells were incubated with anticancer drugs for 24 hours at above mentioned conditions. For negative control untreated cells were included in the experiment.

2.5.1.1 SRB Assay

Sulphorhodamine B abbreviated as SRB assay implies the use of a negatively charged pink amino xanthine dye called Sulphorhodamine B. In the assay the mentioned dye is taken by primary amino acids in cells under screening. The higher number of cells the higher value of amino xanthine will be engrossed by cells and fixed inside. Afterwards, cells are lysed, and the intensity and absorbance of dye is measured (Skehan et al., 1990).

Sulforhodamine B (SRB) cell cytotoxicity assay (Catalog # K943-1000 Biovision) was used for SRB assay. Cells exposed to drug Everolimus and Lorlatinib for 72 hours at 37^0C from (3.10.1) were added with 50uL Fixation solution in each well followed by incubation of well plate for 1 hour at 4^0C. At the end of incubation period Fixation solution was removed and plate was washed three times with 200uL dH_2O without disturbing monolayer of cell. Once the cells were fixed and washed, drying was carried out. 45uL of SRB prepared solution was slightly mixed into separately well of fixed cells plate and were stained for 15 minutes at normal temperature in zero-light condition (as SRB is light sensitive). After staining, solution was removed and washing with 200uL 1X with solution from kit was carried four times. Lastly, washing solution was removed through pipetting and plate was air- dried. 200ul of 1X solubilization solution from kit was added into separately well and plate was shook for 10 minutes using shaker.

Decisively, measurement of O.D at 565nm was performed. Cytotoxicity percentage was calculated using as mentioned below formula

$$\%Cytotoxicity = O.D.DMSO\text{-}O.D\ sample/O.D.DMSO \times 100\%$$

2.6 Cytotoxicity of *Fagonia cretica* against cancer cell line (HepG2)

Extract preparation: An aqueous extract of *Fagonia cretica* was prepared using (Lam, Carmichael, & Griffiths, 2012) protocol. 25g of sample was immerse in 500 ml of distil liquied medium for 5hours at 70^0C with consistanly shaking. The plant extract was finally filtered using (Fisher Scientific, FB59020, UK) Fisherbrand filter paper for removing solid particles and debris. The obtained extract was extraction with ratio 3:1 hexane. The water part was dried using vaccume prior to subjecting for cytotoxicity analysis.

2.7 Cytotoxicity screening of Silver Nanoparticls (AgNPs)

The eco-friendly synthesized of silver nanoparticles (AgNPs) from *Fagonia cretica* were conceal for their cytotoxicity in opposition Human liver cells (HepG2).

To screen cytotoxicity, cell line HepG2 was poured in nighty six well plates. The different concentration of silver nanoparticls was used against cancer cells. Later on cells were exposed to varying concentrations of AgNPs starting from. For positive control 25uM Everolimus and 100μM Lorlatinib treated cells were used for this experiment whereas, for negative control normal cells were used. Cells were exposed for 24 hours with plating density of 15000 cells/well.

CHAPTER 3

RESULTS

3. RESULT

3.1 Thin-Layer chromatographic Technique (TLC):

The thin layer chromatography (TLC) using the methanol: chloroform: acetic acid with the ratio (30:70:0.2). All violet colors were observed using Ultraviolet 254 nanometer with the dot of Retention factor .14, .32 and .46. Brown spots were observed Under Ultraviolet (366nm) Retention factor .14, .32.observed all light pink colour. By using iodine spray all yellow dots were observed the Retention factor .14, .19, and .35. These results clearly indicate that bioactive molecules are present in extract of Fagonia cretica.

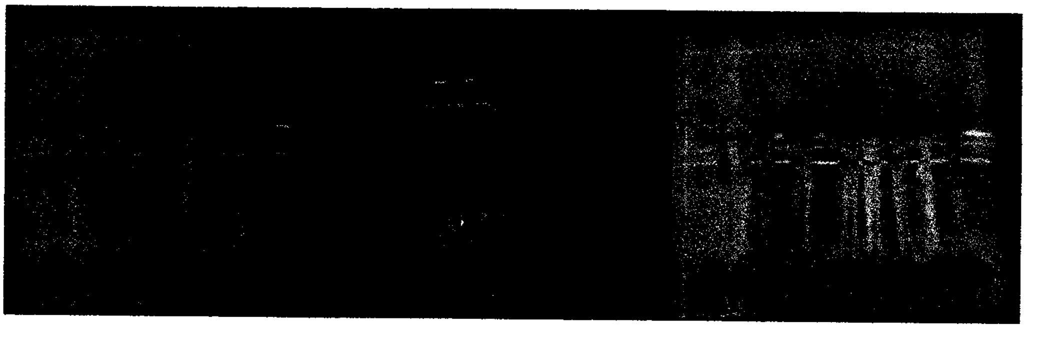

Fig: 3.1 Thin layer chromatography of *Fagonia cretica* extracts

3.2. FTIR of *Fagonia cretica*

The extracts of Fagonia cretica to evaluate possible bioactive molecules were measured by FTIR. The availability of bioactive molecules on the basis of functional in the extract of Fagonia cretica was carried out by using advance instrument FTIR ranging from 500 to 4000 cm^{-1}wavelength as shown in (Fig 3.2 and Table 3.1). The absorption spectrum of different components clearly showed on the graph of the FTIR spectra. In the sample of *Fagonia cretica* it represents an overlap of some characteristic absorption peaks of bioactive molecules on the basis of functional groups. Therefore. *Fagonia cretica* showed the availability of bioactive molecules by using analytical tool of the sample but also we need to more investigation for the pharmaceuticals and therapeutics importance of plants.

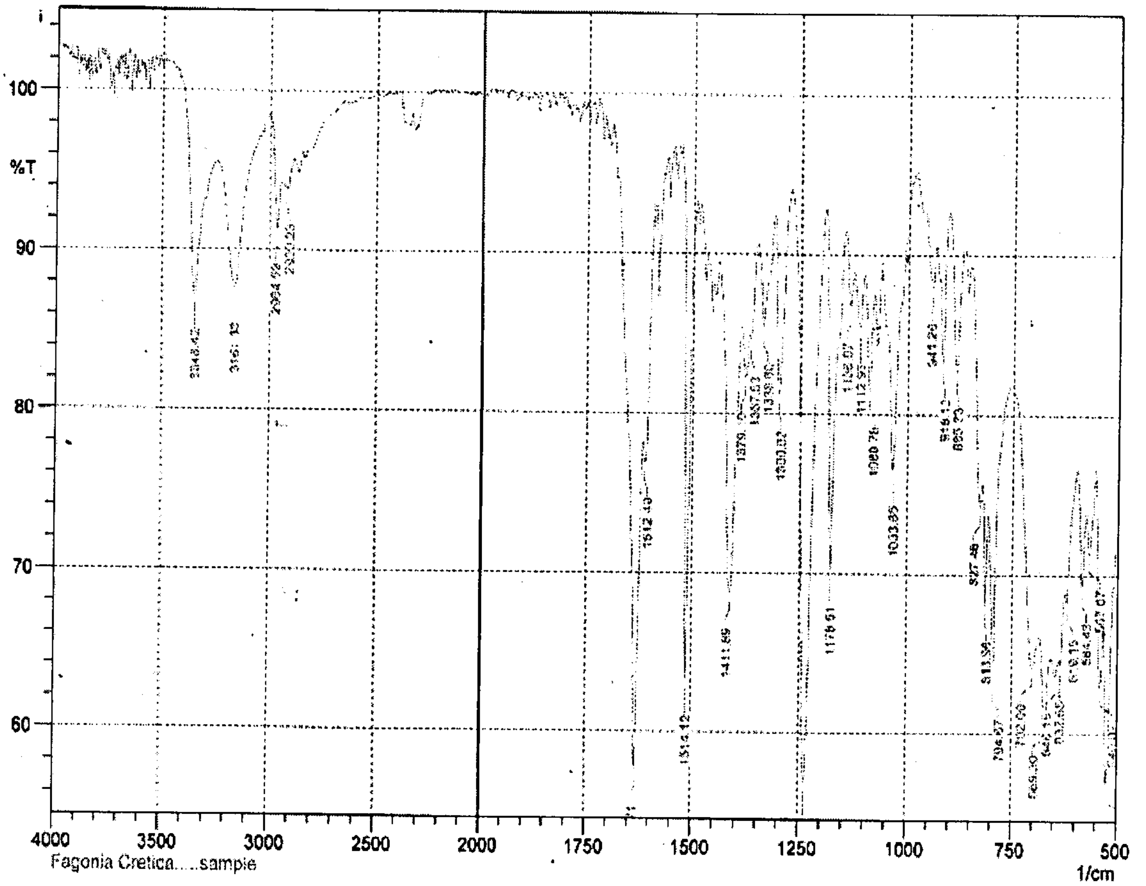

Fig: 3.2 FTIR spectra of *Fagonia cretica* sample

Table 3.1 FTIR spectroscopic data of *Fagonia cretica*

Sr. #	Origin	Frequency Range	Peak value
1	N-H	3,400- 3,200 cm^{-1}	3348.42
2	N-H	3400-3100 cm^{-1}	3161.33
3	C-H	2916-2935 cm^{-1}	2964.59
4	C=O	2865-2845 cm^{-1}	1612.49
5	C=O	1540-1600 cm^{-1}	1514.12
5	N-H	1410-1470 cm^{-1}	1411.89
6	C-O-C	1210-1450 cm^{-1}	1379.1
7	C-O-C	1300-1350 cm^{-1}	1300.02
8	C-O-C	1200-1250 cm^{-1}	1114.29
9	C-o	1250-1000 cm^{-1}	1061.73
10	C-C, C-OH, C-H (rings)	1000-820 cm^{-1}	869.73
11	C-H bending	830-600 cm^{-1}	627.47

3.3 Silver nanoparticle synthesis

The green synthesis of silver nanoparticles by following water extract of *Fagonia cretica* changes their colors in under warmed condition. Earlier, the Fagonia cretica extract had no color change but afterwards it changes to brownish yellow when warm. Upon subsequent inclusion of $AgNO_3$ to warm extract, color is changed again. Reduction of $Ag+$ ions because of heat can lead to color changes and formation of $Ag+$ complex. Silver Nanoparticles were observed due to this color shift. UV-Visible Spectrophotometry is utilized to authenticate the formation of silver nanoparticles.

3.3.1 Confirmation of Silver Nanoparticles (AgNPs)

Green Synthesis of (Ag) nanoparticles was verified by the Ultraviolet-Visual Spectrophotometry.

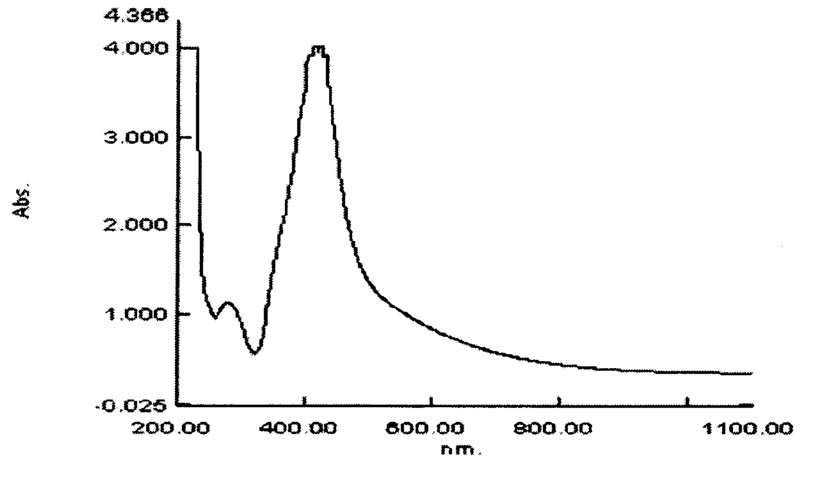

Fig: 3.3 UV-Visual spectrophotometry of experimental group

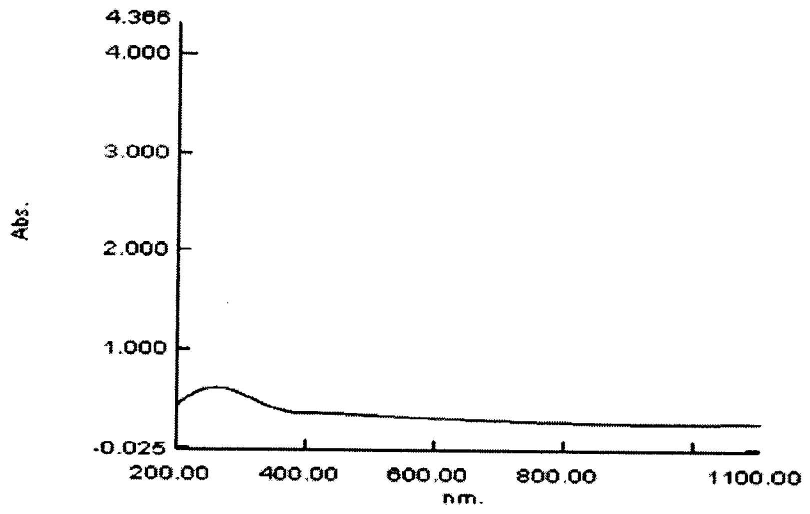

Fig: 3.4 UV-Vis spectrophotometry of control group

3.3.2 Identification of Silver Nanoparticles (AgNPs) by FTIR

Fourier Transform Infrared Spectroscopy helps to identify the molecule structure that exhibits vibrational energetics and bonds. It was carried out to investigate the molecules which confirms the presence of functional groups of silver nanoparticles ranging from 500 to 4000 cm^{-1}wavelength as shown in (Table 3.2).

Green synthesis of AgNPs was revealed by FTIR analysis. The peaks showed at different wavelength number such as 509.21 cm^{-1}, 530.42 cm^{-1}, 584.43 cm^{-1}, 690.52 cm^{-1} and 3244.27 cm^{-1}. The peak absorption was observed at 509.21 cm^{-1} is moved to down area at 530.42 cm^{-1}. The C–N stretch vibrations were observed at peak of 1382 cm^{-1}. The strong bands are due to ether linkages at 1074 cm^{-1} and ensure the availability of bioactive molecules on the upper area of metal nanoparticles. The phenolic groups are placed in the 1315–1037 cm^{-1} and 1456–1600 cm^{-1} . The very strong band is due to C=C at 1592 cm^{-1} due to availability of aromatic group. The silver nanoparticles of O–H observed at 3785 cm^{-1} to 3881 cm^{-1}. The capping and in very short time reduction of ions from silver to silver nanoparticles due to amino acid chain and flavonoids. The flavonoids composite in the aqueous extract of *M. pendans* potentially involved in the reduction of Ag^+ to Ag^0.

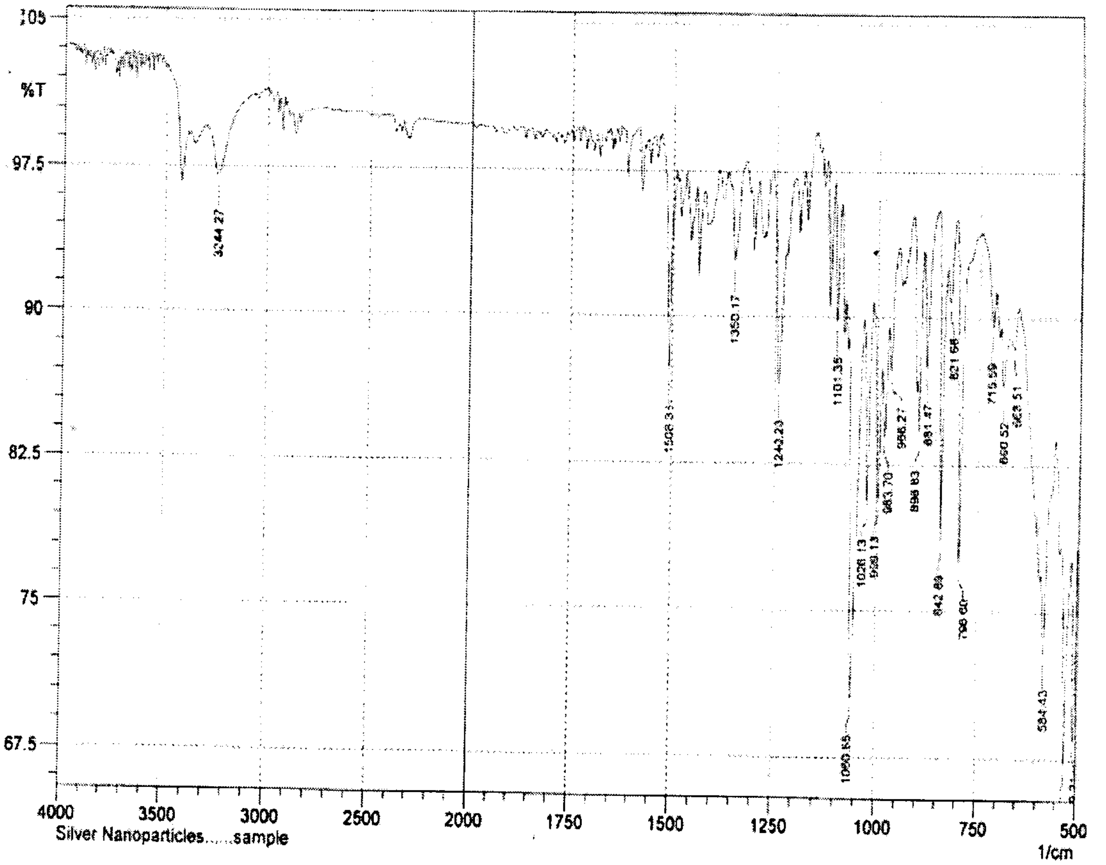

Fig: 3.5 FTIR analysis of silver nanoparticles (AgNPs)

Table 3.2 FT-IR spectroscopic data of silver nanoparticles (AgNPs)

Sr. #	Origin	Frequency Range	Peak value
1	O-H Hydroxyl	3,400- 3,200 cm^{-1}	3244.27
2	N-H	1530-1400 cm^{-1}	1508.33
3	C=O	1380-1235 cm^{-1}	1350.17
4	C-H	1365-1200 cm^{-1}	1240.23
5	P-O	1240-1100 cm^{-1}	1101.35
5	C-H	1110-100cm^{-1}	1060.85
8	C-H	1210-1250 cm^{-1}	1026.13
9	C-C, C-OH	1000-850 cm^{-1}	999.13
10	=C–H	890-800 cm^{-1}	842.89
11	N-H	800-710 cm^{-1}	715.59
12	C-H bending	720-635 cm^{-1}	663.51

3.3.3 Crystallinity by X-ray Diffraction Spectroscopic Technique (XRD)

Crystal form of silver nanoparticles was confirmed from the XRD diffractogram. Different peaks i.e. 2θ of 38°, 45°, 65 and 77°that correlate with the reticulum position of 111, 200, 220 and 311 were observed by the XRD Spectrum of biologically formulated silver nanoparticles. Finer crystallinity of biologically formulated silver nanoparticles is assured due to the observance of more precise diffraction peaks.

The diffraction peaks identified that the availability of crystal and sharp form of biologically synthesized (AgNPs). Due to unique form of biological synthesis silver nanoparticles is not only use for biological application but also so many others applications.

The size of biosynthesis Ag particles is 1.64 nm. In the *Fagonia cretica* extract the availability of capping agent for the formation of silver nanoparticles that maintained the formation to smaller (Zulfiqar *et al,* 2019)

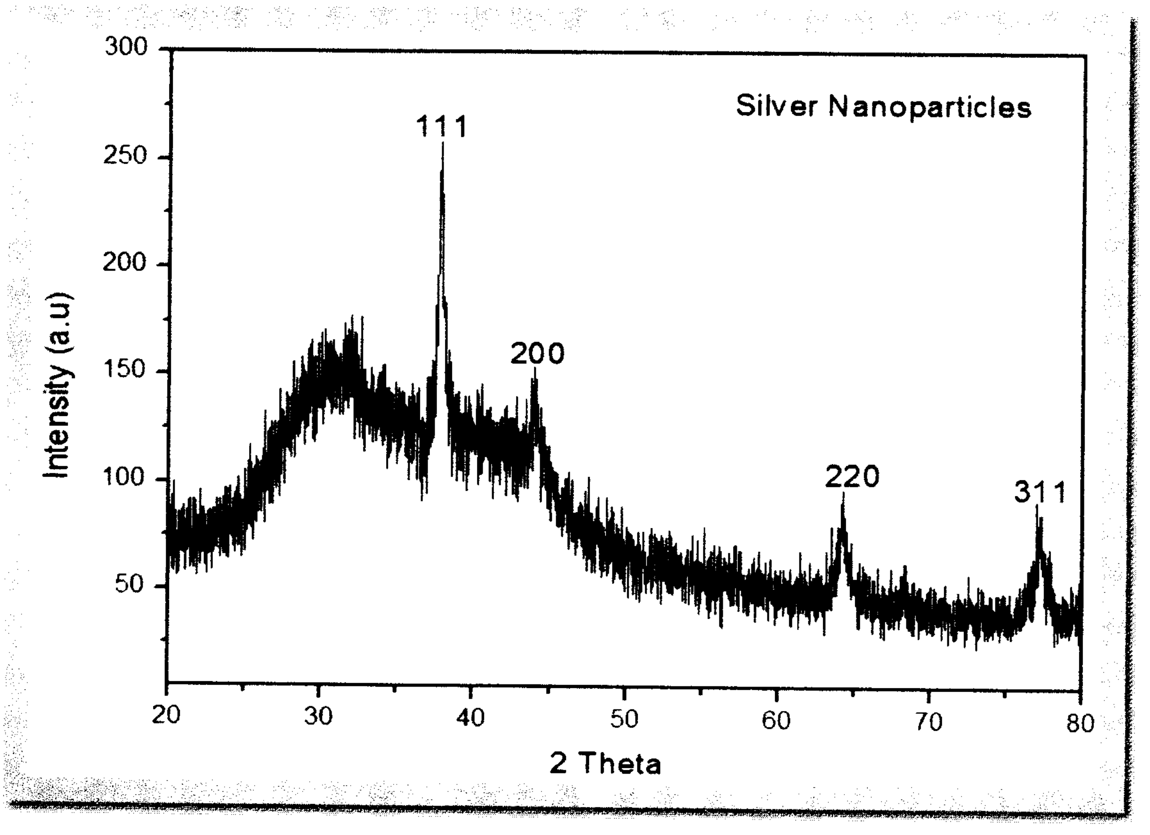

Fig: 3.6 X-Ray Diffraction Peaks of (AgNPs)

3.3.4 Morphological Examination:

In order to know the size and shape of prepared sample, SEM was used with beam of electron beam energy of 15 kv. It can be observed from the image that the prepared samples are nanostructure with the size range of 30 nm and shape of these nanoparticles are spherical.

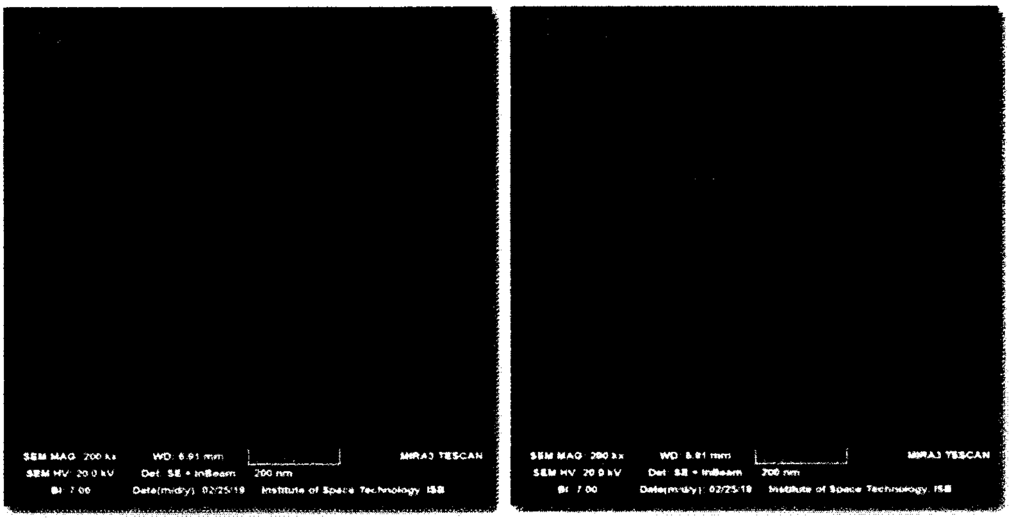

Fig: 3.7 SEM of Silver Nanoparticles

3.5 Anti-Bacterial Potential of Silver nanoparticles (AgNPs):

The biosynthesis of silver nanoparticles was investigated invitro antibacterial potential against on various types of bacteria such as *Enterobacter Klebsiella pneumoniae* and *Streptococcus pyogenes*. The variable quantity of AgNPs was used against three bacteria. The circle of inhibition due to silver nanoparticles was observed against single bacterial media is shown in Fig. 3.8. The clarithromycin drug was used for positive control with the sample of bacterial culture are given in Table 3.3. (Bankar *et al.* 2010)

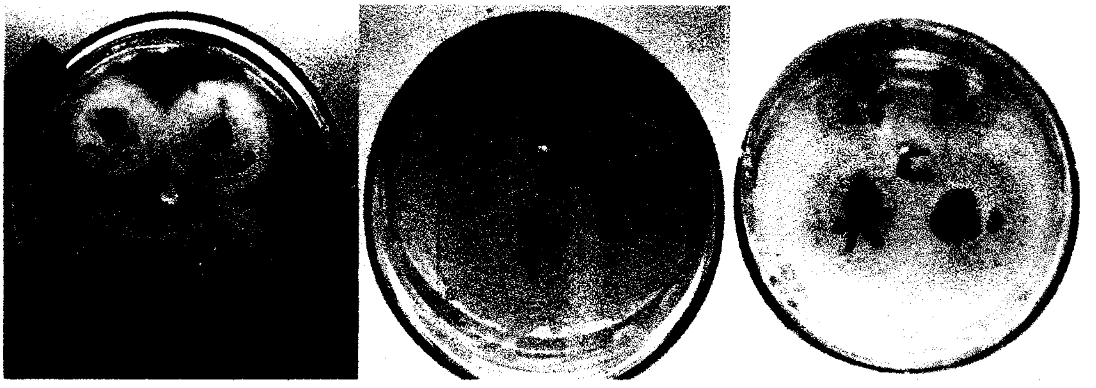

Fig 3.8 Invitro-Antibacterial potential of Silver nanoparticles (AgNPs) in opposition to *Enterobacter Klebsiella pneumoniae* and *Streptococcus pyogenes*

Table 3.3 Circle of Inhibition of green synthesized silver nanoparticles (AgNPs) in opposition to bacteria

Sr. No.	Organisms (Bacteria)	Concentration of Extract (AgNPs)				Control (Clarithromycin)
		25µg	50 µg	75 µg	100 µg	50 µl
1	*Klebsiella pneumoniae*	10±0.02	15±0.02	18±0.02	21±0.02	23±0.02
2	*Enterobacter*	13±0.02	15±0.02	18±0.02	20±0.02	24±0.02
3	*Streptococcus pyogenes*	10±0.02	14±0.02	19±0.02	21±0.02	24±0.02

3.6 Cytotoxicity of silver nanoparticles (AgNPs) against Cancer cell lines (HepG2)

3.6.1 Cytotoxicity evaluation of Everolimus & Lorlatinib

Most of the chemotherapeutics drugs such as Everolimus and Lorlatinib commonly used against cancer are available in the market. Both drugs were used with different concentration in opposition to cancer cell line (HepG2). Due to the use of these anticancer drugs to determine the cytotoxicity and also used for a positive control in experiment.

3.6.2 Cytotoxicity of *Fagonia cretica* plant against cancer cell line (HepG2)

HepG2 cell line was exposed to aqueous extract of *Fagonia cretica.* Cell viability of HepG2 cell line against extract of *Fagonia cretica* was higher. Cytotoxicity of plant extract against cancer cell lines was less effective.

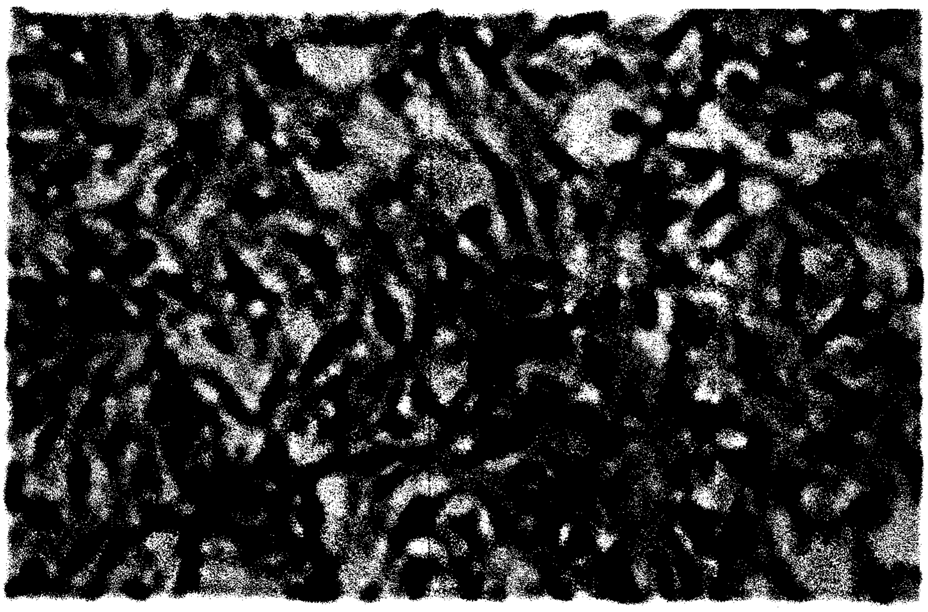

Fig 3.9 Untreated culture of cancer cell lines (HepG2)

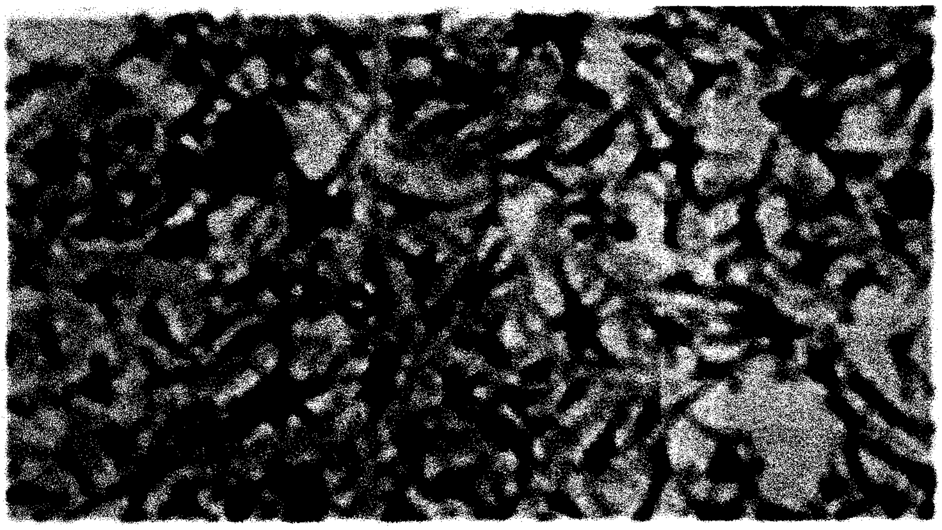

Fig 3.10 Treated culture with *Fagonia Cretica* extract

3.6.3 Cytotoxicity of Silver nanoparticles (AgNPs) against cancer cell line (HepG2)

HepG2 cell line was exposed to AgNPs. Cell viability of HepG2 cell line against green synthesized silver nanoparticles was less. Cytotoxicity of green synthesized silver nanoparticles against cancer cell lines was more effective.

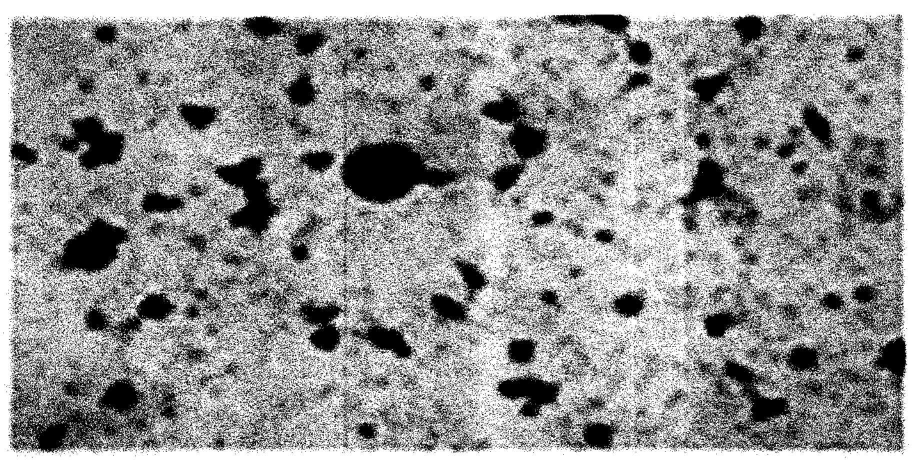

Fig 3.11 Treated culture with Silver nanoparticles (AgNPs)

CHAPTER 4

DISCUSSION

4. DISCUSSION

This study also investigated that Phytochemicals analysis showed presence of Flavonoids Terpenoids, Cardiac Glycosides, Tannins Steroids, and Anthraquinones were available in the Ethanol and Methanol and aqueous extract of *Fagonia cretica.* Phytochemicals ingredients take part in vital role in the field of pharmaceuticals to develop new drugs using these Phytochemicals with other molecules. They also improve the health status of the customer due to the presence of various bioactive molecules (Amala and poonguzhali, 2015)

The results are in agreement with the finding (Mamta and Jyoti, 2012) also studied that the phytochemical screening, was identified the major phytochemical groups.

The thin layer chromatography (TLC) using the methanol: chloroform: acetic acid with the ratio (30:70:0.2). All violet colors were observed using Ultraviolet 254 nanometer with the dot of Retention factor .14, .32 and .46. Brown spots were observed Under Ultraviolet (366nm) Retention factor .14, .32.observed all light pink colour. By using iodine spray all yellow dots were observed the Retention factor .14, .19, and .35. These results clearly indicate that bioactive molecules are present in extract of Fagonia cretica.

Our results are consensus with the finding of (Zschocke *et al,* 2000) studied that the *E. autumnalis* leaves extract by using solvent ethyl acetate, observed different compound under Ultraviolet light 254 and 365nm. The three bands were characterized chromatogram of the extracts at Retention factor .68, .55 and .49, By using anisaldehyde: sulphuric acid reagent dark spots were observed under Ultraviolet 254 and 365 nanometer. The bright blue glow was observed under Ultravoilet 365 nm with the Retention factor rang (0- .85). The TLC results showed that the various parts of the *E. autumnalis* which is not present any major constituent.

FTIR analysis discloses the existence of distinctive functional groups in *fagonia cretica* samples, ranging from 500 to 4000 cm^{-1} wavelength. The FTIR spectra indicated that, each absorption spectrum of various components showed considerable overlap each band represents functional groups existence in the samples.

Our results are in consensus with the discovery of (Bibi *et al,* 2019) who studied that the exchange of AgNO3 to AgNPs by molecules of fresh plant was predict by FTIR analysis in scale of 4000–600 centimeter. The existence of macromolecules in the extracts of leave Fagonia cretica observed at the peaks 612 and 624 cm. The aromatic C-H due to glycosides at the peak 721.87 cm. The tennins, coumarin, and terphenoids of the plants sample were observed at Peak 980. The availability of ester in extracts of plant were observed peaks at 1046.95, 1088.25, 1215.30, and1294.75 indicated the C-O stretch.

The existence of unsaturated alcohols or alkanes in plants was demonstrated by the H-C-H bend which was observed at the peak 1448.94. N-H bend was observed at absorption peak 1528.65 due to amines of steroids. Proteins, steroids, lactones and flavonoids were found as amide stretch C¼O was observed at peak 1662.54. Alkynyl stretch C¼C was observed at peak 2126.54. Alkyl C-H stretch was found at the peaks 2975.81, 2890.30, 2834. 71 because of glycosides. C-h stretch was observed at the peaks 3620.38 due to aliphatic compounds. Amide N-H stretch was found at the peaks 3779.57 because of existence of alkaloids in the respective plants.

The aqueous extract of *Fagonia cretica* changes their colors when warmed. Earlier, the *Fagonia cretica* extract had no color but afterwards it changes to brownish yellow. Upon subsequent inclusion of $AgNO_3$ to warm extract, color is changed again. Reduction of Ag+ ions because of heat can lead to color changes and formation of Ag+ complex. Silver Nanoparticles were observed due to this color shift. UV-Visible Spectrophotometry is utilized to authenticate the manufacturer of (AgNPs).

Our results are in consensus with the discovery of (Bibi *et al,* 2019) reviewed that after the reaction of the plant extract with AgNO3, the visual look of the blend shifted brown color which demonstrates the synthesis of AgNPs. FESEM reviewed the morphology of AgNPs and it was found that the nanoparticles formed were circular in shape ranging between 20-50mm in size.

Possible biomolecules in the silver nanoparticles (AgNPs) were analyzed through FTIR measurements. Existence of different functional groups in Silver nanoparticles samples was found through FTIR data in between 500-4000 cm^{-1} wavelength.

FTIR spectroscopy ranging in between 4000–500 cm^{-1} was conducted to analyze the exchange of AgNO3 to AgNPs by the macromolecules of the plant according to the studies of (Bibi *et al,* 2019) and our results are in consensus with it. Carbohydrates were found as C-H bend was observed in the leaf extract of *Fagonia cretica* at the peaks ranging between 611.01 and 623.73. Aromatic C-H bend was observed at the absorption peak 721.87 because of glycosides. C¼C stretch was found at the peak 978.33 which may be because of presence of coumarin, tennins, and terphenoids of the plant analyzed. C-O stretch was observed at the peaks 1046.95, 1088.25, 1215.30, 1294.75 due to ester in plant extracts. Unsaturated alcohols or alkanes in plant were found as H-C-H bend was observed at the peak 1448.94. N-H bend was found at the peak 1528.65 because of presence of amines of steroids. Amide stretch C¼O was found at the peak 1662.44 suggesting the existence of proteins, steroids, lactones, and flavonoids in plant. C¼C alkynyl stretch was found at the peak 2126.54. Alkyl C-H stretch was found at the peaks at 2975.81, 2890.30, 2834. 71 because of presence of glycosides. C-H stretch was found at the peaks 3620.38 because of open-chain acyclic composite because of fatty acid esters in plant extracts.

Different peaks i.e. 2θ of 38°, 45°, 65 and 77°that correlate with the reticulum position of 111, 200, 220 and 311, were observed by the XRD Spectrum of biologically formulated silver nanoparticles. Finer crystallinity of biologically formulated silver nanoparticles is assured due to the observance of more precise diffraction peaks.

Crystalline form of (AgNPs) was determined from the X-RD diffractogram, therefore our results are fall consensus with the studies of (Bibi *et al,* 2019). The scale of 20-70, robust intensities were found 38.3, 46.6, and 64.8 correlating with 111, 200 & 220 planes for silver nanoparticles. Crystallization of bio-organic phase was also evident by some other relevant peaks that were observed on upper area of silver nanoparticles.

Four distinct concentrations of silver nanoparticles with three microorganisms were used to analyze the antibacterial activity. The circle of inhibition around silver nanoparticles was observed in separate bacterial culture. The widest circle of inhibition was observed around *Klebsiella pneumoniae, Enterobacter* and *Streptococcus pyogenes* at the quantity of 100 μg silver nanoparticles.

According to the discovery of (Qais *et al,* 2019), silver nanoparticles were observed maximum antibacterial potential for reference bacteria and our results are in consensus with this study. Minimum Inhibitory concentration of 32 microgram per ml was observed for silver nanoparticles in case of MRSA and MSSA. Among these strains under study, ECS was observed as most sensitive with minimum inhibitory concentration of 16 microgram per ml.

Along with that, ECESβL2 was observed as highly resistant strain against silver nanoparticles with minimum inhibitory concentration of 64 microgram per ml.

Our investigated results are in accordance with the finding of (Gurunathan *et al,* 2013) studied that the various quantity of silver nanoparticles (1-10ug/ml) were analyzed in opposition to brest cancer for 24 hr time period. AgNPs could inhibit the cell viability due to heavy dose way. The interaction of silver nanoparticles with Cells showed increased reactive oxygen species and hydroxyl radical production. Therefore, the potential of AgNPs in opposition to apoptotic activity was ensured by activation of 3 caspase and DNA N-Fragmentation.

According to discovery of (Gurunathan *et al,* 2013), various quantity of silver nanoparticles (1–10 μg/mL) were used for therapy of MDA-MB-231 breast cancer cells for 24 hours and results demonstrated that AgNPs hinder cells viability due to increase dose and our results are also in consensus with it. Enhanced chemically reaction of oxygen species and hydroxyl radical development was found when cells were made open to silver nanoparticles. Moreover, turning on of pathways like 3 caspase & DNA nuclear dissolution assured apoptotic effects of (AgNPs).

5. Conclusion

Preparation of nanoparticles using green chemistry approach has many benefits as it speeds up the task and economically feasible. By using *Fagonia cretica* plant extract to produce rapid silver nanoparticles with a diameter range 30 nm that are environmental friendly. These silver nanoparticles are spherical and monodispersed. Chemical reagents or surfactant templates were not used in this method which ultimately ensures the eco-friendly bioprocess. Along with the other processes of synthesis of silver nanoparticles (AgNPs), it is relatively a distinctive idea to prepare these nanoparticles using plants.

Invitro antibacterial and cytotoxicity activity in opposition to the bacteria and HepG2 cancer cell line by prepared Silver nanoparticles (AgNPs) high with the high in AgNPs concentration. Results showed a favorable capability of the green manufacture of AgNPs as a cytotoxic product the beneficial application in the field of biomedical especially in the therapy of cancer and in preparation of drugs. The Fagonia cretica extract can be used as a suitable source in preparation of environmental friendly and reasonable cost for the manufacturer of silver nanoparticles (AgNPs) for industrial level synthesis and applied for the use in drugs producing manufacturer units, biomedicine, pharmacokinetics, pharmaceutics and bio-nanotechnology.

Future Perspective

This approach of preparation of silver nanoparticles (AgNPs) using plants will facilitate the investigators is not only to prepare safer nanomaterial, also understanding the health and safety of nanoparticles. Due to different application of (NPs) Nanoparticles, there are good potential to produce nanoparticles at industrial scale in future. On the whole, preparation of nanoparticles using plants is a growing and interesting field if nanotechnology and may have important effect on further advances in nanoscience. Furthermore, field of NPs preparation and synthesis of nanomaterial will be broadened with the access of advance techniques that could be good to produce nanodrugs in future.

CHAPTER 5
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