

Ph.D. Thesis

**Evaluation of Antioxidant Activity of Potential Allelopathic
Medicinal Plants from Moist Temperate Himalayas Pakistan**

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

DEDICATION

I dedicate my work to My Beloved Parents, family members, friends

&

Respected teachers

International Islamic University, Islamabad
Faculty of Sciences
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Dated: 07-04-2023

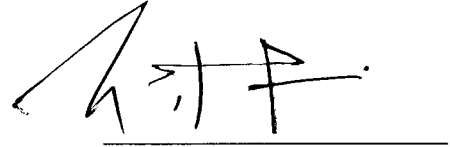
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It is certified that we have read the final thesis entitled “ Evaluation of Antioxidant Activity of Potential Allelopathic Medicinal Plants from Moist Temperate Himalayas Pakistan” submitted by **Mr Syed Anis Ali Jafri** and we judge that this thesis is of sufficient standard to warrant its acceptance by the International Islamic University, Islamabad for the Ph.D. degree in Biotechnology.

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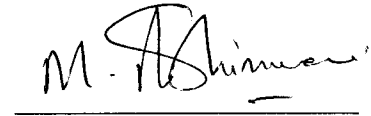
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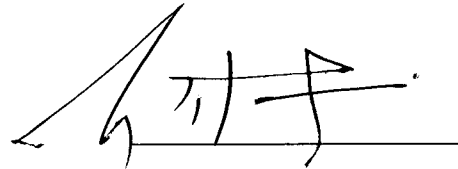
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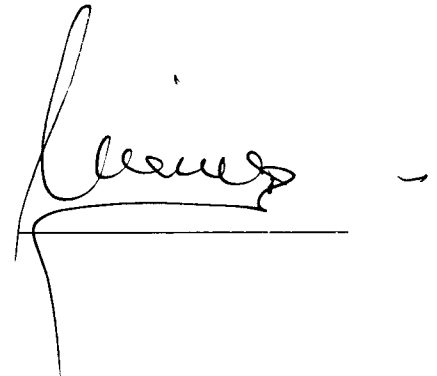
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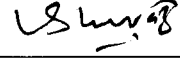
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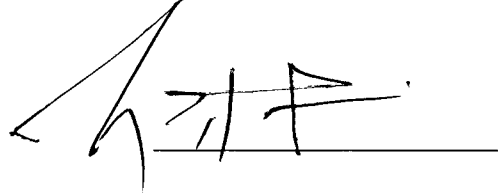
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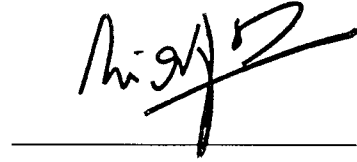
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DECLARATION

I *Syed Anis Ali Jafri* (49-FBAS/PHDBT/F15), student of PhD in Biotechnology (session 2015-22) at department of Biological Sciences, hereby declare that the matter printed in the thesis titled “Evaluation of Antioxidant Activity of Potential Allelopathic Medicinal Plants from Moist Temperate Himalayas Pakistan” is my own work and has not been published or submitted as research work or thesis in any form in any other university or institute in Pakistan or abroad.

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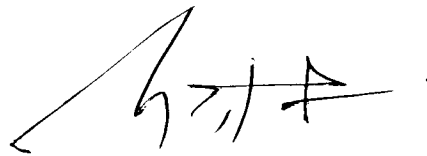


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The thesis entitled “Evaluation of Antioxidant Activity of Potential Allelopathic Medicinal Plants from Moist Temperate Himalayas Pakistan” submitted by Syed Anis Ali Jafri in partial fulfillment of PhD degree in Biotechnology has been completed under my guidance and supervision. I am satisfied with the quality of student’s research work and allow him to submit this thesis for further process to graduate with PhD Degree from Department of Bioinformatics and Biotechnology, as per IIU rules & regulations.

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ABSTRACT

The Allelopathic and antioxidant activity of medicinal plants has been of great importance to obtain the natural, less costly and less damaging products including drugs with reduced side effects. The tested medicinal plants were collected from moist temperate regions of Himalaya, Pakistan. The allelopathic activity was tested using Dish-pack and sandwich methods. The antioxidant potential of various extracts (ethanolic, methanolic and aqueous extracts) were also evaluated using different antioxidant assays such as 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay, FRAP (Ferric Reducing Antioxidant Power) assay and ABTS (2,2-azinobis(-ethylbenzothiozoline-6-sulphonic acid). The higher level of free radical scavenging or antioxidant activity was exhibited by ethanolic extracts of all these medicinal plant samples, followed by methanolic extracts in different assays when compared to the (90.36 ± 1.05 %, 85.48 ± 1.56 % and 88.64 ± 1.22 %) value of the ascorbic acid (standard) in DPPH, FRAP and ABTS assays respectively using UV-spectrophotometer. The highest absorbance was observed in ethanolic extracts of *Euphrasiae stricta* (71.92 ± 1.22 %), (65.77 ± 1.38 %) and (67.88 ± 0.74 %), followed by methanolic extracts 70.14 ± 0.82 %, 64.84 ± 0.74 % and 65.48 ± 1.40 % for DPPH (517nm), FRAP (700nm) and ABTS (734nm), assays respectively. The ethanolic extracts of *Euphorbia platyphyllos* L. showed the antioxidant activity of 69.76 ± 1.48 %, 64.42 ± 0.88 % and 65.54 ± 1.36 % and methanolic extracts 68.00 ± 1.50 %, 62.92 ± 0.64 % and 63.42 ± 0.94 % for DPPH (517nm), FRAP (700nm) and ABTS (734nm), assays respectively. Similarly, the ethanolic extracts of *Epimedium brevicomum* showed the antioxidant potential of 67.46 ± 1.26 %, 63.88 ± 0.54 % and 64.96 ± 0.79 % and methanolic extracts 66.78 ± 0.36 %, 60.68 ± 1.86 % and 62.59 ± 0.73 % for DPPH (517nm), FRAP (700nm) and ABTS (734nm), assays respectively. So, this research suggested that the *Euphrasiae stricta*, *Euphorbia platyphyllos* L. and *Epimedium brevicomum* medicinal plants possess a

significant antioxidant potential and are important source of natural antioxidants and can be effectively used in the treating oxidative stress related widely spreading degenerative diseases like cardiovascular disorders, atherosclerosis, cancer, inflammatory joint disorders, asthma, diabetes, dementia and eyes related degenerative diseases etc.

Key words: Reactive Oxygen Species (ROS), Antioxidant Potential, Free radicals, Antioxidant assays, Phenolic compounds, Ascorbic Acid and Medicinal plants.

CHAPTER 1

1. INTRODUCTION

1.1. Allelopathic Potential of Medicinal Plants

Allelopathy is the process in which plants adopt a competitive advantage by releasing allelochemicals or phyto-toxins into the surrounding environment (Pratley *et al.*, 1996). The increasing interest in allelopathy is believed to be an alternative to harmful synthetic herbicides to control weeds (Romeo *et al.*, 1999), and it is well identified that allelopathy has great potential to manage weeds (Weston and Duke, 2003). For weed management many new techniques have been adopted through genetic control of allelopathy and by the introduction of new allelopathic varieties by (Wu *et al.*, 1999). Research on production of allelopathic crop varieties has been experimented in many field crops, during recent past including, wheat (*Triticum aestivum* L.) (Wu *et al.*, 2000a) and (*Cucumber sativus* L.) (Parvez, 2003). Several varieties of crops have been identified to have strong potential for allelopathy with many weed species growths. The allelopathic plant is termed to as the "donor plant," whereas the plant which is affected by the allelochemicals released by allelopathic or donor plants is called "receiver plant." These two plants can affect one another with competition and allelopathy. The combination of these two terms and their effect is referred as "interference"(Khan, 2009). In allelopathic interference, many phyto-toxins or allelochemicals are secreted by donor allelopathic plants into its surroundings to inhibit the growth of other plants, but in competition effect, one plant absorb or take out one of the sources of growth from the soil which was important for the growth of other plants to reduce the other's growth (Wu *et al.*, 2000b).

1.1.1. Plant to plant allelopathic impacts of medicinal plants.

Many researchers are working with medicinal plants in the search of new allelochemicals. The allelopathic effect can be inhibitory or stimulatory depending on the dynamic and static availability, identity of the active compound and the target species (Keating, 1999). The allelopathic medicinal plants are easy to screen as compared to other plants, perhaps, due to their medicinal use (Fujii *et al.*, 2004; Yadav *et al.*, 2017). In plants with allelopathic activity, there are around 400,000 secondary metabolites and few of them have been examined yet (Swain, 1977). According to Wakdikar, (2004) there are only 12.5% medicinal plants and the unidentified compounds from them may have many promising growth inhibitors.

The allelopathic potential of a certain plant can be simply identified through Laboratory bioassay (Foy *et al.*, 1999). To check the mechanism of allelopathy several bioassays have been used in donor-receiver allelopathic plants (Haugland & Brandsaeter, 1996; Weidenhamer *et al.*, 1989; Shilling and Yoshikawa, 1987; Pederson, 1986; Liu and Lovett, 1993). A very little work has also been done on the development and screening of crops allelopathic potential for herbs to remove weeds. For this reason, more than 60 medicinal plants from Japan were screened for allelopathic potential by adopting water extraction method and it was observed that these medicinal plants possessed strong allelopathy.

1.1.2. Allelopathic effects of medicinal plants on soil rhizosphere

The presence of allelochemicals in the soil has a potential to affect different biotic and abiotic processes through recalcitrant of humic substances, sorption of organic matter, polymerization, microbial and chemical transformation into other compounds

(Hättenschwiler and Vitousek, 2000; Meiners *et al.*, 2017). The degradation of organic acids in the rhizosphere is reduced by allelochemicals but stimulates the biodegradation of xenobiotics (Shaw *et al.*, 2006). The nature of the soil is being affected by the release of different phenolic compounds in the rhizosphere. The knapweed, *Centaurea maculosa* Lam. affected the soil rhizosphere (Blair *et al.*, 2009). Both, type and concentration of these released allelochemicals affect plant physiology, growth rate and other functions of the plants (Cesco *et al.*, 2010). Some allelochemicals released from the roots of a plant reacts with soil organic matter or trapped in it. When the soil of *C. ladanifer* L. was analyzed, five different types of allelochemicals were found in it (Sosa *et al.*, 2010).

Approximately 70 medicinal plants were evaluated to check their allelopathy with the solvent extraction method involving water and methanol (Fujii *et al.*, 1991). The Sandwich method, a newly developed approach, was used to evaluate the allelopathic potential of 239 medicinal plant species. This method is believed to be very efficient and useful and has been used preferably in recent research to check the allelopathy of different medicinal and other plants as it is very less time consuming and large number of samples can be screened in a very short time within the laboratory. The Plant box method is a specific bioassay to check the root samples of plants to screen for their Allelopathic activity (Fujii *et al.*, 2007). The Sandwich method has been used to check the allelopathy of leaf litter leachates of 160 medicinal plants taken from different research stations (Shinwari and Ibrar, 2008).

1.1.3. Effect of allelopathy on soil microorganisms

The microbial abundance is much higher and directly attached to the roots of plants in the soil (Smalla *et al.*, 2006). The chemicals released from plants into the rhizosphere affect microorganisms and making rhizosphere a complex environment (Mukerji *et al.*,

2006; Li *et al.*, 2017). Plant species, soil type, management practices, microbial interactions, and other environmental factors affect the diversity and composition of bacterial taxa in the rhizosphere (Hoitink and Boehm, 1999). Allelochemicals affect the microbial degradation of chemicals in soil (Schoefer *et al.*, 2003). Allelochemicals also attract pathogenic microbes; promote the growth of plants and microbial communities (Cook *et al.*, 1995). Plant growth promoting rhizobacteria is indirectly advantageous for plant growth as they control some biologically harmful microorganisms or root pathogens that inhibit plant growth due to parasitism, decreasing pollutant toxicity, including antibiotic and hydrogen cyanide production, synthesis of extracellular enzymes to hydrolyze the fungal cell wall and competition for nutrients and niches within the rhizosphere (Bhattacharyya and Jha, 2012).

Various diseases have been treated with the use of different types of plants by traditional people; however, the people from different regions may have different recipes for these medicinal plants. Many disorders in the human body have been effectively cured with a rich traditional medicinal plant alone or in different combinations and a lot of indigenous knowledge is there about the use of these medicinal plants (Stevens and Tang, 1985). It was observed that more than 300 different diseases and disorders have been treated with the plants with a wide range of medicinal properties. The importance of medicinal plant sources has increased the human health by recognition of natural products by natural processes. Many medicines have been derived from natural plant resources from the currently available plant (Fabbro *et al.*, 2014).

1.1.4. Effect of allelopathy on environment

At the ecosystem level, non-indigenous plants have altered the geomorphological process, hydrological cycle, and biogeochemical process and at the community level, there is

the change in resource competition (Gordon, 1998). The allelopathic medicinal plants regulate the carbon cycle as well as fix atmospheric carbon dioxide (Haase *et al.*, 2007). The allelopathic medicinal plants have an ecological role in biodiversity conservation. They affect the plant ecology through plant succession, structure of plant communities, growth, diversity, dominance, plant productivity, and occurrence (Ferguson and Rathinasabapathi, 2003). The Algae species *Macrocystis pyrifera* and *Saccharina japonica* are known as underwater forests and build structural habitats within coastal ecosystems. They play a diverse role in ecological services (Tang & Gobler, 2011). The genus *Ulva* alleviates the pollution load and nutrient enrichment. They inhibit the growth of other harmful algal blooms through allelopathy (Tang & Gobler, 2011). Water chemistry has been significantly affected by some aquatic species like *Echhornia crassipes*, *Hydrilla verticillata*, and *Pistia stratioides*. Due to these species, there is an increase in watercolor, turbidity, and dissolved carbon dioxide level, but the decrease in dissolved oxygen, phosphorus, and pH (Schmitz *et al.*, 1993). They also promote the expansion of coastal dead zone by developing anoxic condition (Smetacek & Zingone, 2013). *Microcystis aeruginosa* can inhibit the growth of green algae (*Chlorella pyrenoidosa*, *Scenedesmus quadricauda*) and a diatom (*Cyclotella meneghiniana*), and potentially treat the water and wastewater (Wang *et al.*, 2017). The allelochemicals from *Potamogeton crispus* L. can reduce the eutrophication and effectively improve water quality as well as restore the eutrophic lake (Zhou *et al.*, 2017).

For some reason the habitat degradation and a significant reduction in the wild medicinal population have been observed (Bravo, 1998). So, there is a need for the conservation and sustainable use of the medicinal plant resources. The pressure on wild plants of medicinal plants can be reduced by their conservation in-situ and ex-situ units. Use of these wild plant resources to agriculture is one of the most important ways to conserve

these plant resources. So, there is a need for detailed study to evaluate the chemical effects of these plants on other plants, before they are introduced into agriculture, (Doku *et al.*, 2016).

The Allelopathy is the chemical relationship of plants with one another. The harmful, negative, motivational and beneficial effects of a plant on other species by forming chemical products (end products or by-products) that effect to the environment was termed "allelopathy" (Bandoniene and Murkovic, 2002).

1.1.5. Use of allelopathic plants as a natural herbicide

The medicinal plants affect the growth of weeds and have the potential to use them as natural herbicides (Sodaeizadeh *et al.*, 2009; Li *et al.*, 2009). It is important to note that the allelopathic effect of plant species on each other could be an alternative to pesticides and open a new arena in environmental protection. The phytotoxins can affect shoot growth, germination, root growth, respiration, photosynthesis, metabolic production and results in cell death of the vulnerable plants (Weir *et al.*, 2004). Furthermore, natural allelochemicals can reduce the number of risks from synthetic pesticides, including persistency of pesticides, high cost and environmental consequences (Quaswm and Foy, 2001). *Sorghum bicolor* L. has strong allelopathic potential and released phenolic compounds.

Many scientists conducted Allelopathic experiments in various medicinal plants. The phytochemical effects of three plant species (*Rheum emodi*, *Saussaurea lappa* and *Potentilla fulgens*) were investigated by Riaz *et al.*, (2013). The allelopathic effects on traditional food crops in response to these three herbs were observed. The aqueous extracts of *P. fulgens* and *S. lappa* significantly reduced the growth and germination of all traditional food crops.

1.2. Evaluation of Free Radical Scavenging Activity /Antioxidant Activity

It has been investigated that the reactive oxygen species (ROS) in human body, e.g., H_2O_2 , O^{2-} and OH^- are found in large amounts. These may be primarily because of the reason that almost 5% or more oxygen (O_2) which is inhaled by a human is transformed to these ROS because of univalence reduction of O_2 (Maxwell, 1995; Gupta and Sharma, 2006). The reactive oxygen species (ROS) are free radicals like hydroxyl radical, nitric oxide radical, hydrogen peroxide, superoxide anion radical, hypochlorite radical, lipid peroxides and various singlet oxygen molecules (Hazra *et al.*, 2008). Our body produces certain compounds called free radicals in it by its own during different processes (Pham-Huy *et al.*, 2008).

The normal physiological processes result in lesser production of free radicals in the body but if high abnormal functioning or less antioxidant level results in oxidative stress resulting in generation of free radicals (Valko and Jomova, 2011). These free radicals are the cause of many degenerative and chronic diseases like Parkinson's disease, arthritis, cancers, stroke, Alzheimer's disease, immune suppression, atherosclerosis, ageing, diabetes mellitus, chronic inflammatory diseases, ischemic heart disease and neurodegenerative diseases (Devasagayam *et al.*, 2004). On the other hand, some other factors are also responsible for the increased level of these free radicals; instead of natural causes are alcohol, smoking, ionizing radiation, chronic diseases and environmental pollution (Gupta *et al.*, 2009).

Various important diseases such as diabetes, cancer, cirrhosis, obesity and cardiovascular disorders may be caused by these free radicals (Hertog and Feskens, 1993). So, several enzymatic antioxidant barriers like super oxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) have been efficiently used to neutralize the harmful properties of these free radicals. However, to investigate this oxidative stress, certain factors

like ultraviolet rays, unnecessary NADPH stimulation, and cigarette smoke, environmental contaminants/pollutants exposure, mitochondrial electron transport chain, radiation, some parasitic infections or toxic chemicals have been focused and are responsible in causing the overproduction of the ROS. The oxidative stress is a change in the equilibrium/normal position of an antioxidant or pro-oxidant processes in a living system, resulting in the mutilation/harm to different component like DNA, lipids and membranes proteins and collectively to whole cell structures (Valko *et al.*, 2006; Aliyu *et al.*,2009).

These free radicals are bound by very vital chemical compounds the Antioxidants which reduce and prevent us from the harmful effects on normal cells of the body. Some antioxidants are produced artificially, as butylated hydroxyl-toluene (BHT) and butylated hydroxyl-anisole (BHA) which are available commercially are less stable and are very harmful where natural antioxidants are very safe. That is the reason that the antioxidants have been preferably obtained from natural products (Knight, 1998).The fruits and vegetables, herbs, cereals, sprouts, seeds, and edible mushrooms are the natural sources of food which can be used as an efficient source of these antioxidants to minimize the effects & damage from free radicals (Kaur and Kapoor, 2001).The phyto-constituents that are responsible for antioxidant potential are mainly flavonoids, phenols, anthocyanin, iso-flavones, flavones, lignans, catechins, iso-catechins and coumarins found in the plants were determined by using total phenol and flavonoid estimation TPC and TPF using DPPH, FRAP and ABTS assays (Kanwal *et al.*,2015).

When there was not even the concept of synthetic chemical medicines and surgical management, the plants have been the only source of traditional medicine to man (Cao and Ronald, 1998). It is a fact that indigenous populations have more knowledge about natural resources as plants and other as they are fully dependent on them (Lewis and Elvin-

Lewis, 2003). Many secondary metabolites are also found in Medicinal plants possess that are responsible for their importance as a potential natural source of disease preventing and treating chemical compounds that's why a lot of researchers have been attracted towards the plants to obtain these natural substances important for their medicinal properties (McKinney, 2002). The healing potential of various phytochemicals from plants are flavonoids, tannins, alkaloids and phenolic substances was analyzed in DPPH, FRAP and ABTS antioxidant assay (Rai and Kon, 2013).

Timur or Indian prickly ash, a large spiny shrub or small tree, Botanical name *Zanthoxylum armatum*, is a very important medicinal plant. It is widely spread in Kashmir, throughout India and towards the Bhutan at high altitudes of 2,500 m. It is also found in Nepal, Taiwan, Philippines, China, Japan, Malaysia and in Pakistan from 1,300-1,500 m altitudes (Okwu, 2005).

The ROS diseased conditions can be effectively improved by compounds called antioxidants that can hunt and neutralize the free radicals (Wilson, 1988). An extensive diversity of free radical hunting or antioxidant components like phenols, vitamins, terpenoids and flavonoids have been found in plants which possess high antioxidant potentials (Cai and Sun, 2003). The plant derived polyphenolic constituents might be more useful *in-vivo* with their positive effects as these are proved to be more efficient antioxidants as compared to vitamins E or C *in-vitro* (Rice-Evans and Miller, 1979).

Different medicinal plants had been efficiently applied to treat the ROS and are of great importance having antioxidant potential because of such sort of alimentary radical scavenging diet supplement. The antioxidant potentials of medicinal plants are mainly because of rich source of phyto-nutrients and ingredients like phenols, flavonoids and terpenoids present in them. The antioxidant potentials of many medicinal plants have been

studied (Ghosal *et al.*, 1996) and (Khopde *et al.*, 2001) like anti-cancer and Immunomodulator activity, (Sai *et al.*, 2002), the Hepato-protective benefits, hypolipidemic activity (Mathur *et al.*, 1996).

Hence, the free radical scavenging activity by evaluation and comparison of various medicinal plant with different protocols using spectrophotometer was the main aim of the present study (Gulati *et al.*, 1995; Roy *et al.*, 1991).

Before the coming of the modern pharmacological medicines, the people of Manipur have been using medicinal plants for the treatment of diabetes mellitus. Even today, people not only in the rural areas but those living in the urban areas are also using these traditional medicines and give first preference to herbal treatments by consulting the medicine (Singh, 2011).

The bioactivity of these plant extracts was because of high phenolic and flavonoid contents found in them. A number of oxidizing molecules, like singlet oxygen and numerous other free radicals responsible for different diseases can be effectively scavenged by using the flavonoids (Agati *et al.*, 2012). The reactive oxygen formation and chelate trace elements involved in free-radical production can be effectively suppressed by using these Flavonoids. These can also be used to protect and up-regulate antioxidant defenses and in scavenging of reactive species. Also, the oxidative stress tolerance on plants can be conferred by these phenols and flavonoids. Many plant sources rich in phenols and flavonoids in various plants extracts have been efficiently used in the food industry because of their antioxidant properties and other health assistances e.g., fruits, herbs, vegetables and cereals etc.

1.3- Problem Statement

1.3.1. Allelopathic Study

Herbicides which have been used to control weeds have very harmful effects on other plants, animals, humans, water and overall ecosystem by adding dangerous chemicals in them and making them poisonous and unfit. All the chemicals used in agriculture today, for example herbicides etc. are useful to the ecosystem only if they are properly used. As herbicides Sulfonylurea have been in use excessively in Asia since 1990 as it is very efficient for broad leaves paddy weeds even at very low doses. But many local competitive weeds are observed to have disappeared, such as *Brasenia schreberi* and *Sagittaria aginashi*, affecting the biodiversity because of excessive and repeated use of these herbicides. The cost of weed control can be minimized economically by using allelopathic crops.

- The secondary metabolites released from medicinal plants can affect shoot growth, germination, root growth, respiration, photosynthesis, metabolic production and results in cell death of the vulnerable plants.
- At the same time, the investigation of secondary metabolites can be used as bio pesticides and have a potential to reduce the number of risks from synthetic pesticides, including persistency of pesticides, high cost and environmental consequences.
- The allelopathic potential of medicinal plants of Pakistan is still unexplored. Few basic data is available.

1.3.2. Problem Statement Antioxidant study

The Free Radicals can cause the oxidative damage to living cells resulting in many lethal diseases like Parkinson's disease, arthritis, cancers, stroke, Alzheimer's disease, immune suppression, atherosclerosis, ageing, diabetes mellitus, chronic inflammatory diseases, ischemic heart disease and neurodegenerative diseases etc. Antioxidants are very useful to prevent oxidative damage of cells which is the cause of these lethal diseases. Various antioxidants are excessively found in various medicinal plants, which are the cheap source of treatment and control of various diseases.

1.4. Aims and Objectives

This study was conducted to evaluate the antioxidant activity of various allelopathic medicinal plants from moist temperate Himalayan regions of Pakistan.

The main objectives of this study were,

- To Investigate the allelopathic potential of different medicinal plants from Himalaya.
- To assess the allelopathic potential of selected medicinal plants through bioassay (Sandwich and Dishpack Method).
- The allelopathic medicinal plants were further tested for their potential antioxidant activity.
- Evaluation of the antioxidant activity of plant extracts using Bioassays, e.g., DPPH, FRAP and ABTS
- To determine the Total Phenol Contents (TPC) and Total Flavonoid Contents (TFC)
- To study the Phytochemical screening of medicinal plants
- Identification of plant sources of antioxidants: plants containing high levels of antioxidants, like polyphenols, flavonoids, and carotenoids

1.5. Significance of Study

1.5.1. Significance of Allelopathic study

The plant productivity, growth, development, ecology, occurrence, diversity and succession are largely affected by beneficial as well as negative effects of Allelochemicals. The seedling growth, reduction in root length and inhibition of seed germination under negative as well as positive effects (stimulation of growth) were examined in this study. This study attempted to explore both negative allelopathic effects comprising of reduction root length, inhibition of seed germination and seedling growth as well as positive effects (stimulation of growth). Meanwhile, it had endorsed the application of selected medicinal plants in the soil environment conservation and as a natural herbicide. The application of selected medicinal plants as a natural herbicide also has an important role in the conservation of soil environment. Awareness may also be provided to farmers and rural communities through this study. Also, a new scientific document may be developed as baseline for future researchers.

1.5.2. Significance of Antioxidant study

The growth and development of various diseases in living systems has been mainly caused because of the oxidative stress. The side effects of oxidative damage caused by reactive oxygen species (ROS) can be prevented or treated by combining external antioxidants or strengthening the body's internal antioxidant defenses. So, a wide range of non-enzymatic antioxidants that can reduce ROS oxidation have been efficiently synthesized by various medicinal plants. Various laboratory free radical scavenging protocols were used to test the antioxidant potential of several medicinal plants, and strong antioxidant activity was observed in few medicinal plants. However, the plant antioxidants must undergo many

physiological processes before applying its therapeutic efficacy in-vivo, because the antioxidants potential of studies in the laboratory and in-vivo results are not always the same.

Therefore, the phytochemical activity and antioxidant potential and total phenolic and flavonoid contents of tested medicinal plants were observed in this study. The applications and limitations of allelopathic and antioxidant activity measurement tests and their application to determine the exact pathways for their use that should be followed for future research in the field will also be highlighted and appropriate recommendations will be provided.

1.6. Hypothesis

1. The allelochemicals produced by various plants in the soil affect the growth and development of other plants and soil environment.
2. The medicinal plants are the chief source of antioxidants and phytochemical sources may be useful to reduce chronic disorders like cancer etc.

1.7. Limitations

Although, comprehensive proposed objectives were covered by this study, but it lacks the isolation of secondary metabolites like phenols, flavonoids, alkaloids, isoprenoids, terpenoids and glucosins etc., to carry out their characterization by analytical techniques due to limited resources and time constraints.

CHAPTER 2

2. REVIEW OF LITERATURE

2.1. Allelopathic Activity of Medicinal Plants

An attempt has been made in this article to accumulate and elucidate the literatures about the allelopathic potentiality of medicinal plants, and its role on the development of bio-herbicides for eco-friendly weed management strategies to conserve bio-diversity and ecological balance. *Ochna integerrima*, a deciduous shrub in the family Ochnaceae, was used in this study was carried out with completely randomized design to determine the allelopathic potential of *O. integerrima* leaf extracts in six concentrations against the seedling's growth of dicots plants [garden cress (*Lepidium sativum* L.), lettuce (*Lactuca sativa* L.), alfalfa (*Medicago sativa* L.)], monocot plants [Italian ryegrass (*Lolium multiflorum* Lam.), timothy (*Phleum pratense* L.), and barnyard grass (*Echinochloa crusgalli* L.]. These findings suggested that the leaf extracts of *O. integerrima* may possess allelopathic substances which could be used as a source of bio-herbicide for reducing the weed burden in crop fields. Thus, more research is needed to isolate and identify the allelopathic substances in *O. integerrima* in order to produce bio-herbicide for weed management (Seinn and Kato-Noguchi, 2022).

The allelopathic effect of various concentrations (0, 6.25, 12.5, 50 and 100gL⁻¹) of *Parthenium hysterophorus* Methanol extract on *Cyperus iria* was investigated under laboratory and glasshouse conditions. No seed germination was recorded in the laboratory when *P. hysterophorus* extract was applied at 50gL⁻¹. In the glasshouse, *C. iria* was mostly injured by *P. hysterophorus* extract at 100gL⁻¹. The phytochemical constituents of the Methanol extract of *P. hysterophorus* were analyzed by LC-ESI-QTOF-MS=MS. The results

indicated the presence of phenolic compounds, terpenoids, alkaloids, amino acids, fatty acids, piperazines, benzofuran, indole, amines, azoles, sulfonic acid and other unknown compounds in *P. hysterophorus* methanol extracts (Motmainna *et al.*, 2021).

Natural product-based herbicides could be the effective alternatives to synthetic chemical herbicides for eco-friendly weed management. This research, therefore, was conducted to identify the phytotoxic properties of *Parthenium hysterophorus* L., *Cleome rutidosperma*, and *Borreria alata* with a view to introducing them as a tool for natural herbicide development. The Methanol extracts of these plants were examined on the germination and growth of *Zea mays* L., *Oryza sativa* L., *Abelmoschus esculentus* L. and *Amaranthus gangeticus* L., *Oryza sativa* (Weedy rice), *Echinochloa colona* L., *Euphorbia hirta* L., and *Ageratum conyzoides* L. under laboratory and glass-house conditions. In the laboratory experiment, three plant extracts of 0, 6.25, 12.5, 50, and 100gL⁻¹ were tested on survival rate, hypocotyl, and radicle length of eight test plant species. Among the weeds and crops, *A. conyzoides*, *E. hirta*, *A. esculentus* and *A. gangeticus* were mostly inhibited by *P. hysterophorus* extract at 100gL⁻¹. Based on these results, *P. hysterophorus* was the most phytotoxic among the tested plant extracts and could be used for developing a new natural herbicide for green agriculture (Motmainna *et al.*, 2021).

The experiment was conducted to investigate the allelopathic potential of the aqueous extracts of different parts of *Jatropha curcas* on seven selected field crop species. The experiments were conducted following a completely randomized design with three replications. At the same concentration, *J. curcas* oil extract completely (100%) inhibited the shoot and root growth of all the test crop species except rice. Similarly, seed extract completely inhibited (100%) the shoot and root growth of jute and mustard, whereas around or more than 70% inhibited the shoot and root growth of all the test species except rice.

These results confirm that *J. curcus* has allelopathic properties and may possess allelochemicals (Islam *et al.*, 2020).

The present study investigated the allelopathic potential of sawdust obtained from eleven tropical tree species available in Bangladesh viz., *Azadirachta indica*, *Swietenia macrophylla*, *Acacia auriculiformis*, *Tamarindus indica*, *Eucalyptus camaldulensis*, *Syzygium cumini*, *Mangifera indica*, *Albizia saman*, *Artocarpus heterophyllus*, *Diospyros discolor* and *Tectona grandis*. Four concentrations of aqueous sawdust (1:5, 1:10, 1:15, 1:20 (w/v)) were tested for their potentiality in inhibiting seedling growth of allelopathic sensitive plant *Raphanus sativus* under laboratory condition. Control (distilled water without extract) was also maintained in each case. The results of this experiment showed that *S. macrophylla*, *E. camaldulensis*, *M. indica* and *A. saman* inhibited more than 90% shoot and root growth of *R. sativus*. The results showed that the effect of different sawdust on the weed control varied significantly. Weed growth suppression by the sawdust was increased with the increase in application rate (Islam *et al.*, 2019).

The farmers of different countries use several weed management techniques in order to minimize the deleterious effect of weeds. Among them the most commonly used practice is application of herbicides due to its high efficacy and cost effectiveness, less time-consuming nature, flexibility and easy accessibility, and more rapid out return. Although use of synthetic herbicides in the crop fields has increased the crop production as well as the productivity by reducing the weed infestation, over time it causes a number of environmental hazards. This paper intended to reflect the current state of allelopathic medicinal plants and their potentiality to develop eco-friendly and natural product-based herbicides for sustainable agriculture, and to invite further debate on this issue (Islam *et al.*, 2018).

Allelochemicals are released by different mechanisms, including root exudation, volatilization and decomposition of residues into the soil rhizosphere. Allelopathic plants have diverse ecological role, such as regulation of soil biota, nutrient chelation, and plant defense through low-cost biological control of insects, diseases and pests, decomposition of residues improving nutrients and enhancing the crop diversification by reducing weed infestation. Wheat is used as an allelopathic plant which releases numerous chemicals affecting the soil environment (Aslam *et al.*, 2016).

An experiment was conducted at the Horticulture Farm of Bangladesh Agricultural University, Mymensingh to test the impact of plant growth regulators on growth and yield of summer tomato. In case of combined effect of variety and plant growth regulator, the maximum plant height (87.90 cm), number of flowers and fruits (49.04 and 21.91, respectively) plant⁻¹, individual fruit weight (61.16 g) and fruit yield (27.28 t ha⁻¹) were observed in BARI Hybrid Tomato-8 when treated with 4-CPA + GA₃ together, and the minimum for all these parameters were found in control plants. The results of the present study suggest that both 4-CAP and GA₃ together can be practiced for increasing summer tomato production for both varieties (Rahman *et al.*, 2016).

The present study was conducted to explore the allelopathy of *Hyptis suaveolens*, an important medicinal plant of Lamiaceae family. The aqueous Methanol extracts of this plant at four different concentrations (3, 10, 30 and 100 mg dry weight [DW] equivalent extract/ml), were examined on the seedling growth of eight test plant species, cress (*Lepidum sativum* L.), lettuce (*Lactuca sativa* L.), alfalfa (*Medicago sativa* L.), rapeseed (*Brassica napus* L.), timothy (*Phleum pratense* L.), crabgrass (*Digitaria sanguinalis* L. scop.), barnyard grass (*Echinochloa crus-galli* L.) and Italian ryegrass (*Lolium multiflorum* Lam.) and on the germination of cress and Italian ryegrass. However, isolation and identification of these

allelochemicals from *H. suaveolens* plant extracts could serve as the lead for new natural herbicides development for sustainable weed management strategies (Islam and Hisashi Kato-Noguch, 2013).

It has been shown by Shinwari *et al.*, (2013) who made three-dimensional approach to check and identify the allelopathic activity of 38 medicinal plants and weeds of Japan and Pakistan which were originated from 5 different continents, carried out at Plant Chemical Ecology Laboratory at National Institute of Agro-Environmental Science, Japan from January 2008 to March 2009. The information obtained can also be helpful in the development of new and potent bioactive chemicals from natural products to control environmental risk.

It has been identified by Kundu *et al.*, (2013), who observed the essential oils by Gas chromatography-Mass spectrometry (GC-MS) obtained by hydro-distilling of *Eupatorium adenophorum*. Almost Twenty-six essential oil components were isolated consisting of sesqui-terpenes and monoterpenes. The antioxidant activity of essential oil was comparatively similar to the standards. The essential oil and the cadinene sesquiterpene- rich extract of *E. adenophorum* possess potential antioxidant activities. It is a potential natural antioxidant due to the wide availability of the plant and the potential antioxidant activity.

2.1.1. Allelopathic effects of different plants on other plants and agricultural crops/weeds

The pharmacological and toxicological properties of *Acacia concinna* are well known and it has been used as a traditional medicine. To investigate the allelopathic activity of *Acacia concinna* the pod was extracted with aqueous Menthol. It has been observed that *Acacia concinna* pod extract inhibited the growth of roots and shoots of *Lactuca sativa*, *Brassica napus*, *Lepidium sativum*, *Medicago sativa*, *Lolium multiflorum*, *Phleum pretense*,

Echinochloa crus-gallis and *Vulpia myuros*. With the increase of pod extract there has been a decrease in growth of plants. It has been observed that the test plants show more inhibition in growth as compared to shoots. *Acaccia concinna* pods and their extracts can be applied for weed management and control (Boonmee and Kato-Noguchi, 2017).

A big threat for crop production is the use of synthetic chemicals due to non – degradability, toxicity and negative effect on the environment. The plant architecture is also adversely influenced by the presence of extracts. On the basis of finding, it shows that the seedling of *Brassica napus* shows toxicity and allelopathic effect is due extract of the plant which produce oxidative stress. Besides, *Onosma bracteatum* can be used as a natural herbicide in the form of allelopathic compounds isolated from this plant species or in the form of extract which is the replacement of harmful synthetic herbicide (Ali *et al.*, 2017).

The sunflower (*Helianthus annuus* L.) due to their allelopathic effect can be used for controlling weeds in different crops as well as in sunflower. Due to the presence of phenolic compounds and terpenes which are important allelochemicals present in sunflower. The strong allelopathic potential shows the residues of sunflower plant which can be used for controlling weeds under many agricultural settings either mixing it into the soil or by scattering it in the form of a layer over the Soil. Due to their allelopathic effect different genotypes of sunflower can be cultivated for controlling weeds in sunflower (Quan and Liang, 2017).

2.1.2. Allelopathic effect of various plants on soil

The invasive *Acacia dealbata* had changed the soil characteristics due to the release of allelopathic compounds. The seedling growth of *Acacia dealbata* in native soil had been affected negatively by its leachates and found no interaction between *Acacia dealbata*

leachate and soil microbiota. Soil microbiota had shown a positive effect on the initial growth of *Acacia dealbata* but decreased the seedling growth (Lorenzo and Rodríguez-Echeverría, 2012).

It has been evaluated by Ali *et al.*, (2013) who conducted experiments to check the effects of allelopathy of *Rhynchosia capitata*. Mung bean (*Vigna radiate*) was influenced by it for inhibition of its seed germination and seedling growth and also checking the allelochemicals reason for this activity. The *R. capitata* plant extracts contained a large amount of water-soluble phenolic acids. The leaf extracts had the more content of total phenolic acids than that of the fruit, stem or root extracts. Two phenolic acids involving vanillic acid and 4-(hydroxymethyl) benzoic acid were observed in leaf extracts of *R. capitata*.

2.1.3. Allelopathic effect of some plants on soil microorganism

The activity of Coumarin has been demonstrated against weeds in in- vitro conditions. In each experimental system and treatment, plant growth (mass and length of root and shoot), fungal and bacterial genetic diversity and soil microbial activity and mass has been determined. The results show that the coumarin can be used as a promising natural herbicide and has a potential to apply in sustainable agriculture (Niro *et al.*, 2016).

The relationship among microbes controls the occurrence of unsafe algal blooms which intimidate water quality. These interactions can be influenced by the release of allelochemicals. The research has been conducted to investigate the seasonal changes among bloom forming cyanobacterium *Microcystis aeruginosa* and benthic microbial assemblages. The result shows that the allelopathy and phosphorus (P) competition by the microbial muster differ seasonally and inhibit growth of *M. aeruginosa*.

It was identified by Itani *et al.*, (2013) that because of their bioactive properties, several medicinal plant species are extensively used by traditional population as herbs. This research shows that many herbs possess allelopathy and among them the horseradish has the highest allelopathic activity. The unknown allelopathic substances may be released by living horseradish into the solution. So, the horseradish can be one of the preferable candidates having novel biologically active chemical compounds and may be used to control weeds in variety of agricultural settings for intercropping crops and as a soil additive material.

2.1.4. Anti-microbial activities of allelopathic plants

The use of plants as antifungal is an alternative to synthetic fungicide is an important aspect which is currently in the spotlight. To check the antifungal activity, chemical analysis of 16 essential oils was determined. It has also been observed that oil containing mainly monoterpene alcohols had been more effective and inhibited all fungi tested. Furthermore, it had been decreased the total number of fungi, in situ, using essential oil was evaluated (Stevic *et al.*, 2014).

The secondary products present in plants comprise of different metabolites and reflect the beneficial effects of medicinal plants. The medicinal actions of plants are considered as a unique phenomenon attributed to a taxonomically distinct and specific plant species having a consistent release of combination of secondary products. The screening of the plant species was carried out by ethnobotanical approach, known as a common technique to study antifungal and antimicrobial activities in pharmacology. Several plants have shown antifungal and antibacterial activities (Saranraj and Sivasakthi, 2014).

2.2. Antioxidant Potential of Medicinal Plants

It was checked by Itani *et al.*, (2013) that the different medicinal plants have been excessively used in traditional Indian medication for their antioxidant, anticancer and

possible anti-inflammatory activities. The *H. isora* has potential cytotoxic activity toward the selected cancer was shown toward the target cancer cells such as HL-60 ($30.25 \pm 1.36\%$), HeLa-B75 ($34.21 \pm 0.24\%$), PN-15 ($29.21 \pm 0.52\%$) and HEP-3B ($25.36 \pm 1.78\%$). The discovery of novel anticancer, anti-inflammatory and antioxidant agents in these plants as shown by the results of the present findings strengthen the potential of the selected plants as a source of medicine.

The Joshi *et al.*, (2014) identified that the *Moringa oleifera* plant and identified that its leaf extract possesses anti-oxidative effect on the human KB cells, treating cervical cancer cell line and alveolar cancer cell line A549 cells. In the breast cancer cell lines, the leaf extract showed a dose-dependent and time dependent inhibition on proliferation of cell. In the normal cells the less cytotoxicity was observed, and cellular adhesion is inhibited by it and wound healing in treatment of cancer cells. The anticancer effects on the breast cancer cells induced by the leaf extract from *M. oleifera* were observed. It can be confirmed as an anticancer drug by further studies.

2.2.1. Antimicrobial Activities of Medicinal Plants

The Ganie *et al.*, (2014) observed that the Gaozaban and almost every plant commercially at market with antimicrobial, antibacterial, anti-inflammatory, antifungal and wound-healing properties has *Arnebia benthamii* as a major ingredient. In this study, various extracts of *Arnebia benthamii* were observed *in-vitro* to check their antioxidant and anticancer activity. The Sulphorhod-amine B assay was used to test Cytotoxicity of the extracts ($10\text{--}100\mu\text{g/ml}$) on five human cancer cell lines (prostate, lung, colon, leukemia, and pancreatic cell lines).

The Sharifi-Rad *et al.*, (2015) that the *Salicornia herbacea*, a medicinal plant and prepared the Methanolic extracts from its dry seeds and leaves and were *in-vitro* screened for

their potential allelopathic effects at 2.5, 5 and 10% (v/v) on the seedling parameters and seed germination of medicinal plants *Nigella sativa*, *Hyssopus officinalis* with weeds *Amaranthus retroflexus* and *Taraxacum officinale*. The seed extracts have the same trend. The active compounds or allelo-chemicals in *S. herbacea*, may be the reason for negative effects on both weeds. Although the chemicals for this allelopathic activity are still to be identified, seed and leaf extracts of *S. herbacea* can be applied as an efficient herbicide when applied in concentration of 5 or 10%.

2.2.2. Antitumor/Anticancer Activities of Medicinal Plants

The Iqbal *et al.*, (2015a) who studied the *Goniothalamus velutinus* Airy Shaw from the Annonaceae family, is known to possess antitumor, anticancer, and many other useful qualities. The ABTS and FRAP method were used to measure the Trolox equivalent antioxidant capacity (TEAC). With ABTS method 79 mg and 106 mg trolox equivalent (TE)/g results for bark and leaf extracts were observed respectively. 80 and 89 mg TE/g, results for bark and leaf extracts for FRAP assay, respectively.

The Parray *et al.*, (2015) observed the antioxidant and antibacterial activities of a medicinal plant extracts of *Crocus sativus* L. Kashmirianus. Under *in-vitro* conditions from corm slices the callus was produced on MS medium having BAP (20 μ M) + NAA (15 μ M). As the different parts of plants show diverse pharmacological activities, it was also observed that callus formed in laboratory conditions helps in increased production of secondary metabolites, which releases the pressure from saffron.

The Shameem *et al.*, (2015) investigated the *Arnebia benthamii*, a very important medicinal plant which has been extensively used to treat different harmful prevailing diseases in that area traditionally for many years. Almost the same strength of antioxidant activity of

the shoots and root part extracts, where the roots showed relatively a more antioxidant effects in different concentrations of superoxide anion and hydroxyl radicals.

The Greenwell and Rahman (2015) identified that the globally the human population is extensively affected by cancer disease. So, for the treatment, control, and prevention of this deadly lethal disease there is an intensive need for new strategies. These most recent strategies are nanomedicines using nanoparticles, which are very efficient to increase the drug delivery at the spot of cancer activities through plant-derived drugs which are useful to control release of the compound and to investigate new administration methods. Here, the medicinal plants, the demand for naturally produced chemicals and their properties which contribute to them importance for anticancer treatment potential are discussed.

The Mahboobi and Heidarian (2016), who conducted the study to check the potential for allelopathy of *Plumbago zeylanica* L. which have potential to inhibit the germination of seeds, root and shoot growth of medicinal plant *Andrographis paniculata*. Root extracts was more reduced than leaf and stem extract. These outcomes revealed that the inhibitory and stimulatory effect potentialities are mainly because of some allele-chemicals like as tannins, alkaloids, phenols, steroids and terpenoids etc. in the aqueous leaf, stem, and root extracts of *P. zeylanica* from the present investigation.

CHAPTER 3

3. MATERIALS AND METHODS

3.1. Allelopathic Evaluation of Medicinal Plants

3.1.1. Study Area

More than 200 medicinal plant samples were collected from the localities of moist Himalayan regions of Kashmir, Pakistan and were tested for their allelopathic potential collaboratively within the Ecology and Biodiversity Laboratory, Department of Environmental Sciences (DES), International Islamic University Islamabad (IIUI), at Biology Department Allama Iqbal Open University, (AIOU) Islamabad and antioxidant potentials of these selected medicinal plants were assessed at Department of Biology, University of Virginia (UVa), USA. Several dimensional assessments of allelopathic potential of these Medicinal plants were carried out through analysis of their roots, shoots, leaves or seeds. The Dish-pack and Sandwich methods were used for the screening of Allelopathic potential of this available plant material. The antioxidant activity was evaluated using DPPH free radical Scavenging Assay, FRAP Assay and ABTS antioxidant assay.

3.1.2. Material and Instruments

- Plant samples
- *Lactuca sativa* Seeds (TAKI II Seed G – LEO 1)
- *Triticum aestivum* seeds (Pakistan 2013)
- *Zea mays* seeds (Islamabad Gold)
- Petri dishes
- 6 Well multidishes

- Muslin cloth
- Funnel
- Beakers
- Reagent bottles
- Electric grinder
- Opto-electronical shaker
- Distilled water fully automatic (HWSFA30)
- Filter paper (Whatman No.1)
- Biosafety cabinet (RSBCA 115 MSC Class: II)
- B.O.D. Incubator (NB – 2201 LF)
- Oven (BJPX-SUMMER)
- Vertical autoclave fully automatic (CLASSIC 1050)
- Loading balance (KERN PCB 350 - 3)
- Haier refrigerator (HRF – 420 FDX)
- Ceramic Tweezer
- Agar, powder (Code 01059-85)
- Chemicals (NaOH, HgCl₂)
- Gloves
- Cellophane tape
- Aluminium foil
- Adjustable air-displacement pipette (Z58141N)
- Sterilized syringes
- Polythene bags

- Soil (NARC)
- Paper bags and newspapers
- Logarithmic graph

3.1.3. Sample collection and Preparation

The medicinal plants were collected from various locations of Himalayan regions of Pakistan. The fresh voucher samples were collected, separately placed in paper bags, labeled and were identified by Dr. Samar Naseer, Department of Biology at AIOU and handed over to the research supervisor Prof. Dr. Khalid Mehmood Khan at Department of Biological Sciences, International Islamic University Islamabad. The samples were transported to Ecology Laboratory, Environmental Laboratories Complex for further experimentation. An herbarium sheet of each plant sample was developed for the identification and then oven dried at 60°C for 48 hours. The samples were stored in an airtight box to avoid contamination and ensure chain of custody. The Soxhlet Extraction method was used for plant extraction which involves the use of a Soxhlet extractor, which is a type of apparatus that allows for the continuous extraction of plant materials using a solvent. The plant material is placed in a thimble, and the solvent is circulated through the extractor over a period of several hours. The solvent is then evaporated to yield the extracted compounds. An analysis of the allelopathic potential of collecting plant species was performed through leaf litter analysis using Sandwich and Dish Pack method (Fuji, 2004; Shinwari *et.al*, 2013; Appiah *et al.*, 2015 and Ou *et al.*, 2002).

3.1.4. Media Preparation

Agar powder was used at a temperature of 30-31 °C in this method. The 0.75% w/v of Agar solution was prepared in-vitro and sterilization was achieved at 121 °C and 15 psi for about 15 minutes. The agar medium contains all essential nutrients for the growth of seeds.

The dried leaves in different concentrations were placed in various 6 well dishes. Each concentration was used in three replications. In the first layer of agar, 5 mg of dried leaves sample was applied, so the dried plant material was removed and allowed to dry, following the other layers of agar with lettuce seeds. In each dish well, seven lettuce seeds were added on the agar. The aluminum foil was used to cover these dishes to prevent them from light. These dishes were then shifted to a growth chamber at 25 °C for the time period of 72 hours. The Radicle length and hypocotyl length were measured of each seedling after 3 days.

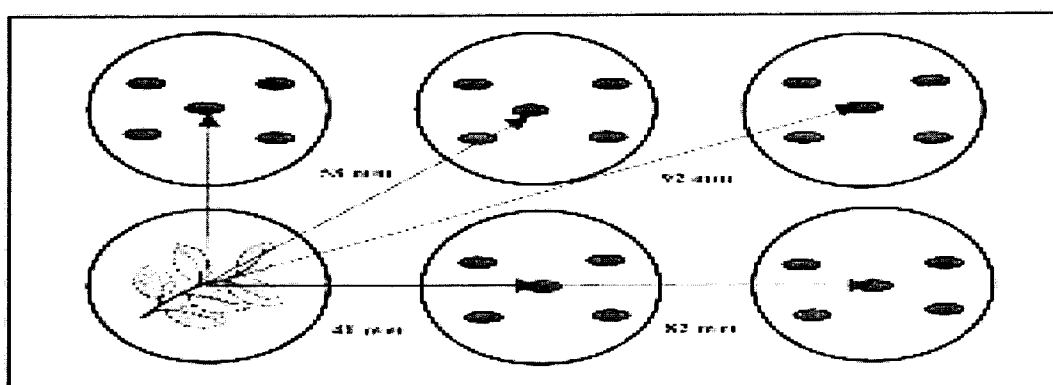


Fig 3.1. Method of Bioassay for Volatile Allelochemical

Methods used for evaluation of Allelopathic activity of medicinal plants.

3.1.5. Dish-pack method

The presence of volatile allelochemicals released from plant species has achieved attention in recent research. The dish pack method has been adopted as a valuable technique to determine the presence of volatile allelochemicals from plants. This study screened 200 medicinal plant species and evaluated them for possible allelopathic effects due to volatile substances. The experimentation was carried out in multi-well plastic dishes (6 well) using distilled water, plant material and lettuce seeds. The distances between the well containing plant sample and other wells were 41, 58, 82 and 92mm respectively (Fig. 3.2). The distilled

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water (0.75 ml) was enumerated in each well having filter paper except source well, which contain only 200 mg of oven-dried plant material. Like the sandwich method, the control sample did not hold any plant sample. In each well, seven lettuce seeds were implied in the filter paper. The multi-well dishes were wrapped with aluminum foil, tightly sealed with cellophane tape (circumvent of volatile substances) and kept in incubator at 25°C for 72 hours. The hypocotyl and radicle lengths were recorded after the incubation to assess growth inhibition and stimulation as well as determine the degree of inhibition (Fujii *et al.*, 2005; Amini *et al.*, 2014; Halimshah *et al.*, 2016; Nurul *et al.*, 2016; Gilani *et al.*, 2017, Sothearith *et al.*, 2021).

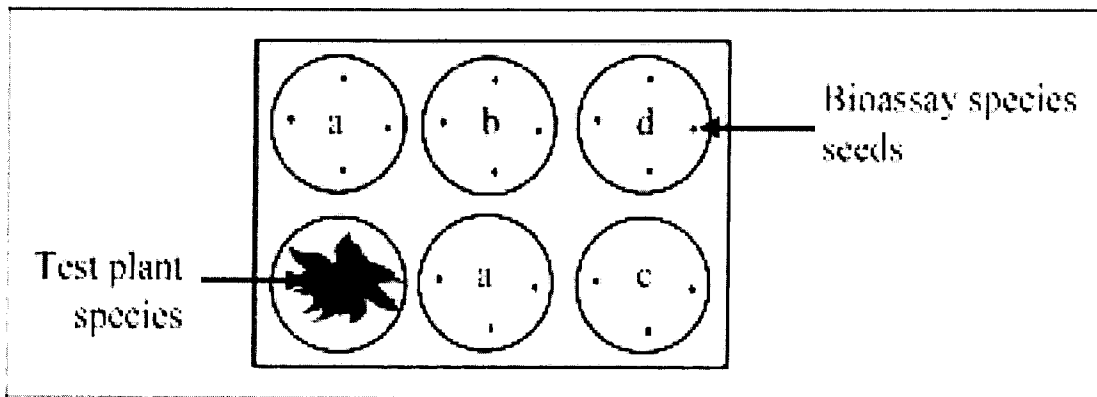


Fig. 3.2. View from top of multi-welled plastic plate used to test for plant allelopathy through volatile substances.

3.1.6. Sandwich method

The agar medium was prepared in a beaker by adding 7.5 g agar powder in 1000 ml of distilled water. The beaker was placed in an autoclave at a temperature of 120 °C, 115 pa pressure for 20 minutes as well as cooled down to 40 °C (Appiah *et al.*, 2015). The dried plant sample (10 or 50 mg) was added in 5ml plus 5ml agar, two layers in each well of multi-well plastic plates (six wells. Furthermore, 5 seeds of Lettuce (*Lactuca Sativa* Var.) were vertically placed in each Well. The plates were concealed with plastic tape, labelled, and

wrapped with aluminum foil for incubation in dark condition (Fujii et al., 2004) (Fig. 3.3). The sandwich method is known as productive and reported a technique to evaluate to allelopathic effect under laboratory condition. This study screened 200 medicinal plants as well as determined the allelopathic activity of leachates from collected donor plant's leaves and a control (only Lettuce in agar). The experiments were replicated three times to ensure accuracy and average values represent reported data. An incubator (BGPX/Summer) containing multi-plastic tubes were used at 25 °C for 72 hours for incubation, which followed by hypocotyl and radicle length measurement (Shiraishi et al., 2002; Fujii *et al.*, 2003; Fujii *et al.*, 2004; Morikawa *et al.*, 2012; Shinwari et al., 2010; Appiah *et al.*, 2015).

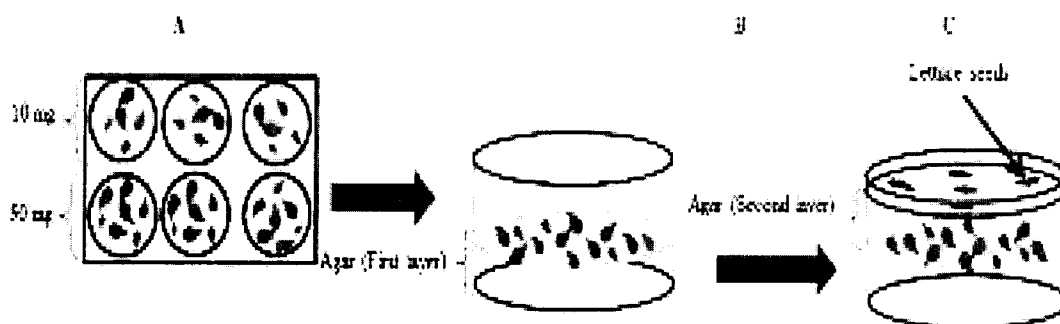


Fig 3.3: Sandwich Method for allelopathic activity of leachates from the plant's leaves.

3.2. Evaluation of Antioxidant Potential of medicinal plants

3.2.1. Plant Material

The Medicinal plant samples were collected from Himalayan regions of Pakistan and where dried samples were carried out to University of Virginia (UVa), Department of Biology, USA where the evaluation for antioxidant properties of the medicinal plant samples was achieved under the supervision of Professor Dr. Michael P. Timko. The chemicals,

solvents and reagents used in the preparation of plant extracts were Distilled water, Ascorbic acid, DPPH (1, 1-diphenyl, 2-picryl hydrazyl), Methanol and Ethanol.

3.2.2. Preparation of Medicinal Plant Extracts

For preparing the Methanolic extracts, 100 mg of dried powdered plant leaf of 200 medicinal plant samples were taken in 1.5 ml of aqueous Methanol (40%) in Eppendorf tubes. The samples were shaken well to dissolve to a greater extent with the help of stirrer/shaker. All samples were then incubated in the dark at 5°C for 24 hours. The extracts were then filtered to remove undissolved solid particles in the form of pellet and the supernatant was obtained for further analysis.

3.2.3. Preparation of 1mM DPPH solution

For preparing 1mM of DPPH solution, 3.94mg of DPPH was added in 100 ml Methanol in a 200 ml flask and was well mixed with the help of stirrer. DPPH gave purple color when dissolved in Methanol. The incubation was then done at room temperature for 20 min. The series of dilution were made for less concentrated DPPH solution, as highly concentrated solution does not give accurate absorbance value by blocking most of light in spectrophotometer. Different concentrations of plant extract (5ug, 25ug, 50 ug, 75 ug and 100 ug,) were added in diluted DPPH solution. The samples were kept in incubation for 30 min in the dark again. The purple color of DPPH in Methanol solution faded or completely disappeared because of the activity of antioxidants from medicinal plant samples. The free radical hunting or scavenging activity was measured by taking the absorbance reading of samples at 517 nm on spectrophotometer.

The Methanol, as the basic and the DPPH were used as a blank and negative control respectively. Ascorbic Acid was taken as standard because of its high Antioxidant activity.

The increased absorbance indicated the high antioxidant activity of samples. The formula used to calculate the reduction percentage was,

$$\text{Reduction (\%)} = \frac{A_c - A_s}{A_c} * 100$$

3.2.4. Preparation of Ascorbic Acid Stock solution and Ascorbic Acid Curve Graph

To prepare the standard Ascorbic Acid stock standard solution, 0.5mg of Ascorbic Acid was mixed in 1 ml of Methanol. The percent reduction in absorbance was observed using different concentrations of Ascorbic Acid in sample solution to construct an Ascorbic Acid curve. The Eppendorf tubes were taken with 40ul of DPPH in 1.5ml of Methanol by mixing it well on stirrer. The initial absorbance reading was taken at 517 nm.

To measure the absorbance difference, different ranges of this solution i.e., (10-100ul), were added to DPPH sample solution. The DPPH reagent solution was taken as a control in separate Eppendorf tubes. The absorbance value of the samples was then measured at 517 nm, after 5 minutes of incubation at room temperature using spectrophotometer.

The percentage reduction/inhibition was calculated by using following formulae,

$$\text{Inhibition (\%)} = \frac{A_c - A_s}{A_c} * 100$$

In this formula A_c = Absorbance of control and A_s = Absorbance of Sample

3.2.5. IC₅₀ value:

The Inhibition concentration or IC₅₀ is the value which shows the half or 50% inhibition/reduction in initial absorbance by an antioxidant in DPPH Assay. It was calculated by plotting different concentration of extracts and by reading their % inhibition/reduction. The lower IC₅₀ indicated the antioxidant potential of the sample.

Calculation of IC₅₀ value in Antioxidant assays.

It was a simple calculation consisting of the following steps.

1. A scattered graph was made in excel (where X axis was taken as concentration and Y axis as Percentage Inhibition activity)

2. The slope equation used was

$$(Y=mx+c \text{ or } Y=mx-c)$$

Where the value of “Y” in IC₅₀ equation was Y=50 or (0.5) as it means the half or 50% inhibition/reduction in absorbance of samples, where this equation already has the values of M and C itself in the curve graph.

3. The equation was solved, and the value of "X" was found which was IC₅₀ value.

4. The difference was then calculated by plotting a graph between various ascorbic acid concentrations and DPPH Absorbance reduction with the addition of Ascorbic acid and IC₅₀ (ascorbic acid concentration required to reduce absorbance value to half or 50%) are calculated from the equation as below,

$$y = 0.5822(X) + 28.262$$

$$50 = 0.5822(X) + 28.262$$

$$X = 50 - 28.262 / 0.5822 = 37.337$$

As in this equation the “X” is “IC₅₀” so, IC₅₀ = 37.337

In next step, the IC₅₀ of Methanolic, ethanolic and aqueous extracts of all medicinal plant samples was also measured following same DPPH protocol for Methanol (2 mg/ml), 0.5 mg/ml of ethanol and water (2 mg/ml) with the addition of increasing concentration i.e., Methanol (5-100 µl), Ethanol (10-50 µl) and water (5-100 µl) in Methanol and DPPH reagent solution in already prepared Eppendorf tubes.

3.3. Evaluation of Total Phenolic Contents (TPC) and Total Flavonoid Contents (TFC)

The antioxidant activity of medicinal plants is usually because of the presence of Phenolic and Flavonoid contents in them. The antioxidant potential is primarily because of

redox properties possessed by Phenolic compounds in the medicinal plants or other plants and fruits (Pekal and Pyrzyńska, 2014). In this research the ethanolic extracts of *Euphrasia stricta*, showed the higher total phenol contents, measured as 58.19 ± 1.74 followed by 46.05 ± 1.10 and 51.93 ± 1.72 gallic acid equivalents/g for ethanolic extracts of *Euphorbia platyphyllos* L. and *Epimedium brevicomum* Maxim respectively. As the hydroxyl groups (OH) are responsible for the free radical scavenging ability in them. So, rapid screening of antioxidant activity can be effectively applied by using on the total phenolic contents of these medicinal plants.

Similarly, various plant secondary metabolites like flavanols, flavones and abbreviated tannins are present in flavonoids. Plant flavonoids can be used as potential antioxidant in vitro as well as in-vivo (Soobrattee *et al.*, 2005); (Geetha *et al.*, 2003). As the antioxidant activity of various medicinal plants in this study was investigated for the first time so a detailed phytochemical investigation to recognize the potential phenolic and flavonoid contents of these medicinal plants should be achieved before their application in oxidative stress related diseases.

3.3.1. Estimation of Total Phenol Contents (TPC)

The plant extract preparation for Total phenol estimation was as followed,

1. The Folin Ciocalteu reagent was used to measure the TPC of these samples at 765nm using Uv-spectrophotometer (Soobrattee *et al.*, 2005).
2. The dried and powdered plant samples of about 100g were taken in separate flasks and were extracted with 100ml of 70% ethanol, 40% Methanol or distilled water using stirrer.
3. The filtration was achieved using Muslin cloth, solutions were then centrifuged, and rotary evaporator was used for sample drying through evaporation.

4. The air-tight plastic vials were used to store these collected dried plant samples for further antioxidant studies.
5. The dilutions of all these ethanolic and Methanolic extracts (0.5 ml of 1mg/ml or standard phenolic compound Gallic acid was mixed with Folin Ciocalteu reagent solution, dilution was made by using distilled water (5ml, 1:10 dilution) and 4 ml of 1M aqueous sodium carbonate.
6. The mixture was kept for 30 minutes and then the absorbance was measured at 765nm for total phenol estimation using spectrophotometer.
7. The Gallic acid equivalent (mg/g of dry mass) a common reference compound was used to express the total phenol contents.
8. All experiments were achieved as triplicates test and the data was analyzed by average of three values.

3.3.2. Estimation of Total Flavonoid Contents (TFC)

The TFC determination was carried out by Aluminum Chloride assay (Pekal A and Pyrzynska, 2014).

1. 10 ml volumetric flasks containing 4ml of water were added with different plant extracts (1.0mg/ml) and various dilutions (10-1000 ug/ml) from Rutin (Standard).
2. Then 0.3 ml of 5% NaNO₂ was added to the above mixture.
3. 1M NaOH (2ml) was added after 6 min and 10ml total volume was made by using distilled water.
4. The solution was mixed well, and absorbance was taken at 510nm.
5. The Rutin equivalent (mg/gm of dry mass) a common reference compound was used to express the total Flavonoid contents.

6. All experiments were achieved as triplicates test and the data was analyzed by an average of three values.

3.4. Evaluation of Antioxidant potential of Medicinal plants

Various experiments were conducted to evaluate the antioxidant activity of selected medicinal plants at Biology Laboratory, Department of Biological Sciences, International Islamic University Islamabad and Timko's Biology Laboratory. Department of Biology, University of Virginia, Charlottesville, Virginia USA. Three assays, the DPPH, FRAP and ABTS antioxidant assays were used to test the antioxidant potential of these selected medicinal plants.

3.4.1. Chemicals and Reagents

DPPH, Methanol, Ethanol, Trichloroacetic acid, Gallic Acid, Quercetin, Rutin, Butylated hydroxy toluene, 2-deoxy-2-ribose, Trichloroacetic acid, EDTA, Hydrogen peroxide (H₂O₂), Ascorbic Acid, 2-thiobarbiluric acid (TBA), Folin Ciocalteu reagent, Nitro blue-tetrazolium (NBT), Riboflavin, Potassium chloride, Aluminium chloride, Sodium carbonate, Sodium nitrate, Sodium hydroxide (NaOH), Hydrochloric acid, Disodium hydrogen phosphate, Sodium dihydrogen phosphate Sodium chloride, Potassium chloride, Deionized water, Potassium dihydrogen phosphate, Ferric chloride, Potassium ferricyanide, Deionized water, distilled water, Water bath, Pipette, Centrifuge tubes, Centrifuge, incubator, Vortex shaker and UV-Spectrophotometer. All reagents, chemicals and HPLC standards used for this study were purchased from Sigma Chemicals (USA).

3.4.2. Preparation of sample solution

The dry plant powder was accurately weighed and about 0.10 g was taken in test tubes. The aqueous ethanol (70%), Methanol (40%), 10.0 ml was added in these test tubes. The test

tubes were then incubated for about 24 hours at 5°C in an incubator. The tube sample contents were filtered through filter paper and the further analysis was done with the filtrate.

3.4.3. DPPH Free Radical Scavenging Assay (RSA)

The antioxidant or radical scavenging properties of various plant samples were investigated by applying DPPH (2, 2-diphenyl-1-picrylhydrazyl) antioxidant assay with some modifications in Roberta *et al.*, (2006) and Sharma (2009) protocols. About 1 ml of test solution was dissolved to equivalent amount of DPPH solution (0.1 mM) in ethanol, Methanol and water. The increase in DPPH absorbance of tested samples was measured after 20 min incubation at room temperature, by taking the absorbance at 517nm. Standard Ascorbic acid (1mM) showed a maximum absorbance of $90.36 \pm 1.05 \mu\text{g/ml}$ and was taken as a reference solution in this DPPH antioxidant assay. The DPPH is a stable free radical chemical with purple color that absorbs at 517 nm. The ethanolic, Methanolic and aqueous extracts of selected medicinal plants were prepared to check their antioxidant potential at a concentration of 1 mg/ml. The ethanol, Methanol or deionized water was used as a blank and control for their respective sample extracts while Ethanol/Methanol/Water + DPPH + sample [100 μl extract or (1 mg/ml)] was used as a test.

3.4.4. Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP test (which reduces the ability of ferric antioxidants) is very important for assessing the antioxidant activity of medicinal plants. The test consists of phases of antioxidant FRAP: a) sample preparation, b) responses c) final measurement of absorption of all samples and parameters using a spectrometer at 700 nm wavelength. The sample consisted of Methanolic extracts of various medicinal plants and ascorbic acid as a standard. The experiment was provided with a buffer solution with pH 6.6, and some reagents like

trichloroacetic acid (10%), 0.1% ferric chloride solutions and potassium ferricyanide (1%) solution. The FRAP (Ferric Reducing Antioxidant Power) assay can be used to determine the free radical scavenging activity or antioxidant activity of medicinal plants. The antioxidant activity is mainly because of the ability to form compounds of metal atoms, like iron and copper by flavonoids and phenolic acids presents in medicinal plants. A colored compound was formed in the mixture, resulted from reaction of antioxidants with ferric chloride, trichloroacetic acid and potassium ferricyanide. The absorbance value of this solution is then measured at 700 nm by spectrophotometer.

3.4.4.1. Material and Chemicals

Trichloroacetic acid, Hydrochloric acid, Disodium hydrogen phosphate, Sodium chloride, Potassium chloride, Deionized water, Potassium dihydrogen phosphate, Ferric chloride, Potassium ferricyanide, Water bath, Pipette, Centrifuge tubes, Centrifuge, Vortex shaker and UV-Spectrophotometer.

3.4.4.2. Preparation of Reagents

1. Preparation of Phosphate Buffer (0.2 M):

The Phosphate Buffer was prepared by taking 800 ml of water in 1000 ml graduated flask and added with 8 g of NaCl, 1.44 g of disodium hydrogen Phosphate, 0.2 g of Potassium Chloride, Potassium dihydrogen phosphate 0.24 g and pH was adjusted to 6.6 using HCl and the volume was adjusted using deionized water.

2. Preparation of 1% Potassium cyanide: Potassium ferricyanide (1g) was added in 100 ml of deionized or distilled water.

3). 10% Trichloroacetic acid: Trichloroacetic acid (10g) was added in 100 ml of deionized or distilled water.

4). 0.1% Ferric Chloride: The Ferric chloride (100 mg) was mixed in 100 ml of dH₂O.

5). Preparation of 0.1% Ascorbic Acid: Ascorbic Acid (1 mg) was mixed in 1 ml of water.

3.4.4.3. Methodology:

1. Various concentrations of Methanolic extracts of different medicinal plant samples were prepared e.g., (10-50 mg/ml) and were added with sodium phosphate buffer (2.5 ml) and potassium cyanide (2.5 ml) (Berker *et al.*, 2007).
2. The reaction mixture was mixed well using vortex and then were incubated at vortex shaker (50°C) for 20 min.
3. Then trichloroacetic acid (2.5ml) was mixed in the solution and centrifugation was done for 10 minutes at 3000 rpm.
4. The solution was filtered, and the supernatant (2.5 ml) was dissolved in the deionized water (2.5 ml) and 0.5 ml of ferric chloride.
5. The colored solution absorbance was observed at 700 nm compared to the blank as a reference to the standard (Ascorbic Acid) using spectrophotometer. The sample's reducing power was compared to the ascorbic acid, a reference standard here.

3.4.5.2.2. Azinobis-(3-ethylbenzothiazoline-6-sulfonate)] Assay/ ABTS assay:

The ABTS radical cation decolorization assay was also used to investigate the free radical scavenging potential of plant samples (Re *et al.* 1999). The 7 mM ABTS in water and 2.45 mM potassium persulfate (1:1) were used in this experiment which when mixed and stored in the dark at room temperature for 12-16 hours before use, resulted in the formation of ABTS⁺ cation radical by chemical reaction between them.

The dilution of ABTS⁺ solution was prepared with Methanol to attain an absorbance value of 0.700 at 734 nm. Then 3.995 ml of diluted ABTS⁺ solution was added with 5 µl of plant extract and after 30 min incubation in dark from initial mixing, the absorbance was

measured. Each assay was accompanied by running an appropriate solvent blank. The experiment was conducted in triplicates and the measurements were taken at least three times. The formula used to calculate the Percent (%) inhibition of absorbance at 734 nm was,

$$\text{ABTS}^+ \text{ inhibition/scavenging effect (\%)} = ((A_B - A_A)/A_B) \times 100$$

Where, A_B denotes the absorbance of ABTS radical + Methanol; A_A represents the absorbance of ABTS radical + sample extract/standard. The standard substance used in this experiment was trolox.

3.4.5.1. Assay Procedure:

1. The ABTS solution was allowed to come at the room temperature after it was removed from the refrigerator (Re *et al.* 1999).

2. Trolox standard solution was prepared as followed:

The thawed contents of the 1.5 mM Trolox standard tube were briefly spinned down and was added with 80 μl of Assay Buffer into the given 1.5 mM Trolox standard tube using Pipette and was mixed well on vortex. A 300 μM diluted stock Trolox standard was produced. A series of dilution were made by using the newly diluted stock Trolox solution. Before going on to the next step, each new dilution was thoroughly mixed. The assay buffer was taken as the zero standard whereas 300 μM stock dilution was taken as the highest standard.

3. The medicinal plant extracts in various solvents (Ethanol, Methanol and water) were prepared and were briefly spinned down. About 25 μl of these extracts were added to the Dilution Buffer and was slowly inverted. The working solution was placed in freezer until next use.

4. 10 μl of assay buffer was added to separate tubes as a negative control and 10 μl of samples or Trolox standards was also added to the given individual wells of the assay tubes.

5. The solution was again added with 20 μ l sample or trolox to all tubes with standards and samples from step 4.
6. About 100 μ l of the ABTS solution was added to each tube. The tubes were then placed on plate shaker at room temperature. The reaction was allowed to proceed for about 5 min. 50 μ l of Stop Solution per tube was added to stop the reaction.
7. The absorbance of each sample was measured at a wavelength of 734 nm.

3.5. Statistical analysis

The results have three replications, randomized design and acquire statistical analysis. This study had evaluated the means, standard deviation, variance (SDV) and standard deviation (SD) using Microsoft Excel 2016. The equations used are given below:

The equation used for calculating Allelopathy were,

$$\text{Inhibitory (\%)} = 100 - \frac{\text{Average length of treatment Radicle/Hypocotyl}}{\text{Average length of control radicle/hypocotyl}}$$

$$\text{Elongation (\%)} = 100 + \frac{\text{Average length of treatment Radicle/Hypocotyl}}{\text{Average length of control radicle/hypocotyl}}$$

CHAPTER 4

4. RESULTS:

4.1. Allelopathic Potentials of Medicinal Plants

4.1.1. Allelopathic Potentials using Dish-pack Method.

The complete data for all tested medicinal plants using Dish-pack Method is given in Table 4.1. The highest allelopathic potential using dish-pack method was shown by *Rheum officinale* L., *Cassia tora*, *Ophiopogon japonicas* L., *Picrorhiza scrophulariiflora* and *Trifolium repense* L. The effect of 5 mg concentration of *Rheum officinale* and *Ophiopogon japonicus* inhibited the maximum root growth. Less hypocotyl growth was also observed with 5 mg of leaves of *Thymus vulgaris*, *Mentha piperita* and *Hibisci sabdariffae*. So, these medicinal plants with relatively high allelopathic potential can be efficiently used in future agriculture systems to control weeds by use of natural resources. However, some plants showed very low or same allelopathic effects on radicle and hypocotyl lengths with these different concentrations. For example, the *Angelica sylvestris*, *Nepeta cataria* and *Pteris multifida* showed no effect of any leaf concentration on root or hypocotyl growth.

Table 4.1: The Allelopathic potential of various traditionally used medicinal plant species from Himalayan regions of Pakistan using Dish-pack method.

Sr. No.	Plant Samples	Allelopathic Potential (%)		Criterion
		Radicle length	Hypocotyl length	
1	<i>Rheum officinale</i> L.	48.65	85.71	***
2	<i>Cassia tora</i> L.	48.81	80.68	***
3	<i>Ophiopogon japonicas</i> L.	49.22	82.57	***
4	<i>Picrorhiza scrophulariiflora</i>	49.64	92.65	***

5	<i>Trifolium repense</i> L.	50.26	91.76	***
6	<i>Artemisia alba</i>	51.16	91.57	**
7	<i>Thymus vulgaris</i>	52.12	99.85	**
8	<i>Mentha piperita</i>	52.76	83.67	**
9	<i>Hibisci sabdariffae</i>	54.12	110.31	**
10	<i>Dioscorea opposita</i>	54.57	99.92	**
11	<i>Veronicae officinalis</i>	54.66	87.14	**
12	<i>Origanum vulgare</i> L.	54.86	87.93	**
13	<i>Salicis cortex</i>	55.32	93.13	**
14	<i>Primula officinalis</i>	56.33	90.41	**
15	<i>Satureja hortensis</i>	56.62	90.46	**
16	<i>Polygonum Hydropiper</i>	57.72	101.24	**
17	<i>Polygonum aviculare</i>	57.93	99.74	**
18	<i>Satureja montana</i>	58.47	92.81	**
19	<i>Glycyrrhiza uralensis</i>	58.66	92.97	**
20	<i>Bidens pilosa</i> L.	58.73	81.42	**
21	<i>Fagopyrum cymosum</i>	59.35	97.15	**
22	<i>Polygonum cuspidatum</i>	59.38	82.23	**
23	<i>Gunnera perpensa</i>	59.42	85.54	**
24	<i>Tephrosia purpurea</i> L.	59.88	87.03	**
25	<i>Solanum torvum</i> L.	59.92	90.14	**
26	<i>Thymus vulgaris</i>	60.25	98.81	*
27	<i>Psoralea corylifolia</i> L.	60.35	91.81	*
28	<i>Geranii robertiani</i>	60.45	85.91	*
29	<i>Arctium lappa</i> L.	60.48	91.25	*
30	<i>Pteris multifida</i>	60.52	94.26	*
31	<i>Ocimum basilicum</i>	61.24	97.77	*
32	<i>Vitex rotundifolia</i>	61.31	89.28	*
33	<i>Salvia officinalis</i>	61.36	86.54	*
34	<i>Galium verum</i> L.	61.52	95.58	*

35	<i>Solanum nigrum</i> L.	61.65	96.53	*
36	<i>Teraxaci folium</i>	61.82	91.73	*
37	<i>Paris polyphilla</i>	62.26	95.33	*
38	<i>Pygeum africanum</i>	62.73	104.44	*
39	<i>Teucrium chamaedrys</i>	62.84	96.12	*
40	<i>Urticae dioica</i> L.	63.11	88.27	*
41	<i>Portulaca oleracea</i> L.	63.25	91.62	*
42	<i>Crataegus monogyna</i>	63.4	90.02	*
43	<i>Polygoni avicularis</i>	63.53	92.86	*
44	<i>Tinospora cordifolia</i>	63.66	93.38	*
45	<i>Lonicera japonica</i>	63.87	105.4	*
46	<i>Viscum album</i>	63.92	98.34	*
47	<i>Eclipta prostrata</i>	64.16	96.27	*
48	<i>Solidaginis virgaureae</i>	64.33	91.52	*
49	<i>Melissae officinalis</i>	64.56	98.51	*
50	<i>Iris potaninii</i>	64.87	110.62	*
51	<i>Cirsium setosum</i>	65.04	90.84	
52	<i>Violae tricoloris</i>	65.26	118.45	
53	<i>Indigofera tinctoria</i>	65.42	113.33	
54	<i>Melissa officinalis</i>	65.52	92.26	
55	<i>Apium graveolens</i>	65.86	97.77	
56	<i>Dianthus superbus</i> L.	66.19	97.04	
57	<i>Fragaria vesca</i>	66.31	95.36	
58	<i>Euphrasiae stricta</i>	66.58	97.92	
59	<i>Thymus serpyllum</i> L.	66.73	98.55	
60	<i>Plantago lanceolata</i> L.	66.97	111.22	
61	<i>Larrea tridentate</i>	67.24	90.85	
62	<i>Dendrobium nobile</i>	67.46	94.38	
63	<i>Combretum caffrum</i>	67.62	96.61	
64	<i>Papever somniferum</i>	67.8	89.26	

65	<i>Vitex trifolia</i> L.	68.15	90.06	
66	<i>Cannabis sativa</i> L.	68.34	97.71	
67	<i>Achillea Millefolium</i>	68.53	96.44	
68	<i>Morus alba</i> L.	69.56	99.35	
69	<i>Withania somnifera</i>	69.44	90.72	
70	<i>Asperula odorata</i>	69.62	96.54	
71	<i>Artemisia absinthium</i> L.	69.78	116.26	
72	<i>Euphorbia platyphyllos</i> L.	70.14	94.81	
73	<i>Citrullus colocynthis</i> L.	70.16	95.71	
74	<i>Glycyrrhiza glabra</i>	70.44	90.71	
75	<i>Rehmannia glutinosa</i>	70.48	103.63	
76	<i>Ephedra sinica</i>	71.14	90.84	
77	<i>Eclipta alba</i> L.	71.19	92.94	
78	<i>Marrubium vulgare</i>	71.35	99.15	
79	<i>Punica granatum</i>	71.69	95.23	
80	<i>Cynarae folium</i>	71.86	102.42	
81	<i>Hyoscyamus niger</i> L.	71.95	95.68	
82	<i>Prunella vulgaris</i>	72.23	108.58	
83	<i>Cichorium intybus</i>	72.27	108.45	
84	<i>Chellidonium majus</i> L.	72.69	101.22	
85	<i>Eucommia ulmoides</i>	72.91	142.13	
86	<i>Tragia involucrata</i> L.	73.21	105.81	
87	<i>Utazi afang</i>	73.88	112.42	
88	<i>Ailanthus altissima</i>	74.57	118.12	
89	<i>Veronica officinalis</i>	74.62	116.5	
90	<i>Dipsacus japonicus</i>	74.62	102.93	
91	<i>Imperata cylindrica</i> L.	74.69	92.28	
92	<i>Digitalis purpurea</i> L.	75.61	94.69	
93	<i>Angelica sinensis</i>	75.66	103.12	
94	<i>Bursera simaruba</i>	75.68	123.51	

95	<i>Justicia procumbens</i>	76.01	123.79	
96	<i>Ocimum gratissimum</i> L.	76.03	93.68	
97	<i>Lauri folium</i>	76.55	96.86	
98	<i>Saussurea lappa</i>	77.64	101.46	
99	<i>Houttuynia cordata</i>	77.66	95.98	
100	<i>Ocimum tenuiflorum</i> L.	77.94	99.44	
101	<i>Xanthium sibiricum</i>	78.31	98.82	
102	<i>Patrinia scabiosifolia</i>	78.36	94.22	
103	<i>Nothapodytes nimmoniana</i>	78.94	110.26	
104	<i>Rhus glabra</i>	78.64	124.52	
105	<i>Justicia adhatoda</i> L.	78.51	99.72	
106	<i>Asparagus cochinchinensis</i>	79.23	104.19	
107	<i>Phellodendron amurense</i>	79.44	92.58	
108	<i>Erythroxylum pervillei</i>	79.36	124.44	
109	<i>Podophyllum hexandrum</i>	79.64	96.22	
110	<i>Fritillaria thunbergii</i>	80.38	139.62	
111	<i>Doronicum pardalianches</i>	80.67	98.49	
112	<i>Cleistanthus collinus</i>	81.46	109.13	
113	<i>Cimicifuga racemosa</i>	81.83	96.92	
114	<i>Rhus chinensis</i>	81.95	117.78	
115	<i>Verbenae officinalis</i>	82.51	108.33	
116	<i>Rhyncosia minima</i>	83.04	95.57	
117	<i>Emilia sonchifolia</i>	84.14	89.6	
118	<i>Lavandulae angustifolia</i> L.	84.43	147.41	
119	<i>Sanguisorba officinalis</i> L.	84.53	87.34	
120	<i>Rhodiola sacra</i>	84.58	111.66	
121	<i>Hedyotis diffusa</i>	85.78	101.86	
122	<i>Farfarae folium</i>	86.06	121.29	
123	<i>Rubus ellipticus</i>	86.45	99.17	
124	<i>Plantago asiatica</i> L.	86.52	114.63	

125	<i>Dysoxylum binectariferum</i>	86.66	131.95	
126	<i>Epilobium parvifolium</i>	86.73	105.94	
127	<i>Melia azedarach</i> L.	87.56	104.46	
128	<i>Hederae folium</i>	87.4	156.84	
129	<i>Pestemon deustus</i>	87.84	141.48	
130	<i>Astragalus complanatus</i>	88.01	95.44	
131	<i>Lycopus lucidus</i>	88.42	108.21	
132	<i>Ipomoea aquatica</i>	88.52	103.41	
133	<i>Teucrii Montana</i>	88.62	96.84	
134	<i>Taraxacum mongolicum</i>	89.36	105.21	
135	<i>Indigofera aspalathoides</i>	90.09	92.96	
136	<i>Cuscuta chinensis</i> L.	90.59	161.39	
137	<i>Salviae officinalis</i>	90.68	125.3	
138	<i>Curculigo orchioides</i>	90.76	116.32	
139	<i>Betulae folium</i>	91.13	142.82	
140	<i>Equiseti arvense</i>	91.24	151.33	
141	<i>Salvia miltiorrhiza</i>	91.53	118.42	
142	<i>Rubia cordifolia</i>	91.61	98.56	
143	<i>Bardanae folium</i>	91.82	131.6	
144	<i>Tilia cordata</i>	93.17	114.72	
145	<i>Sambucus nigra</i> L.	93.24	108.17	
146	<i>Sophora tonkinensis</i>	93.26	95.54	
147	<i>Andrographis paniculata</i>	93.74	94.66	
148	<i>Epimedium brevicomum</i>	94.11	153.73	
149	<i>Caesalpinia sappan</i> L.	94.38	95.88	
150	<i>Trollius europaeus</i>	94.48	139.66	
151	<i>Centaurium erythraea</i>	95.05	143.74	
152	<i>Isatis indigotica</i>	95.36	99.54	
153	<i>Ipomoea squamosa</i>	95.86	94.46	
154	<i>Viola reichenbachiana</i>	96.47	100.19	

155	<i>Diphylleia grayi</i>	96.71	110.48	
156	<i>Hyperii Herba</i>	96.87	109.58	
157	<i>Rubia cordifolia</i> L.	97.65	92.44	
158	<i>Bupleurum falcatum</i>	98.16	111.82	
159	<i>Campotheca acuminata</i>	98.25	139.63	
160	<i>Zanthoxylum nitidum</i>	98.27	94.56	
161	<i>Acacia catechu</i>	98.48	96.53	
162	<i>Drynaria fortune</i>	98.81	105.36	
163	<i>Rubi idaei</i>	99.28	155.51	
164	<i>Coptis chinensis</i>	99.52	121.64	
165	<i>Galium verum</i> L.	99.7	113.89	
166	<i>Centipeda minima</i>	99.74	102.48	
167	<i>Fraxini folium</i>	100.54	91.88	
168	<i>Cinnamomum cassia</i>	101.68	98.72	
169	<i>Uvae ursi</i>	102.43	151.14	
170	<i>Agrimonia pilosa</i>	102.55	117.86	
171	<i>Polygala tenuifolia</i>	103.4	98.21	
172	<i>Asparagus racemosus</i>	104.62	92.26	
173	<i>Gynostemma pentaphyllum</i>	106.25	95.76	
174	<i>Pulsatilla chinensis</i>	106.66	97.55	
175	<i>Vernonia cinerea</i>	107.64	97.08	
176	<i>Bauhinia variegata</i>	108.46	94.54	
177	<i>Uncaria macrophylla</i>	109.58	105.69	
178	<i>Actaea racimosa</i> L.	109.76	114.63	
179	<i>Matteuccia struthiopteris</i>	110.48	102.6	
180	<i>Satureiae montana</i>	110.86	94.86	
181	<i>Stemona sessilifolia</i>	111.55	102.96	
182	<i>Sennae folium</i>	112.09	99.65	
183	<i>Amaranthus viridis</i> L.	112.3	97.63	
184	<i>Tripterygium wilfordii</i>	112.91	144.52	

185	<i>Oleae folium</i>	113.25	154.43	
186	<i>Trichosanthes kirilowii</i>	113.61	120.27	
187	<i>Rosmarinus officinalis</i>	114.21	98.42	
188	<i>Tiliae cordata</i>	115.63	103.88	
189	<i>Morus nigra</i> L.	116.56	110.64	
190	<i>Sedum sarmentosum</i>	116.89	104.42	
191	<i>Croton setigerus</i> L.	117.33	91.69	
192	<i>Scutellaria baicalensis</i>	118.99	98.56	
193	<i>Viscum coloratum</i>	120.16	94.52	
194	<i>Geocrypta galii</i>	120.78	108.49	
195	<i>Smilax glabra</i> L.	123.81	98.79	
196	<i>Rubus chingii</i>	123.71	105.92	
197	<i>Inula Britannica</i> L.	124.44	111.34	
198	<i>Calycopteris floribunda</i>	128.16	109.48	
199	<i>Angelica sylvestris</i>	129.86	111.91	
200	<i>Nepeta cataria</i>	132.29	91.53	
	Mean	81.178	104.007	
	SD	19.695	16.319	
	Mean SD	61.482	87.687	
	Mean-1.5 SD	79.678	102.507	
	Mean-2 SD	79.178	102.007	
	Mean-2.5 SD	78.678	101.507	

The table 4.1 showed the controlled compared percentage of growth rate and stronger inhibitory activity in the radicle was measured as *M-1(σ) for minimum, for great **M-1.5(σ) and for maximum ***M-2(σ). σ (SD):standard deviation of radicle. M:mean of radicle.

The lettuce seeds were exposed to volatile compounds released from 200 plants to assess inhibitory or stimulatory effect in 6 well multi-dishes containing the dry leaves (oven

dried) and distilled water. Additionally, the control was grown without plant material in a dish. The detail and allelopathic effect of all selected plants (200) is demonstrated in Table 4.1. The results revealed that the volatile compounds of different plants had exhibited inhibitory or stimulatory effect on the germination and growth of the hypocotyl and radicle of the lettuce in a different manner. The seedling growth of lettuce was completely inhibited by one specie, 5 species reflected strong inhibition, 8 species indicated medium inhibition, 18 species revealed least inhibitory effect and 112 species showed insignificant allelopathic effects. Contemporary, 64 species least promoted the growth while 15 species presented strong stimulatory allelopathic effect on lettuce seeds. The complete inhibitory effect was noticed by *Rheum officinale* L. while strong inhibitory effect was observed by *Cassia tora* L., *Ophiopogon japonicas* L., *Picrorhiza scrophulariiflora* and *Trifolium repense* L. followed by medium inhibitory effect from *Artemisia alba*, *Thymus vulgaris*, *Mentha piperita*, *Hibisci sabdariffae*, *Dioscorea opposita.*, *Veronicae officinalis*, *Origanum vulgare* L. and *Salicis cortex*. However, *Trichosanthes kirilowii*, *Rosmarinus officinalis*, *Tiliae cordata*, *Morus nigra* L., *Sedum sarmentosum*, *Croton setigerus* L. *Scutellaria baicalensis*, *Viscum coloratum*, *Geocrypta galii*, *Geocrypta galii*, *Smilax glabra* L., *Rubus chingii*, *Inula Britannica* L., *Calycopteris floribunda*, *Angelica sylvestris* and *Nepeta cataria* exhibited strong stimulatory allelopathic effect on lettuce radicle growth as compared to control. It is notable to mention that *Trichosanthes kirilowii* indicated an exceptional stimulatory allelopathic effect on radicle growth of lettuce.

However, some plants showed an increase in the root growth but suppressed the hypocotyl growth. For example, in the case of *Teucree Montana*, *Erythroxylum pervillei*, *Rhus chinensis* and *Taraxacum mongolicum*, a decrease in growth of hypocotyls was observed with the higher concentrations of leaves but an improvement in growth was observed with

reduced concentrations. Similarly, some plants like *Citrullus colocynthis* L., *Ephedra sinica*, *Punica granatum* and *Prunella vulgaris* showed an increase in hypocotyl growth, but suppression was observed in the radicle growth. Some of the tested medicinal plants improved the length of hypocotyls and radicles, while some plants showed no effects on radicle growth. The dish pack method can effectively identify the allelopathic effects of volatile compounds from plants on lettuce (Appiah *et al.*, 2015). Likewise in the following study the medicinal plants (two hundred) were collected from different regions of the country, identified their families, genera, and specie as well screen them prior to assess allelopathic effects. The allelopathic effect of two hundred medicinal plants was also tested and enlisted them based on their allelopathic effect.

4.1.2. Allelopathic Potentials of medicinal plants using Sandwich Method.

In the second step the allelopathic potential of the tested medicinal plants were also observed using the Sandwich method (Sothearith *et al.*, 2021). The highest allelopathic potential was shown by *Mentha piperita*, *Satureja hortensis* L., *Bidens pilosa* L., *Satureja montana*, *Thymus vulgaris* L. and *Artemisia alba* using sandwich method as shown in table 4.2. The effect of 5 mg concentration of *Rheum officinale* and *Ophiopogon japonicus* inhibited the maximum root growth. Less hypocotyl growth was also observed with 5 mg of leaves of *Rheum officinale* L., *Trifolium repense* L. and *Hibisci sabdariffae*. So, these medicinal plants with relatively high allelopathic potential can be efficiently used in future agriculture systems to control weeds by use of natural resources. The current research particularly focused on collection, screening, identification, and allelopathic investigation of medicinal plants. The allelopathic effects of some medicinal plants used in this study were less or seldom reported previously. Hence, this study will share more knowledge and

contribute to effective utilization of medicinal plants in agriculture and industry in addition to medicinal use.

Table 4.2: The Allelopathic potential of various traditionally used medicinal plant species from Himalayan regions of Pakistan using Sandwich method.

Sr. No.	Scientific Name	Extension (%)		Criterion
		Radicle 10mg	Hypocotyl 10mg	Radicle 10mg
1	<i>Mentha piperita</i>	2.18	6.36	***
2	<i>Satureja hortensis</i> L.	2.98	12.4	***
3	<i>Bidens pilosa</i> L.	3.39	8.87	***
4	<i>Satureja montana</i>	3.77	11.7	***
5	<i>Thymus vulgaris</i> L.	4.84	6.58	***
6	<i>Artemisia alba</i> L.	5.6	13.2	**
7	<i>Trifolium repense</i> L.	6.45	10.45	**
8	<i>Rheum officinale</i> L.	6.8	12.72	**
9	<i>Hibisci sabdariffae</i>	7.2	11.26	**
10	<i>Dioscorea opposita</i>	7.46	18.12	**
11	<i>Veronicae officinalis</i>	7.83	12.28	**
12	<i>Origanum vulgare</i> L.	8.09	16.46	**
13	<i>Cassia tora</i> L.	9.36	19.34	**
14	<i>Primula officinalis</i>	9.7	15.4	**
15	<i>Salicis cortex</i>	10.48	22.9	**
16	<i>Polygonum hydropiper</i>	10.8	18.22	**
17	<i>Polygonum aviculare</i>	11.26	13.6	**
18	<i>Azadirachta indica</i> L.	11.71	20.11	**
19	<i>Glycyrrhiza uralensis</i>	12.02	26.48	**
20	<i>Ophiopogon japonicus</i> L.	12.48	21.5	**
21	<i>Fagopyrum cymosum</i>	12.94	18.64	**
22	<i>Polygonum cuspidatum</i>	13.34	14.7	**

23	<i>Gunnera perpensa</i>	13.63	19.15	**
24	<i>Tephrosia purpurea</i> L.	14.4	24.55	**
25	<i>Solanum torvum</i> L.	14.86	22.2	**
26	<i>Thymus vulgaris</i> L.	15.38	26.08	*
27	<i>Psoralea corylifolia</i> L.	15.55	22.16	*
28	<i>Geranii robertiani</i>	16.3	37.3	*
29	<i>Arctium lappa</i> L.	16.63	24.47	*
30	<i>Pteris multifida</i>	16.7	42.08	*
31	<i>Ocimum basilicum</i>	17.44	22.24	*
32	<i>Vitex rotundifolia</i>	17.68	40.12	*
33	<i>Salvia officinalis</i>	18.01	32.28	*
34	<i>Galium verum</i> L.	18.61	48.4	*
35	<i>Solanum nigrum</i> L.	18.75	33.26	*
36	<i>Teraxaci folium</i>	19.05	52.25	*
37	<i>Paris polyphilla</i>	19.43	39.3	*
38	<i>Pygeum africanum</i>	19.66	31.92	*
39	<i>Teucrium chamaedrys</i>	20.09	45.18	*
40	<i>Urticae dioica</i> L.	20.23	28.62	*
41	<i>Portulaca oleracea</i> L.	20.51	44.36	*
42	<i>Crataegus monogyna</i>	20.6	36.54	*
43	<i>Polygoni avicularis</i>	21.08	60.7	*
44	<i>Tinospora cordifolia</i>	21.38	48.53	*
45	<i>Lonicera japonica</i>	21.65	35.48	*
46	<i>Viscum album</i>	22.45	52.24	*
47	<i>Eclipta prostrata</i>	23.39	49.9	*
48	<i>Solidaginis virgaureae</i>	23.64	37.28	*
49	<i>Melissae officinalis</i>	23.78	54.86	*
50	<i>Iris potaninii</i>	24.66	61.04	*
51	<i>Cirsium setosum</i>	25.3	41.29	*
52	<i>Violae tricoloris</i>	26.05	43.14	

53	<i>Indigofera tinctoria</i>	27.86	60.13	
54	<i>Melissa officinalis</i>	28.2	46.33	
55	<i>Apium graveolens</i>	28.98	59.25	
56	<i>Dianthus superbus</i> L.	29.12	64.81	
57	<i>Fragaria vesca</i>	29.53	50.2	
58	<i>Euphrasiae stricta</i>	29.74	44.58	
59	<i>Thymus serpyllum</i> L.	30.22	67.35	
60	<i>Plantago lanceolata</i> L.	30.46	48.52	
61	<i>Larrea tridentate</i>	30.64	74.17	
62	<i>Dendrobium nobile</i>	31.36	59.55	
63	<i>Combretum caffrum</i>	31.5	47.08	
64	<i>Papever somniferum</i>	31.69	58.9	
65	<i>Vitex trifolia</i> L.	32.32	39.84	
66	<i>Cannabis sativa</i> L.	32.64	72.02	
67	<i>Achillea Millefolium</i>	33.23	60.84	
68	<i>Morus alba</i> L.	33.76	51.05	
69	<i>Withania somnifera</i>	35.08	46.38	
70	<i>Asperula odorata</i>	35.44	66.62	
71	<i>Artemisia absinthium</i> L.	35.58	54.17	
72	<i>Euphorbia platyphyllos</i> L.	36.29	82.33	
73	<i>Citrullus colocynthis</i> L.	36.56	91.04	
74	<i>Glycyrrhiza glabra</i>	37.04	64.5	
75	<i>Rehmannia glutinosa</i>	37.62	45.23	
76	<i>Ephedra sinica</i>	37.7	95.58	
77	<i>Eclipta alba</i> L.	38.1	73.22	
78	<i>Marrubium vulgare</i>	38.35	52.48	
79	<i>Punica granatum</i>	38.52	89.02	
80	<i>Cynarae folium</i>	38.73	54.09	
81	<i>Hyoscyamus niger</i> L.	39.16	65.39	
82	<i>Prunella vulgaris</i>	39.48	90.28	

83	<i>Cichorium intybus</i>	39.71	62.74	
84	<i>Chellidonium majus</i> L.	40.9	88.6	
85	<i>Eucommia ulmoides</i>	40.31	46.72	
86	<i>Tragia involucrata</i> L.	40.5	96.12	
87	<i>Utazi afang</i>	40.74	65.27	
88	<i>Ailanthus altissima</i>	41.26	86.25	
89	<i>Veronica officinalis</i>	41.67	67.42	
90	<i>Dipsacus japonicus</i>	42.21	74.3	
91	<i>Imperata cylindrica</i> L.	42.61	99.51	
92	<i>Digitalis purpurea</i> L.	43.18	83.16	
93	<i>Angelica sinensis</i>	43.55	56.21	
94	<i>Bursera simaruba</i>	43.86	75.54	
95	<i>Justicia procumbens</i>	44.21	82.2	
96	<i>Ocimum gratissimum</i> L.	44.88	62.06	
97	<i>Lauri folium</i>	44.9	96.64	
98	<i>Saussurea lappa</i>	45.5	72.36	
99	<i>Houttuynia cordata</i>	45.64	80.7	
100	<i>Ocimum tenuiflorum</i> L.	46.31	42.26	
101	<i>Xanthium sibiricum</i>	46.4	92.48	
102	<i>Patrinia scabiosifolia</i>	46.58	66.4	
103	<i>Nothapodytes nimmoniana</i>	46.64	85.08	
104	<i>Rhus glabra</i>	46.81	73.51	
105	<i>Justicia adhatoda</i> L.	47.01	68.14	
106	<i>Asparagus cochinchinensis</i>	47.3	97.35	
107	<i>Phellodendron amurense</i>	47.62	71.2	
108	<i>Erythroxylum pervillei</i>	47.9	55.62	
109	<i>Podophyllum hexandrum</i>	48.15	90.44	
110	<i>Fritillaria thunbergii</i>	48.49	64.09	
111	<i>Doronicum pardalianches</i>	48.93	42.84	
112	<i>Cleistanthus collinus</i>	49.31	70.16	

113	<i>Cimicifuga racemosa</i>	49.42	98.63	
114	<i>Rhus chinensis</i>	49.72	57.32	
115	<i>Verbenae officinalis</i>	49.8	80.02	
116	<i>Rhynchosia minima</i>	50.7	87.76	
117	<i>Emilia sonchifolia</i>	51.33	81.49	
118	<i>Lavandulae angustifolia</i> L.	51.55	67.34	
119	<i>Sanguisorba officinalis</i> L.	52.4	84.43	
120	<i>Rhodiola sacra</i>	52.63	92.22	
121	<i>Hedyotis diffusa</i>	53.25	98.5	
122	<i>Farfarae folium</i>	53.65	60.41	
123	<i>Rubus ellipticus</i>	53.7	72.49	
124	<i>Plantago asiatica</i> L.	54.28	86.62	
125	<i>Dysoxylum binectariferum</i>	54.49	96.6	
126	<i>Epilobium parvifolium</i>	54.94	78.26	
127	<i>Melia azedarach</i> L.	55.2	94.18	
128	<i>Hederae folium</i>	55.54	65.08	
129	<i>Pestemon deustus</i>	55.76	95.76	
130	<i>Astragalus complanatus</i>	55.94	82.25	
131	<i>Lycopus lucidus</i>	56.37	99.46	
132	<i>Ipomoea aquatica</i>	56.72	81.24	
133	<i>Teucrii Montana</i>	57.06	60.6	
134	<i>Taraxacum mongolicum</i>	57.4	72.57	
135	<i>Indigofera aspalathoides</i>	57.82	80.74	
136	<i>Cuscuta chinensis</i> L.	58.18	99.8	
137	<i>Salviae officinalis</i>	58.52	72.48	
138	<i>Curculigo orchiooides</i>	58.94	85.26	
139	<i>Betulae folium</i>	59.3	76.04	
140	<i>Equiseti arvense</i>	59.46	93.44	
141	<i>Salvia miltiorrhiza</i>	59.58	65.8	
142	<i>Rubia cordifolia</i>	59.8	89.06	

143	<i>Bardanae folium</i>	60.35	81.66	
144	<i>Tilia cordata</i>	60.61	91.58	
145	<i>Sambucus nigra</i> L.	60.88	74.9	
146	<i>Sophora tonkinensis</i>	61.43	99.4	
147	<i>Andrographis paniculata</i>	61.48	95.3	
148	<i>Epimedium brevicomum</i>	62.24	76.08	
149	<i>Caesalpinia sappan</i> L.	62.53	64.71	
150	<i>Trollius europaeus</i>	62.77	92.88	
151	<i>Centaurium erythraea</i>	63.39	82.7	
152	<i>Isatis indigotica</i>	64.3	73.36	
153	<i>Ipomoea squamosa</i>	64.66	81.54	
154	<i>Viola reichenbachiana</i>	64.71	78.38	
155	<i>Diphylleia grayi</i>	65.23	98.18	
156	<i>Hyperii Herba</i>	65.7	93.44	
157	<i>Rubia cordifolia</i> L.	68.32	95.08	
158	<i>Bupleurum falcatum</i>	68.58	92.23	
159	<i>Campotheca acuminata</i>	68.65	88.49	
160	<i>Zanthoxylum nitidum</i>	68.8	104.2	
161	<i>Acacia catechu</i>	69.26	92.56	
162	<i>Drynaria fortune</i>	69.46	75.88	
163	<i>Rubi idaei</i>	69.75	107.16	
164	<i>Coptis chinensis</i>	70.32	86.48	
165	<i>Galium verum</i> L.	70.44	126.55	
166	<i>Centipeda minima</i>	70.58	78.55	
167	<i>Fraxini folium</i>	71.34	98.84	
168	<i>Cinnamomum cassia</i>	72.47	112.7	
169	<i>Uvae ursi</i>	72.62	81.32	
170	<i>Agrimonia pilosa</i>	73.57	98.05	
171	<i>Polygala tenuifolia</i>	73.7	80.54	
172	<i>Asparagus racemosus</i>	74.01	106.24	

173	<i>Gynostemma pentaphyllum</i>	74.36	99.6	
174	<i>Pulsatilla chinensis</i>	76.68	109.54	
175	<i>Vernonia cinerea</i>	77.39	108.37	
176	<i>Bauhinia variegata</i>	79.85	128.08	
177	<i>Uncaria macrophylla</i>	80.16	109.12	
178	<i>Actaea racemosa</i> L.	80.48	92.46	
179	<i>Matteuccia struthiopteris</i>	80.62	130.3	
180	<i>Satureia montana</i>	81.78	102.36	
181	<i>Stemona sessilifolia</i>	82.48	110.22	
182	<i>Sennae folium</i>	84.16	96.7	
183	<i>Picrorhiza scrophulariiflora</i>	87.66	121.48	
184	<i>Tripterygium wilfordii</i>	88.28	111.74	
185	<i>Oleae folium</i>	89.56	93.48	
186	<i>Trichosanthes kirilowii</i>	90.25	107.14	
187	<i>Rosmarinus officinalis</i>	90.63	136.2	
188	<i>Tilia cordata</i>	90.65	90.92	
189	<i>Morus nigra</i> L.	91.58	115.45	
190	<i>Sedum sarmentosum</i>	94.42	104.86	
191	<i>Croton setigerus</i> L.	94.88	125.04	
192	<i>Scutellaria baicalensis</i>	95.3	102.28	
193	<i>Viscum coloratum</i>	96.55	119.7	
194	<i>Geocrypta galii</i>	97.49	106.42	
195	<i>Smilax glabra</i> L.	99.66	110.16	
196	<i>Rubus chingii</i>	100.44	132.38	
197	<i>Inula Britannica</i> L.	103.38	127.4	
198	<i>Calycopteris floribunda</i>	110.62	103.66	
199	<i>Angelica sylvestris</i>	111.57	140.08	
200	<i>Nepeta cataria</i>	118.7	129.74	
	Mean	46.703	69.541	
	SD	26.034	31.745	

	Mean-1SD	20.669	37.795	
	Mean-1.5SD	45.203	68.041	
	Mean-2SD	44.703	67.541	
	Mean-2.5SD	44.203	67.041	

The above table 4.2 showed the Strong inhibitory effect on the radicle by deviation value:

*M-1(SD), **M-1.5(SD), ***M-2(SD) and M-2.5(SD). SD: standard deviation of radicle.

M: mean of radicle.

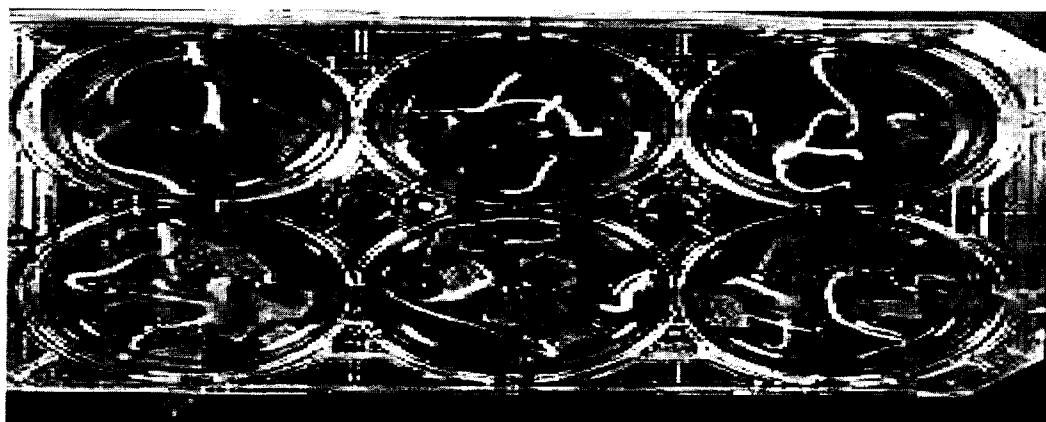


Fig. 4.1. Lettuce seed germination under medicinal plant influence in Sandwich method

The growth and germination of lettuce seeds portray the allelopathic effect in sandwich method due to leachates released from different parts of medicinal plants. The results demonstrated that 5 plants exhibited a strong inhibitory effect, 14 plants presented medium inhibitory, and 13 plants showed the low inhibitory effect on the lettuce seeds. Contemporary, the 7 plants substantially stimulate the growth of lettuce seeds in sandwich method. The details of plant species, their families and allelopathic effects were illustrated in table 4.2. The *Mentha piperita*, *Satureja hortensis* L., *Bidens pilosa* L., *Satureja montana* and *Thymus vulgaris* L. showed a strong inhibitory effect on the germination and seedling growth. The results pointed out the medium inhibitory effect on 14 species, including *Artemisia alba* L., *Trifolium repense* L. *Rheum officinale* L. *Hibisci sabdariffae*, *Dioscorea*

opposita., *Veronicae officinalis*, *Origanum vulgare* L., *Cassia tora* L., *Primula officinalis* and *Salicis cortex*.

However, the *Trichosanthes kirilowii*, *Rosmarinus officinalis*, *Tiliae officinalis*, *Morus nigra* L., *Sedum sarmentosum*, *Croton setigerus* L. *Scutellaria baicalensis*, *Viscum coloratum*, *Geocrypta galii*, *Smilax glabra* L., *Rubus chingii*, *Inula Britannica* L., *Calycopteris floribunda*, *Angelica sylvestris* and *Nepeta cataria* showed low inhibitory effect on the germination and growth of lettuce seeds. Meanwhile, the stimulatory allelopathic effects were recorded from the dry leaves of *Nepeta cataria*, *Angelica sylvestris*, *Calycopteris floribunda*, *Inula Britannica* L., *Smilax glabra* L., *Rubus cchingii*, and *Geocrypta galii*, on lettuce germination and seedling growth (hypocotyl and radicle length).

However, some plants showed very lower or same allelopathic effects on radicle and hypocotyl lengths with these different concentrations. For example, the *Angelica sylvestris*, *Nepeta cataria* and *Pteris multifida* showed no effect of any leaf concentration on root or hypocotyl growth as shown in Table 4.2. On the other hand, some plants showed an increase in the root growth but suppressed the hypocotyl growth. For example, in the case of *Teucrii Montana*, *Erythroxylum pervillei*, *Rhus chinensis* and *Taraxacum mongolicum*, a decrease in growth of hypocotyls was observed with the higher concentrations of leaves but an improvement in growth was observed with reduced concentrations.

Similarly, some plants like *Citrullus colocynthis* L., *Ephedra sinica*, *Punica granatum* and *Prunella vulgaris* showed an increase in hypocotyl growth, but suppression was observed in the radicle growth. Some of the tested medicinal plants improved the length of hypocotyls and radicles, while some plants showed no effects on radicle growth.

The *Rheum officinale* and *Cassia tora* L. showed inhibitory effects at the concentration of 50 mg leaves on the root/radicle growth up to 80-90% and followed by

Ophiopogon japonicus, *Azadirachta indica* and *Trifolium repense*. Other plants also showed diverse inhibitory effects on growth of radicle and hypocotyl of Lettuce. The lowest inhibitory effects at this concentration were observed in *Angelica sylvestris*, *Nepeta cataria* and *Pteris multifida* as shown in table 4.2.

4.2. Antioxidant Potential of Medicinal Plants

The bioactivity of these plant extracts was because of high phenolic and flavonoid contents found in them. A number of oxidizing molecules, like singlet oxygen and numerous other free radicals responsible for different diseases can be effectively scavenged by using the flavonoids. The ROS formation and chelate trace elements involved in free-radical production can be effectively suppressed by using these flavonoids. These can also be used to protect antioxidant defenses and in scavenging of reactive species. Also, the oxidative stress tolerance on plants can be conferred by these phenols and flavonoids (Aswatha *et al.*, 2008).

4.2.1. Ascorbic Acid Curve

The DPPH solution was used in this experiment because it is never affected by some side reactions, like enzymatic suppression or metal ion chelation, comparing with the other free radicals like superoxide oxides and hydroxyl ions (Gulcin *et al.*, 2010). The deep purple color was shown by freshly prepared DPPH solution with maximum absorbance at 517nm. The purple color of DPPH faded up or almost disappeared because of the antioxidant's activity present in these plant extracts. Therefore, the free radicals in DPPH can be neutralized by antioxidant molecules (i.e., by providing hydrogen atoms or donating electrons, possibly through an attack on the free radicals present in DPPH molecule) and thus resulting in purple to colorless change in color (e.g., by converting to 2, 2-diphenyl-1-hydrazine, or by replacing corresponding hydrazine molecule), which showed increase in the

absorbance at 517nm. This DPPH Assay is also very useful as the increase in absorbance of the sample solution can be directly measured by a continuous spectrophotometry in the reaction medium at any time. The consistent information regarding the antioxidant potential of these tested plant samples has been efficiently measured using DPPH assay (Aswatha *et al.*, 2008).

Table 4.3. Reduction in the percentage of DPPH absorbance values of samples at 517 nm with the addition of Ascorbic Acid.

Sr. No.	Concentration (ug/ml)	Absorbance	Inhibition (%)	IC ₅₀ (ug/ml)	R ²
1	10	0.944	34.44		
2	20	0.884	38.62		
3	30	0.752	47.77		
4	40	0.691	52.11		
5	50	0.616	57.22	37.337	0.989
6	60	0.575	60.06		
7	70	0.426	70.41		
8	80	0.394	72.69		
9	90	0.242	83.19		
10	100	0.198	86.25		
Blank (Methanol)		1.44			

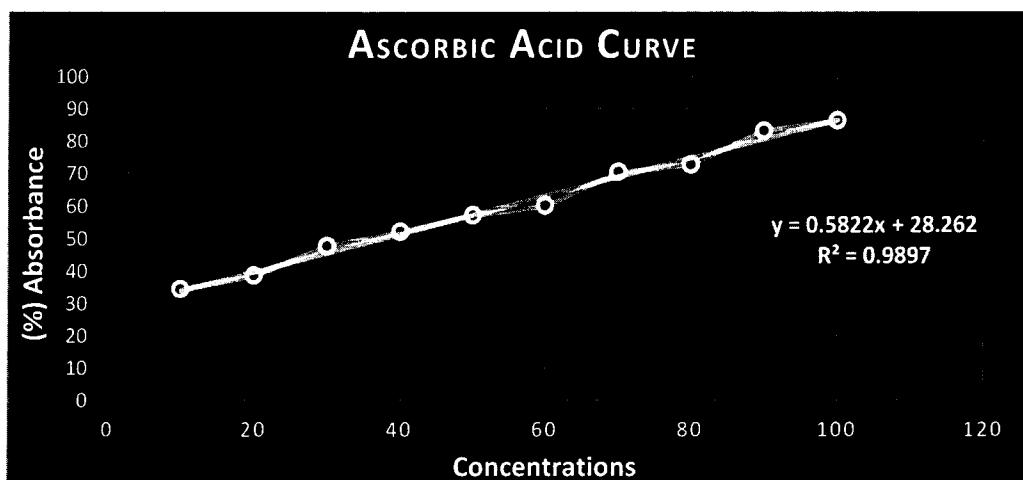


Fig. 4.2: Graphical presentation of Standard Ascorbic Acid Absorbance at 517nm

Table 4.4: Free Radical Scavenging Activity of Medicinal Plant samples using different Extract Solutions.

Sr. No.	Plant Sample	Sample Extracts	Radical Scavenging Activity (RSA) IC ₅₀ (ug/ml)	R ²
1	<i>Euphrasiae stricta</i> L.	Ethanol	38.972	0.968
		Methanol	43.665	0.962
		Water	110.057	0.985
2	<i>Euphorbia platyphyllos</i> L.	Ethanol	40.817	0.983
		Methanol	42.988	0.979
		Water	121.512	0.997
3	<i>Epimedium brevicomum</i> L.	Ethanol	46.265	0.978
		Methanol	51.249	0.996
		Water	98.605	0.981
4	<i>Viscum album</i>	Ethanol	52.279	0.984
		Methanol	54.463	0.991
		Water	141.227	0.979
5	<i>Psoralea corylifolia</i> L.	Ethanol	51.821	0.988
		Methanol	52.665	0.995
		Water	124.134	0.999
6	<i>Equiseti arvense</i>	Ethanol	55.246	0.958
		Methanol	58.781	0.993
		Water	128.427	0.983
7	<i>Veronica officinalis</i>	Ethanol	51.594	0.998
		Methanol	54.159	0.983
		Water	113.361	0.984
8	<i>Artemisia alba</i>	Ethanol	53.036	0.954
		Methanol	55.514	0.996

		Water	107.481	0.976
9	<i>Fagopyrum cymosum</i>	Ethanol	54.801	0.981
		Methanol	57.882	0.991
		Water	125.838	0.981
10	<i>Prunella vulgaris</i>	Ethanol	53.667	0.985
		Methanol	54.531	0.996
		Water	104.822	0.964
11	<i>Hederae folium</i>	Ethanol	56.797	0.982
		Methanol	59.797	0.998
		Water	109.527	0.981
12	<i>Salvia divinorum</i>	Ethanol	60.961	0.979
		Methanol	65.356	0.999
		Water	111.201	0.977
13	<i>Thymus serpyllum</i> L.	Ethanol	52.559	0.996
		Methanol	57.329	0.994
		Water	134.297	0.972
14	<i>Melissae officinalis</i>	Ethanol	51.768	0.952
		Methanol	59.436	0.997
		Water	59.436	0.995
15	<i>Cassia tora</i> L.	Ethanol	54.768	0.991
		Methanol	58.971	0.998
		Water	149.891	0.982
16	<i>Saussurea lappa</i>	Ethanol	55.289	0.988
		Methanol	58.978	0.999
		Water	112.452	0.995
17	<i>Epilobium parvifolium</i>	Ethanol	57.381	0.982
		Methanol	59.831	0.999
		Water	116.097	0.994
18	<i>Satureja montana</i>	Ethanol	57.335	0.991
		Methanol	61.972	0.996
		Water	113.874	0.987
19	<i>Asperula odorata</i>	Ethanol	64.561	0.973
		Methanol	66.287	0.974
		Water	117.113	0.988
20	<i>Gunnera perpensa</i>	Ethanol	57.705	0.987
		Methanol	59.522	0.998
		Water	144.375	0.974
21	<i>Fritillaria thunbergii</i>	Ethanol	55.551	0.997
		Methanol	58.624	0.998
		Water	123.577	0.977
22	<i>Melissa flava</i>	Ethanol	52.658	0.987
		Methanol	54.856	0.999
		Water	114.268	0.982
23	<i>Ocimum basilicum</i>	Ethanol	58.759	0.984
		Methanol	60.446	0.999
		Water	91.913	0.986

24	<i>Achillea millefolium</i>	Ethanol Methanol Water	57.279 58.336 118.324	0.979 0.981 0.998
25	<i>Urticae dioica L.</i>	Ethanol Methanol Water	51.986 53.122 126.186	0.996 0.997 0.991
26	<i>Polygonum aviculare L.</i>	Ethanol Methanol Water	57.452 62.838 132.052	0.994 0.998 0.979
27	<i>Lonicera japonica L.</i>	Ethanol Methanol Water	66.194 82.037 123.601	0.992 0.991 0.969
28	<i>Tinospora cordifolia L.</i>	Ethanol Methanol Water	50.966 53.372 127.955	0.989 0.998 0.991
29	<i>Paris polyphilla L.</i>	Ethanol Methanol Water	55.081 56.098 137.321	0.979 0.997 0.999
30	<i>Mentha piperita</i>	Ethanol Methanol Water	51.739 52.919 120.492	0.995 0.999 0.986
31	<i>Tephrosia purpurea L.</i>	Ethanol Methanol Water	50.955 54.951 117.435	0.981 0.999 0.981
32	<i>Marrubium vulgare</i>	Ethanol Methanol Water	55.541 65.398 113.849	0.998 0.997 0.983
33	<i>Lantana camara</i>	Ethanol Methanol Water	51.546 53.722 131.394	0.992 0.992 0.983
34	<i>Betulae folium</i>	Ethanol Methanol Water	52.297 56.297 129.645	0.991 0.991 0.999
35	<i>Teraxaci folium</i>	Ethanol Methanol Water	57.496 61.474 117.948	0.989 0.998 0.985
36	<i>Rubi idaei</i>	Ethanol Methanol Water	58.889 63.138 110.017	0.988 0.999 0.995
37	<i>Hedyotis diffusa</i>	Ethanol Methanol Water	54.642 58.837 121.351	0.987 0.999 0.989
38	<i>Smilax glabra</i>	Ethanol Methanol Water	56.638 60.301 109.511	0.994 0.998 0.999
	<i>Trifolium repense L.</i>	Ethanol	52.734	0.998

39		Methanol	56.442	0.999
		Water	123.492	0.973
40	<i>Centaurium erythraea</i>	Ethanol	52.487	0.992
		Methanol	58.836	0.998
		Water	134.361	0.981
	Standard Ascorbic Acid	Methanol	37.337	0.989

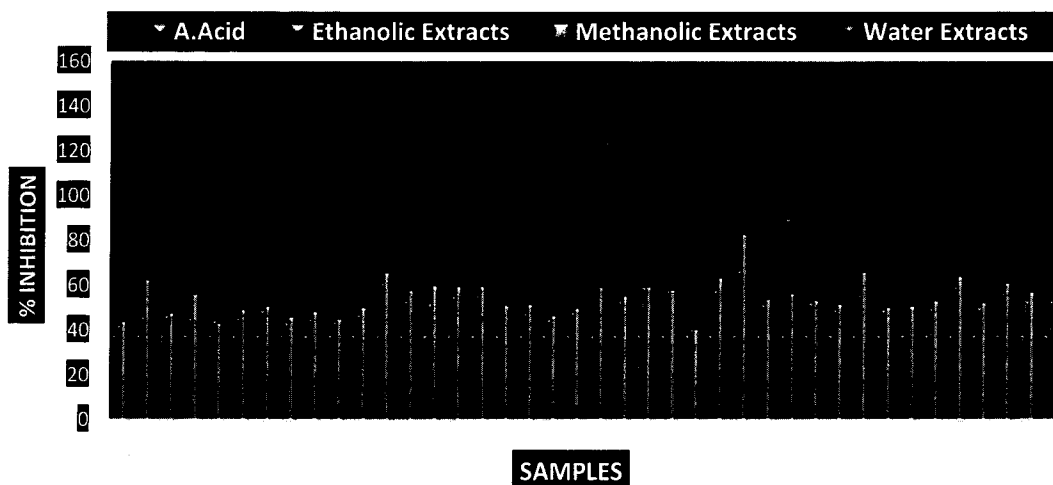


Fig. 4.3: Graphical presentation of Free Radical Scavenging potential of various plant extracts of various Medicinal plant samples in comparison with Ascorbic Acid.

In this research, the ethanolic, Methanol and aqueous plant extracts of 40 medicinal plants were experimented to test their free radical scavenging potential using DPPH assay. The “Table 4.3” and “Figure 4.3” showed the IC_{50} (the test solution concentration required to increase the absorbance of a sample by 50% comparing to the blank solution) for different medicinal plants in different extracts. The results showed that ethanolic extracts of these medicinal plant extracts showed the higher level of free radical scavenging or antioxidant properties, followed by Methanolic extracts in comparison with the $IC_{50} = 37.337 \mu\text{g/ml}$ of standard ascorbic acid. The ethanolic and Methanolic extracts *Euphrasiae stricta* L. showed the highest antioxidant potential of 38.972 $\mu\text{g/ml}$ and 43.665 $\mu\text{g/ml}$ respectively followed by the *Euphorbia platyphyllos* L. (40.817 $\mu\text{g/ml}$ and 42.988 $\mu\text{g/ml}$) and *Epimedium brevicomum*

Maxim (46.265ug/ml and 51.249ug/ml) as compared to the Ascorbic acid IC_{50} = 37.337ug/ml. The aqueous extracts showed almost similar results for all samples regarding %age inhibition of free radicals as shown in “Table 4.4”.

The *Viscum album* L. and *Psoralea corylifolia* L. showed the radical scavenging activity of 52.279 ug/ml and 51.821 ug/ml for its ethanolic and 54.463 ug/ml and 51.821 ug/ml for Methanolic extracts respectively followed by *Equiseti arvense*, *Veronica officinalis*, *Artemisia alba*, *Fagopyrum cymosum*, *Prunella vulgaris*, *Hederae folium*, *Salvia divinorum*, *Thymus serpyllum* L., *Melissae officinalis* and *Cassia tora* as compared to the Standard L-ascorbic acid.

The *Tephrosia purpurea* L., *Marrubium vulgare*, *Lantana camara*, *Betulae folium*, *Teraxaci folium*, *Rubi idaei*, *Hedyotis diffusa*, *Smilax glabra* L., *Trifolium repense* L. and *Centaurium erythraea* showed relatively less antioxidant potential as compared to other tested medicinal plants using DPPH free radical assay as shown in “Table 4.4” and “Figure 4.4”.

The similar studies were conducted by Yin and Chuang, (2000) for the antioxidant potentials of *C. tora* L. aqueous extracts investigated in their study. It was noted that at a dose of 0.2 mg / ml, the *C. tora* (unroasted) showed 94% hang-up of linoleic acid peroxidation even greater than the alpha-tocopherol (82%). The roasting of water extracts of *C. tora* L. was achieved at 200 ° C for 5 minutes and 175 ° C for 5 minutes with inhibition of linoleic acid peroxidation results of 82% and 83%, respectively.

Thus, these medicinal plants with the higher antioxidant potentials can be efficiently used as an efficient source of natural antioxidants to treat various oxidative stress related problems like cancer and other cardiovascular disorders. A quality control protocol may also be designed by following this study obtained by DPPH assay method and might be useful to

develop an efficient protocol to investigate the antioxidant, anticancer or phytochemical activities of traditionally used medicinal plants.

4.2.2. Antioxidant Activity of Medicinal Plants

4.2.2.1. Total Phenolic Contents (TPC) and Total Flavonoid Content (TFC)

4.2.2.1.1. Total Phenolic Contents (TPC)

The bioactivity of these plant extracts was because of high phenolic and flavonoid contents found in them. A number of oxidizing molecules, like singlet oxygen and numerous other free radicals responsible for different diseases can be effectively scavenged by using the flavonoids. The reactive oxygen formation and chelate trace elements involved in free-radical production can be effectively suppressed by using these Flavonoids. The antioxidant activity of medicinal plants is primarily because of the presence of the Phenolic and Flavonoid contents found in them. The antioxidant potential is primarily because of redox properties possessed by Phenolic compounds in the medicinal plants or other plants and fruits. These plants can also be used to protect and up-regulate antioxidant defenses and in scavenging of reactive species. Also, the oxidative stress tolerance on plants can be conferred by these phenols and flavonoids.

Table 4.5. The Total Phenolic Contents (TPC) of top medicinal plants

Sr. No.	Plant Sample	Total Phenolic Contents at 765 nm		
		Mean \pm SD (mg/ml)		
		Ethanollic Ext.	Methanolic Ext.	Aqueous Ext.
1	<i>Euphrasiae stricta</i> L.	58.19 \pm 1.74	45.70 \pm 1.48	13.00 \pm 1.20
2	<i>Euphorbia platyphyllos</i> L.	46.05 \pm 1.10	42.00 \pm 1.54	12.84 \pm 1.24
3	<i>Epimedium brevicomum</i>	51.93 \pm 1.72	44.06 \pm 0.64	13.26 \pm 0.44
4	<i>Viscum album</i> L.	42.84 \pm 0.48	42.50 \pm 0.56	11.08 \pm 0.92

5	<i>Psoralea corylifolia</i> L.	37.70 ± 0.62	38.12 ± 0.06	8.54 ± 0.88
6	<i>Equiseti arvense</i>	40.56 ± 0.32	36.90 ± 0.18	11.49 ± 0.70
7	<i>Veronica officinalis</i>	44.28 ± 2.00	39.02 ± 1.24	13.56 ± 1.20
8	<i>Artemisia alba</i>	42.24 ± 0.36	37.16 ± 0.82	13.04 ± 0.18
9	<i>Fagopyrum cymosum</i>	41.82 ± 1.87	40.30 ± 1.41	10.03 ± 1.24
10	<i>Prunella vulgaris</i>	36.77 ± 1.84	34.68 ± 0.54	11.26 ± 0.69
11	<i>Hederae folium</i>	34.62 ± 1.63	32.76 ± 1.06	14.40 ± 0.56
12	<i>Salvia divinorum</i>	39.40 ± 1.76	37.08 ± 0.96	12.00 ± 1.66
13	<i>Thymus serpyllum</i> L.	35.23 ± 1.00	33.28 ± 1.44	10.03 ± 0.74
14	<i>Melissae officinalis</i>	42.00 ± 1.36	38.12 ± 1.28	11.20 ± 1.31
15	<i>Cassia tora</i> L.	40.94 ± 1.82	38.74 ± 0.46	13.02 ± 0.46
16	<i>Saussurea lappa</i>	35.79 ± 1.74	34.33 ± 1.04	12.20 ± 1.58
17	<i>Epilobium parvifolium</i>	40.52 ± 1.90	37.96 ± 0.06	9.00 ± 1.64
18	<i>Satureja montana</i>	44.30 ± 1.34	42.12 ± 1.00	13.44 ± 0.80
19	<i>Asperula odorata</i>	38.26 ± 0.53	36.05 ± 0.44	11.56 ± 0.68
20	<i>Gunnera perperisa</i>	34.15 ± 1.72	33.46 ± 1.16	14.66 ± 1.40
21	<i>Fritillaria thunbergii</i>	43.72 ± 1.84	40.78 ± 1.26	12.14 ± 1.56
22	<i>Melissa flava</i>	41.56 ± 0.88	38.42 ± 0.34	9.26 ± 0.68
23	<i>Ocimum basilicum</i>	34.49 ± 1.44	32.80 ± 1.56	11.33 ± 1.78
24	<i>Achillea millefolium</i>	39.35 ± 0.77	36.76 ± 0.34	8.82 ± 0.33
25	<i>Urticae dioica</i> L.	37.06 ± 1.15	34.76 ± 0.55	14.53 ± 0.52
26	<i>Polygonum aviculare</i>	41.98 ± 1.86	40.00 ± 0.98	12.50 ± 1.80
27	<i>Lonicera japonica</i>	36.75 ± 1.59	51.64 ± 1.62	11.00 ± 1.4
28	<i>Tinospora cordifolia</i>	39.54 ± 1.88	37.08 ± 0.76	12.36 ± 1.22
29	<i>Paris polyphilla</i>	35.21 ± 0.88	34.62 ± 1.42	13.44 ± 1.68
30	<i>Mentha piperita</i>	42.92 ± 0.46	41.36 ± 1.24	10.31 ± 0.60
31	<i>Tephrosia purpurea</i> L.	33.83 ± 0.57	31.08 ± 0.92	14.48 ± 0.43
32	<i>Marrubium vulgare</i>	40.78 ± 0.44	39.64 ± 0.88	13.81 ± 0.66
33	<i>Lantana camara</i>	34.56 ± 1.65	32.50 ± 1.81	10.04 ± 1.90
34	<i>Betulae folium</i>	39.47 ± 0.46	36.82 ± 0.53	8.45 ± 0.94

35	<i>Teraxaci folium</i>	35.42 ± 1.56	34.16 ± 2.12	8.56 ± 1.48
36	<i>Rubi idaei</i>	43.33 ± 1.74	42.02 ± 0.58	14.00 ± 1.90
37	<i>Hedyotis diffusa</i>	40.92 ± 1.78	40.50 ± 1.82	13.57 ± 0.74
38	<i>Smilax glabra</i> L.	36.80 ± 1.76	35.63 ± 1.51	10.88 ± 1.64
39	<i>Trifolium repense</i>	41.45 ± 1.75	38.59 ± 1.76	11.24 ± 1.92
40	<i>Centaurium erythraea</i>	38.24 ± 0.24	36.18 ± 0.92	12.44 ± 0.88

In this research the ethanolic extracts of *Euphrasiae stricta*, showed the higher total phenol contents, measured as 58.19 ± 1.74 followed by 46.05 ± 1.10 and 51.93 ± 1.72 gallic acid equivalents/g for ethanolic extracts of *Euphorbia platyphyllos* L. and *Epimedium brevicomum* Maxim respectively as shown in table 4.5. The Methanolic extracts of *E. stricta*, *E. platyphyllos* and *E. brevicomum* showed the Total phenol contents in range of 45.70 ± 1.48 , 42.00 ± 1.54 and 44.06 ± 0.64 gallic acid equivalents/g respectively.

The *Viscum album* L. and *Psoralea corylifolia* L. showed the radical scavenging activity of 42.84 ± 0.48 and 37.70 ± 0.62 gallic acid equivalents/g for its ethanolic and 37.70 ± 0.62 and 38.12 ± 0.06 gallic acid equivalents/g for Methanolic extracts respectively followed by *Equiseti arvense*, *Veronica officinalis*, *Artemisia alba*, *Fagopyrum cymosum*, *Prunella vulgaris*, *Hederae folium*, *Salvia divinorum*, *Thymus serpyllum* L., *Melissae officinalis* and *Cassia tora* as compared to the Standard L-ascorbic acid.

The *Tephrosia purpurea* L., *Marrubium vulgare*, *Lantana camara*, *Betulae folium*, *Teraxaci folium*, *Rubi idaei*, *Hedyotis diffusa*, *Smilax glabra* L., *Trifolium repense* L. and *Centaurium erythraea* showed relatively less antioxidant potential as compared to other tested medicinal plants as shown in “Table 4.5” and “Figure 4.5”.

4.2.2.1. Total Flavonoid Content (TFC)

Various plant secondary metabolites like flavanols, flavones and abbreviated tannins were present in flavonoids. The presence of free OH groups, especially 3-OH groups, is mainly responsible for the antioxidant activity of these flavonoids. Plant flavonoids can be used as potential antioxidant in vitro as well as in-vivo. As the antioxidant activity of various medicinal plants in this study was investigated for the first time so a detailed phytochemical investigation to recognize the potential phenolic and flavonoid contents of these medicinal plants should be achieved before their application in oxidative stress related diseases evaluated the total phenol content (TPC) and flavonoids (TFC) from each extract of *E. pinnatum* extracts through spectral methods. It was observed that the higher antioxidant and antimicrobial activity was shown by the Methanolic extract as compared to the ethanolic or water extracts (Satyajit *et al.*, 2014).

Table 4.6. The Total Flavonoid Contents (TFC) of top medicinal plants

Sr. No.	Plant Sample	Total Flavonoid Contents at 510 nm		
		Mean \pm SD (mg/ml)		
		Ethanolic Ext.	Methanolic Ext.	Aqueous Ext.
1	<i>Euphrasiae stricta</i> L.	42.44 \pm 1.26	39.18 \pm 0.74	12.10 \pm 1.62
2	<i>Euphorbia platyphyllos</i> L.	36.39 \pm 1.05	35.88 \pm 1.34	10.23 \pm 0.44
3	<i>Epimedium brevicomum</i>	39.21 \pm 1.76	38.62 \pm 1.98	11.90 \pm 1.72
4	<i>Viscum album</i>	40.92 \pm 1.80	38.96 \pm 0.16	11.72 \pm 0.88
5	<i>Psoralea corylifolia</i> L.	28.29 \pm 1.34	26.73 \pm 1.91	9.62 \pm 1.54
6	<i>Equiseti arvense</i>	37.80 \pm 1.98	34.26 \pm 0.69	10.06 \pm 1.88
7	<i>Veronica officinalis</i>	33.71 \pm 1.36	31.42 \pm 1.50	8.64 \pm 0.96
8	<i>Artemisia alba</i>	25.85 \pm 0.43	25.15 \pm 0.46	14.46 \pm 0.62
9	<i>Fagopyrum cymosum</i>	37.53 \pm 1.74	35.76 \pm 1.14	10.16 \pm 1.92

10	<i>Prunella vulgaris</i>	26.12 ± 1.65	25.38 ± 1.74	9.94 ± 1.76
11	<i>Hederae folium</i>	24.36 ± 0.82	22.16 ± 0.40	11.66 ± 0.98
12	<i>Salvia divinorum</i>	28.93 ± 1.24	27.88 ± 1.59	10.33 ± 1.34
13	<i>Thymus serpyllum</i> L.	24.44 ± 0.77	23.26 ± 0.94	10.85 ± 0.48
14	<i>Melissae officinalis</i>	30.21 ± 1.56	28.57 ± 0.25	12.43 ± 0.55
15	<i>Cassia tora</i> L.	33.78 ± 1.63	31.00 ± 0.78	12.58 ± 1.26
16	<i>Saussurea lappa</i>	29.35 ± 1.51	26.94 ± 1.12	9.00 ± 1.31
17	<i>Epilobium parvifolium</i>	25.56 ± 1.28	24.04 ± 0.44	10.76 ± 1.86
18	<i>Satureja montana</i>	32.41 ± 0.83	50.72 ± 1.52	6.64 ± 1.64
19	<i>Asperula odorata</i>	27.82 ± 0.96	25.46 ± 1.64	9.33 ± 0.40
20	<i>Gunnera perperna</i>	21.60 ± 0.52	20.58 ± 0.32	10.49 ± 0.93
21	<i>Fritillaria thunbergii</i>	35.28 ± 0.46	33.44 ± 0.86	8.61 ± 0.79
22	<i>Melissa flava</i>	32.66 ± 1.35	28.80 ± 1.41	6.44 ± 1.96
23	<i>Ocimum basilicum</i>	26.31 ± 0.93	25.74 ± 0.63	8.85 ± 0.38
24	<i>Achillea millefolium</i>	28.84 ± 1.66	24.36 ± 1.22	10.56 ± 1.86
25	<i>Urticae dioica</i> L.	24.38 ± 1.14	22.04 ± 0.54	13.34 ± 1.00
26	<i>Polygonum aviculare</i>	29.52 ± 1.44	27.56 ± 1.68	12.76 ± 0.58
27	<i>Lonicera japonica</i>	26.60 ± 1.96	23.64 ± 1.42	11.58 ± 1.46
28	<i>Tinospora cordifolia</i>	24.85 ± 1.72	22.29 ± 1.96	11.64 ± 1.98
29	<i>Paris polyphilla</i>	28.44 ± 0.84	27.88 ± 0.12	9.51 ± 0.62
30	<i>Mentha piperita</i>	32.16 ± 0.56	30.33 ± 1.44	11.00 ± 1.18
31	<i>Tephrosia purpurea</i> L.	24.39 ± 1.88	23.96 ± 0.80	13.52 ± 0.86
32	<i>Marrubium vulgare</i>	31.72 ± 1.64	28.58 ± 1.35	11.84 ± 1.54
33	<i>Lantana camara</i>	26.30 ± 1.46	25.06 ± 0.86	8.40 ± 1.66
34	<i>Betulae folium</i>	25.82 ± 0.70	23.69 ± 0.53	10.36 ± 1.48
35	<i>Teraxaci folium</i>	24.46 ± 1.58	21.27 ± 0.67	12.64 ± 0.76
36	<i>Rubi idaei</i>	34.62 ± 1.97	32.65 ± 0.54	10.33 ± 1.64
37	<i>Hedyotis diffusa</i>	30.54 ± 0.69	29.37 ± 0.96	10.74 ± 0.41
38	<i>Smilax glabra</i> L.	28.87 ± 0.78	24.54 ± 0.45	8.22 ± 1.20
39	<i>Trifolium repense</i>	22.40 ± 1.42	20.16 ± 1.94	11.08 ± 0.52

40	<i>Centaurium erythraea</i>	26.63 ± 0.86	25.94 ± 0.22	9.70 ± 0.82
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In this table the ethanolic extracts of *Euphrasiae stricta*, showed the higher total phenol contents, measured as 42.44 ± 1.26 followed by 36.39 ± 1.05 and 39.21 ± 1.76 gallic acid equivalents/g for ethanolic extracts of *Euphorbia platyphyllos* L. and *Epimedium brevicomum* Maxim respectively as shown in table 4.6. The Methanolic extracts of *E. stricta*, *E. platyphyllos* and *E. brevicomum* showed the Total phenol contents in range of 39.18 ± 0.74 , 35.88 ± 1.34 and 38.62 ± 1.98 gallic acid equivalents/g respectively. As the antioxidant activity of various medicinal plants in this study was investigated for the first time so a detailed phytochemical investigation to recognize the potential phenolic and flavonoid contents of these medicinal plants should be achieved before their application in oxidative stress related diseases.

The *Viscum album* L. and *Psoralea corylifolia* L. showed the radical scavenging activity of 40.92 ± 1.80 and 28.29 ± 1.34 gallic acid equivalents/g for its ethanolic and 38.96 ± 0.16 and 26.73 ± 1.91 gallic acid equivalents/g for Methanolic extracts respectively followed by *Equiseti arvense*, *Veronica officinalis*, *Artemisia alba*, *Fagopyrum cymosum*, *Prunella vulgaris*, *Hederae folium*, *Salvia divinorum*, *Thymus serpyllum* L., *Melissae officinalis* and *Cassia tora* as compared to the Standard L-ascorbic acid.

The *Tephrosia purpurea* L., *Marrubium vulgare*, *Lantana camara*, *Betulae folium*, *Teraxaci folium*, *Rubi idaei*, *Hedyotis diffusa*, *Smilax glabra* L., *Trifolium repense* L. and *Centaurium erythraea* showed relatively less antioxidant potential as compared to other tested medicinal plants as shown in “Table 4.6” and “Figure 4.6”.

The similar findings were observed by Satyajit *et al.*, (2014) who evaluated the total phenol content (TPC) and flavonoids (TFC) from each extract of *E. pinnatum* and *Euphorbia*

platyphyllos extracts through spectral methods. It was observed that the higher antioxidant and antimicrobial activity was shown by the Methanolic extract as compared to the ethanolic or water extracts. The Methanol extract also showed high value of (149 and 36.6 mg /g) for TPC and TFC of *E. pinnatum* extracts.

The *Epimedium brevicomum* was also evaluated for its total phenol content, antioxidant capacity of flavonoids, free radicals and potential effects of high blood pressure were studied for the water extract. The absorption activity of nitrogen oxide in vitro was 1 mg /L TE 63.43% showing an IC₅₀ value of 122.36 µg/ml. It was noticed that in all experimental mice, after treatment with TE the heart index was the same. Thus, these results are in accordance with this present study. (Mihailovic *et al.*, 2013).

The similar findings were observed for the ethanolic extracts of leaves and bark of *Eucommia olmoides* (Oliv.) were studied by Xu *et al.*, (2014), for the investigation of their total phenol content (TPC) and total flavonoid content (TFC). It was found that the extract of de Chung plant contains the highest content of TPC (94.45 +/- 1.17 mg equivalent of hard galic acid / g) and TFC content (60.35 +/- 0.60 mg of cation equivalent/g of solid extraction). In all three previous antioxidant testing systems, the plant extract also showed the strongest anti-oxidant capacity, followed by the extraction of crispy crust and seed extraction. So, it was suggested by this study that the plant extract may be a potential source of natural antioxidants.

4.2.2.2. Evaluation of Antioxidant potential of medicinal plants using DPPH free radical scavenging assay

About 1 ml of test solution was dissolved to equivalent amount of DPPH solution (0.1 mM) in ethanol, Methanol and water. The increase in DPPH absorbance of tested samples

was measured after 20 min incubation at room temperature, by taking the absorbance at 517nm. Standard Ascorbic acid (1mM) showed a maximum absorbance of $90.36 \pm 1.05 \mu\text{g/ml}$ and was taken as a reference solution in this DPPH antioxidant assay.

Table 4.7: The Percentage Free Radical Scavenging Activity (RSA)/Antioxidant activity of medicinal plants using DPPH Assay.

Sr. No.	Plant Sample	Free Radical Scavenging Activity at 517 nm (%)		
		Mean \pm SD ($\mu\text{g/ml}$)		
		Ethanollic Ext.	Methanolic Ext.	Aqueous Ext.
1	<i>Euphrasiae stricta</i>	72.56 \pm 0.46	69.22 \pm 1.94	33.16 \pm 0.55
2	<i>Euphorbia platyphyllos L.</i>	71.92 \pm 1.22	70.14 \pm 0.82	12.08 \pm 0.96
3	<i>Epimedium brevicomum</i>	70.63 \pm 0.49	68.38 \pm 1.22	12.84 \pm 0.60
4	<i>Viscum album</i>	70.24 \pm 1.6	66.13 \pm 0.42	23.8 \pm 0.28
5	<i>Psoralea corylifolia L.</i>	69.76 \pm 1.48	68.00 \pm 1.50	18.34 \pm 1.84
6	<i>Equiseti arvense</i>	69.55 \pm 0.44	66.31 \pm 2.26	27.15 \pm 0.92
7	<i>Veronica officinalis</i>	69.31 \pm 0.69	67.40 \pm 0.78	18.40 \pm 0.34
8	<i>Artemisia alba</i>	67.96 \pm 1.67	64.04 \pm 1.32	13.66 \pm 1.52
9	<i>Fagopyrum cymosum</i>	67.58 \pm 0.72	65.18 \pm 1.44	26.30 \pm 0.96
10	<i>Prunella vulgaris</i>	67.46 \pm 1.26	66.78 \pm 0.36	13.34 \pm 0.25
11	<i>Origanum vulgare</i>	67.12 \pm 1.05	63.64 \pm 0.25	23.36 \pm 0.74
12	<i>Camptotheca acuminata</i>	65.88 \pm 1.96	63.08 \pm 0.36	10.70 \pm 1.56
13	<i>Matteuccia struthiopteris</i>	65.82 \pm 1.18	64.54 \pm 0.74	25.36 \pm 0.51
14	<i>Nothapodytes nimmoniana</i>	65.75 \pm 0.53	63.66 \pm 1.34	25.24 \pm 0.88
15	<i>Viscum articulatum</i>	65.44 \pm 1.14	62.18 \pm 0.66	15.06 \pm 1.40
16	<i>Justicia procumbens</i>	65.22 \pm 0.66	64.22 \pm 0.34	10.86 \pm 0.74
17	<i>Citrullus colocynthis L.</i>	64.95 \pm 0.26	62.39 \pm 0.75	24.83 \pm 0.62
18	<i>Bauhinia variegata</i>	64.77 \pm 1.55	61.68 \pm 0.66	22.31 \pm 0.27
19	<i>Cimicifuga dahurica</i>	64.63 \pm 1.22	63.35 \pm 0.56	20.63 \pm 0.24
20	<i>Psoralea corylifolia L.</i>	64.50 \pm 1.48	62.52 \pm 1.66	20.50 \pm 1.41
21	<i>Acacia catechu</i>	63.80 \pm 0.53	60.58 \pm 1.76	26.2 \pm 0.56

22	<i>Geranii robertiani</i>	63.66 ± 1.58	62.14 ± 0.22	10.90 ± 0.88
23	<i>Equiseti arvense</i>	63.58 ± 1.28	61.86 ± 0.55	15.90 ± 1.12
24	<i>Veronica officinalis</i>	62.74 ± 1.66	60.04 ± 0.56	12.26 ± 1.78
25	<i>Artemisia alba</i>	62.67 ± 0.54	59.37 ± 0.28	9.44 ± 0.44
26	<i>Rubia cordifolia</i>	62.53 ± 0.32	61.86 ± 1.94	9.28 ± 0.40
27	<i>Patrinia scabiosifolia</i>	62.44 ± 1.32	60.25 ± 1.82	20.19 ± 1.48
28	<i>Salviae officinalis</i>	62.27 ± 0.62	61.32 ± 0.94	18.62 ± 1.58
29	<i>Lycopus lucidus</i>	62.04 ± 0.32	59.70 ± 0.90	28.86 ± 0.38
30	<i>Glycyrrhiza uralensis</i>	61.77 ± 0.81	59.06 ± 0.36	17.56 ± 0.46
31	<i>Satureja bachtiarica</i>	61.46 ± 0.68	58.28 ± 0.44	29.96 ± 0.79
32	<i>Bidens pilosa</i> L.	61.04 ± 2.00	59.80 ± 1.33	26.00 ± 1.05
33	<i>Fagopyrum cymosum</i>	60.93 ± 0.85	59.43 ± 0.76	25.06 ± 1.42
34	<i>Prunella vulgaris</i>	60.79 ± 1.22	57.66 ± 0.82	10.08 ± 1.16
35	<i>Rehmannia glutinosa</i>	59.96 ± 1.56	58.68 ± 1.06	18.02 ± 1.00
36	<i>Hederae folium</i>	59.92 ± 1.40	56.86 ± 0.62	17.58 ± 1.6
37	<i>Portulaca oleracea</i> L.	59.68 ± 1.34	57.62 ± 1.82	28.16 ± 1.76
38	<i>Cuscuta chinensis</i> L.	59.60 ± 1.22	58.91 ± 1.92	19.56 ± 0.52
39	<i>Asparagus cochinchinensis</i>	59.42 ± 0.68	56.06 ± 1.14	10.66 ± 0.38
40	<i>Veronicae officinalis</i>	59.20 ± 0.78	57.40 ± 0.78	17.08 ± 0.82
41	<i>Ephedra sinica</i>	58.84 ± 0.69	56.71 ± 0.33	26.19 ± 0.62
42	<i>Eclipta prostrata</i>	58.42 ± 1.30	55.20 ± 1.82	19.00 ± 1.88
43	<i>Cynarae folium</i>	58.19 ± 1.74	57.70 ± 1.48	14.00 ± 1.20
44	<i>Caesalpinia sappan</i> L.	58.05 ± 1.10	55.00 ± 1.54	20.84 ± 1.24
45	<i>Morus alba</i> L.	57.93 ± 1.72	55.06 ± 0.64	13.26 ± 0.44
46	<i>Salvia divinorum</i>	57.84 ± 0.48	54.80 ± 0.56	10.08 ± 0.90
47	<i>Rubus chingii</i>	57.70 ± 0.62	54.11 ± 0.06	28.54 ± 0.88
48	<i>Rosmarinus officinalis</i>	57.56 ± 0.32	56.90 ± 0.18	31.49 ± 0.70
49	<i>Picrorhiza scrophulariiflora</i>	57.28 ± 2.00	54.02 ± 1.24	23.56 ± 1.20
50	<i>Thymus serpyllum</i> L.	57.24 ± 0.36	55.16 ± 0.82	16.04 ± 0.18
51	<i>Rubia cordifolia</i> L.	56.82 ± 1.87	53.30 ± 1.41	22.03 ± 1.24

52	<i>Melissae officinalis</i>	56.77 ± 1.84	55.68 ± 0.54	19.26 ± 0.69
53	<i>Podophyllum hexandrum</i>	56.62 ± 1.63	54.76 ± 1.06	15.40 ± 0.56
54	<i>Lavandulae angustifolia</i> L.	56.40 ± 1.76	53.08 ± 0.96	12.00 ± 1.66
55	<i>Cassia tora</i> L.	56.23 ± 1.00	55.28 ± 1.44	18.03 ± 0.74
56	<i>Arctium lappa</i> L.	56.00 ± 1.36	54.12 ± 1.28	11.20 ± 1.31
57	<i>Saussurea lappa</i>	55.94 ± 1.82	53.74 ± 0.46	15.02 ± 0.78
58	<i>Bursera simaruba</i>	55.79 ± 1.74	53.33 ± 1.04	15.20 ± 1.58
59	<i>Viola reichenbachiana</i>	55.52 ± 1.90	54.96 ± 0.06	25.00 ± 1.64
60	<i>Zanthoxylum nitidum</i>	55.30 ± 1.34	54.12 ± 1.00	23.44 ± 0.80
61	<i>Solanum nigrum</i> L.	55.26 ± 0.53	52.05 ± 0.44	24.56 ± 0.68
62	<i>Bupleurum falcatum</i>	55.15 ± 1.72	54.46 ± 1.16	19.66 ± 1.40
63	<i>Isatis indigotica</i>	54.72 ± 1.84	53.78 ± 1.26	24.14 ± 1.56
64	<i>Epilobium parvifolium</i>	54.56 ± 0.88	53.42 ± 0.34	16.26 ± 0.68
65	<i>Punica granatum</i>	54.49 ± 1.44	52.80 ± 1.56	22.33 ± 1.78
66	<i>Angelica sylvestris</i>	54.35 ± 0.77	52.76 ± 0.34	19.82 ± 0.33
67	<i>Satureja montana</i>	54.06 ± 1.15	51.76 ± 0.55	14.03 ± 0.5
68	<i>Asperula odorata</i>	53.98 ± 1.86	52.00 ± 0.98	16.50 ± 1.80
69	<i>Gunnera perpensa</i>	53.75 ± 1.59	51.64 ± 1.62	14.00 ± 1.4
70	<i>Rhus glabra</i>	53.54 ± 1.88	52.08 ± 0.76	12.36 ± 1.22
71	<i>Fritillaria thunbergii</i>	53.21 ± 0.88	50.62 ± 1.42	13.64 ± 1.63
72	<i>Melissa flava</i>	52.92 ± 0.46	50.36 ± 1.24	20.31 ± 0.60
73	<i>Glycyrrhiza glabra</i>	52.83 ± 0.57	49.08 ± 0.92	14.48 ± 0.43
74	<i>Angelica sinensis</i>	52.78 ± 0.44	51.64 ± 0.88	24.81 ± 0.66
75	<i>Rhus chinensis</i>	52.56 ± 1.65	48.50 ± 1.81	24.04 ± 1.90
76	<i>Morus nigra</i> L.	52.47 ± 0.46	49.82 ± 0.53	18.45 ± 0.94
77	<i>Sennae folium</i>	52.42 ± 1.56	50.16 ± 2.12	18.56 ± 1.48
78	<i>Centaurium erythraea</i>	52.33 ± 1.74	49.02 ± 0.58	14.00 ± 1.90
79	<i>Apium graveolens</i>	51.92 ± 1.78	48.50 ± 1.82	13.57 ± 0.74
80	<i>Ocimum basilicum</i>	51.80 ± 1.76	50.63 ± 1.51	13.38 ± 1.94
81	<i>Fragaria vesca</i>	51.45 ± 1.75	48.59 ± 1.76	11.24 ± 1.92

82	<i>Rhynchosia minima</i>	51.24 ± 0.24	49.18 ± 0.92	16.44 ± 0.88
83	<i>Achillea millefolium</i>	51.18 ± 0.86	48.30 ± 1.40	24.00 ± 1.78
84	<i>Satureja subspicata</i>	50.82 ± 1.18	47.46 ± 0.50	18.53 ± 0.82
85	<i>Phellodendron amurense</i>	50.61 ± 1.84	48.88 ± 1.75	21.83 ± 1.64
86	<i>Urticae dioica</i> L.	50.60 ± 1.47	49.04 ± 0.83	13.00 ± 1.76
87	<i>Ocimum tenuiflorum</i> L.	50.55 ± 0.50	47.29 ± 0.62	22.06 ± 1.22
88	<i>Astragalus complanatus</i>	50.39 ± 0.48	48.67 ± 0.74	14.81 ± 0.38
89	<i>Salvia miltiorrhiza</i>	50.32 ± 1.77	48.35 ± 0.55	22.83 ± 0.60
90	<i>Tragia involucrata</i> L.	49.94 ± 0.62	46.07 ± 0.56	18.14 ± 0.42
91	<i>Ailanthus altissima</i>	49.87 ± 0.77	47.94 ± 0.65	21.02 ± 1.34
92	<i>Dendrobium nobile</i>	49.80 ± 1.48	46.66 ± 1.84	12.68 ± 0.92
93	<i>Bardanae folium</i>	49.64 ± 0.56	48.04 ± 0.82	22.51 ± 0.68
94	<i>Indigofera aspalathoides</i>	49.59 ± 0.64	47.23 ± 0.46	22.96 ± 1.05
95	<i>Papever somniferum</i>	49.48 ± 0.65	48.15 ± 0.98	26.84 ± 0.72
96	<i>Coptis chinensis</i>	49.45 ± 0.55	46.09 ± 0.58	22.51 ± 0.26
97	<i>Inula Britannica</i> L.	48.91 ± 0.16	46.58 ± 0.40	20.06 ± 0.34
98	<i>Calycopteris floribunda</i>	48.63 ± 0.51	47.17 ± 0.50	27.13 ± 0.58
99	<i>Solanum torvum</i> L.	48.26 ± 0.89	45.33 ± 0.55	25.61 ± 0.42
100	<i>Scutellaria baicalensis</i>	47.72 ± 1.04	46.5 ± 1.64	17.81 ± 1.55
101	<i>Salvia fruticosa</i>	47.54 ± 0.55	45.87 ± 1.96	22.10 ± 0.78
102	<i>Sedum sarmentosum</i>	47.37 ± 0.32	46.15 ± 0.70	24.60 ± 0.94
103	<i>Vernonia cinerea</i>	47.00 ± 0.44	45.30 ± 1.04	26.16 ± 1.56
104	<i>Ocimum gratissimum</i> L.	46.86 ± 1.16	43.06 ± 0.28	17.84 ± 0.46
105	<i>Lonicera japonica</i>	46.18 ± 0.42	44.84 ± 0.58	18.33 ± 0.29
106	<i>Juniperus communis</i>	45.84 ± 1.70	42.00 ± 0.86	14.04 ± 1.62
107	<i>Cinnamomum cassia</i>	45.66 ± 1.18	44.44 ± 1.00	22.08 ± 1.72
108	<i>Withania somnifera</i>	45.22 ± 1.61	43.00 ± 1.37	23.12 ± 1.94
109	<i>Agrimonia pilosa</i>	44.88 ± 0.38	42.77 ± 0.20	19.44 ± 0.96
110	<i>Plantago lanceolata</i> L.	44.81 ± 1.74	41.56 ± 0.30	20.85 ± 0.72
111	<i>Asparagus racemosus</i>	44.74 ± 1.87	43.40 ± 1.43	21.66 ± 1.34

112	<i>Emilia sonchifolia</i>	44.22 ± 1.6	41.64 ± 1.22	25.46 ± 1.50
113	<i>Tinospora cordifolia</i>	44.02 ± 0.78	42.96 ± 1.88	20.06 ± 0.64
114	<i>Larrea tridentata</i>	43.82 ± 0.44	41.16 ± 0.84	22.08 ± 0.74
115	<i>Andrographis paniculata</i>	43.68 ± 0.85	40.13 ± 0.28	20.37 ± 1.60
116	<i>Cirsium setosum</i>	43.51 ± 0.44	42.06 ± 0.54	23.58 ± 0.45
117	<i>Gynostemma pentaphyllum</i>	42.98 ± 1.55	40.24 ± 0.61	27.38 ± 0.60
118	<i>Paris polyphilla</i>	42.92 ± 1.86	41.06 ± 0.24	18.28 ± 1.82
119	<i>Mentha piperita</i>	42.80 ± 0.58	39.55 ± 0.36	16.40 ± 1.24
120	<i>Gnetum africanum</i>	42.56 ± 0.74	41.34 ± 1.04	19.26 ± 0.88
121	<i>Croton setigerus</i> L.	42.49 ± 1.01	39.00 ± 1.49	18.62 ± 1.91
122	<i>Taraxacum mongolicum</i>	42.24 ± 0.86	41.32 ± 1.78	24.67 ± 0.36
123	<i>Tephrosia purpurea</i> L.	42.06 ± 0.40	40.38 ± 1.76	13.70 ± 0.46
124	<i>Ipomoea squamosa</i>	41.79 ± 0.60	39.22 ± 0.84	19.89 ± 0.66
125	<i>Rubus ellipticus</i>	41.60 ± 1.80	39.00 ± 1.36	24.10 ± 1.87
126	<i>Marrubium vulgare</i>	41.54 ± 1.62	38.46 ± 0.16	20.14 ± 0.56
127	<i>Gongronema latifolium</i>	41.32 ± 2.52	40.55 ± 0.74	23.41 ± 0.38
128	<i>Polygala tenuifolia</i>	40.77 ± 0.58	37.06 ± 0.74	21.42 ± 2.16
129	<i>Rheum officinale</i>	40.40 ± 0.28	39.9 ± 0.72	15.27 ± 0.58
130	<i>Vitex trifolia</i> L.	40.32 ± 0.58	38.41 ± 2.02	16.04 ± 0.12
131	<i>Artemisia absinthium</i> L.	39.95 ± 0.64	37.81 ± 1.06	14.46 ± 0.28
132	<i>Centipeda minima</i>	39.56 ± 0.58	38.36 ± 0.40	26.22 ± 0.74
133	<i>Solanum idicum</i> L.	39.14 ± 1.92	36.60 ± 1.57	26.62 ± 1.23
134	<i>Curculigo orchioides.</i>	38.86 ± 0.64	36.18 ± 1.32	19.04 ± 0.94
135	<i>Tiliae cordata</i>	38.63 ± 0.6	37.56 ± 0.54	27.29 ± 0.68
136	<i>Eucommia ulmoides</i>	38.50 ± 1.55	35.04 ± 1.30	16.60 ± 1.48
137	<i>Digitalis purpurea</i> L.	38.25 ± 1.94	34.48 ± 0.66	21.34 ± 0.74
138	<i>Nepeta cataria</i>	38.16 ± 0.51	35.36 ± 0.40	23.33 ± 0.81
139	<i>Betulae folium</i>	37.78 ± 0.42	36.44 ± 1.8	12.14 ± 0.82
140	<i>Teraxaci folium</i>	37.45 ± 0.73	35.52 ± 0.75	15.45 ± 0.62
141	<i>Rubi idaei</i>	36.84 ± 1.55	34.6 ± 1.76	12.80 ± 0.33

142	<i>Viscum coloratum</i>	36.44 ± 0.28	35.90 ± 0.48	18.10 ± 0.60
143	<i>Plantago asiatica</i> L.	36.20 ± 0.62	33.04 ± 1.86	21.78 ± 0.57
144	<i>Nepeta trachonitica</i>	36.06 ± 0.22	34.25 ± 0.43	21.72 ± 0.26
145	<i>Eclipta alba</i> L.	35.92 ± 1.56	34.51 ± 1.67	15.40 ± 1.51
146	<i>Hedyotis diffusa</i>	35.68 ± 1.76	32.36 ± 0.94	18.54 ± 1.14
147	<i>Pygeum africanum</i>	35.14 ± 0.54	33.36 ± 1.28	19.44 ± 0.50
148	<i>Satureja hortensis</i>	34.84 ± 1.34	32.6 ± 1.85	10.88 ± 1.70
149	<i>Ipomoea aquatica</i>	34.60 ± 1.77	33.94 ± 1.55	15.12 ± 1.32
150	<i>Houttuynia cordata</i> L.	34.46 ± 0.63	31.45 ± 0.81	17.64 ± 0.92
151	<i>Dianthus superbis</i> L.	34.13 ± 0.92	32.28 ± 0.54	14.14 ± 0.82
152	<i>Justicia adhatoda</i> L.	33.96 ± 1.05	32.35 ± 0.20	16.63 ± 1.55
153	<i>Chellidonium majus</i> L.	33.58 ± 0.44	30.12 ± 1.76	15.00 ± 0.70
154	<i>Galium verum</i> L.	33.24 ± 0.94	31.56 ± 1.58	12.74 ± 0.16
155	<i>Sanguisorba officinalis</i> L.	31.53 ± 0.21	30.35 ± 0.09	10.22 ± 0.52
156	<i>Smilax glabra</i> L.	30.82 ± 0.8	27.87 ± 0.63	11.55 ± 0.76
157	<i>Melia azedarach</i> L.	27.68 ± 1.81	25.00 ± 1.54	12.62 ± 1.22
158	<i>Trichosanthes kirilowii</i>	22.63 ± 1.44	21.59 ± 1.51	10.96 ± 1.07
159	<i>Trifolium repense</i> L.	20.56 ± 1.54	18.72 ± 1.09	12.21 ± 1.86
160	<i>Actaea racemosa</i> L.	19.46 ± 0.45	18.66 ± 0.84	9.76 ± 0.62
161	<i>Sophora tonkinensis</i>	9.94 ± 0.48	7.42 ± 1.14	1.26 ± 0.80
162	<i>Uncaria macrophylla</i>	9.70 ± 1.42	7.20 ± 1.32	1.04 ± 1.52
163	<i>Fraxini folium</i>	9.46 ± 1.44	6.38 ± 1.64	1.66 ± 1.28
164	<i>Trollius europaeus</i>	9.40 ± 0.54	6.15 ± 0.34	1.61 ± 0.73
165	<i>Polygoni avicularis</i>	9.28 ± 1.42	4.00 ± 1.07	0.85 ± 0.76
166	<i>Dipsacus japonicus</i>	8.74 ± 0.72	3.40 ± 0.88	1.66 ± 0.51
167	<i>Tripterygium wilfordii</i>	8.62 ± 0.25	5.45 ± 0.84	1.36 ± 0.48
168	<i>Primula officinalis</i>	8.38 ± 0.50	4.68 ± 0.9	0.46 ± 0.44
169	<i>Salicis cortex</i>	8.22 ± 0.48	4.76 ± 0.14	0.23 ± 0.60
170	<i>Dysoxylum binectariferum</i>	8.06 ± 0.64	5.78 ± 0.5	0.06 ± 0.26
171	<i>Tilia cordata</i>	7.96 ± 1.56	5.14 ± 0.45	1.66 ± 1.02

172	<i>Echinochloa crus-galli</i>	7.70 ± 1.82	4.26 ± 1.56	0.60 ± 1.84
173	<i>Pulsatilla chinensis</i>	7.63 ± 0.77	5.50 ± 0.52	0.26 ± 1.02
174	<i>Stemona sessilifolia</i>	7.50 ± 1.41	5.23 ± 1.41	1.61 ± 1.49
175	<i>Iris potaninii</i>	7.42 ± 1.4	5.34 ± 1.08	1.00 ± 0.16
176	<i>Uvae ursi</i>	7.34 ± 1.4	4.60 ± 1.12	0.60 ± 0.76
177	<i>Pteris multifida</i>	7.00 ± 1.08	4.12 ± 0.24	1.04 ± 1.48
178	<i>Lauri folium</i>	6.82 ± 1.28	4.60 ± 1.78	1.42 ± 0.71
179	<i>Crataegus monogyna</i>	6.64 ± 1.87	4.93 ± 0.46	1.39 ± 0.54
180	<i>Xanthium sibiricum</i>	6.46 ± 0.58	4.36 ± 1.52	0.28 ± 0.94
181	<i>Teucrii Montana</i>	6.23 ± 0.70	5.48 ± 0.34	1.06 ± 1.52
182	<i>Sambucus nigra L.</i>	6.21 ± 0.32	4.60 ± 1.19	0.20 ± 0.24
183	<i>Erythroxylum pervillei</i>	6.12 ± 0.52	5.44 ± 0.54	0.33 ± 0.29
184	<i>Ophiopogon japonicus</i>	6.10 ± 1.42	4.58 ± 1.92	0.22 ± 1.04
185	<i>Indigofera tinctoria</i>	6.03 ± 0.94	4.60 ± 0.86	1.02 ± 0.78
186	<i>Dioscorea opposita</i>	5.90 ± 0.64	4.88 ± 0.54	0.44 ± 0.82
187	<i>Drynaria fortune</i>	5.88 ± 1.92	4.52 ± 1.68	0.75 ± 1.26
188	<i>Hyperii Herba</i>	5.74 ± 0.45	3.94 ± 0.80	0.44 ± 1.02
189	<i>Canabis sativa L.</i>	5.70 ± 1.54	4.16 ± 1.98	0.88 ± 1.56
190	<i>Cichorium intybus</i>	5.58 ± 1.8	3.42 ± 1.28	0.52 ± 0.54
191	<i>Verbenae officinalis</i>	5.49 ± 1.18	3.98 ± 1.34	1.00 ± 1.32
192	<i>Polygonum hydropiper</i>	5.48 ± 1.47	2.00 ± 1.17	0.24 ± 1.07
193	<i>Azadirachta indica</i>	5.33 ± 1.41	3.38 ± 0.12	0.80 ± 1.95
194	<i>Hyoscyamus niger L.</i>	5.29 ± 0.12	3.72 ± 1.42	0.07 ± 0.36
195	<i>Pestemon deustus</i>	4.66 ± 1.44	2.94 ± 1.00	0.42 ± 0.30
196	<i>Cleistanthus collinus</i>	4.53 ± 0.54	3.44 ± 0.4	0.30 ± 0.46
197	<i>Diphylleia grayi</i>	4.44 ± 1.48	2.76 ± 0.62	0.84 ± 0.78
198	<i>Doronicum pardalianches L.</i>	4.39 ± 0.26	3.32 ± 0.53	0.68 ± 1.32
199	<i>Farfarae folium</i>	4.35 ± 1.34	3.34 ± 2.26	0.80 ± 1.48
200	<i>Rhodiola sacra</i>	4.22 ± 1.32	3.14 ± 0.20	0.56 ± 0.64
	Ascorbic Acid (Standard)		90.36 ± 1.05	

The highest antioxidant potential was observed in the ethanolic extracts of *Euphrasia stricta* L. The radical scavenging activity of ethanolic, Methanolic and aqueous extract of various medicinal plant extracts was examined as shown in Table 4.7. The standard antioxidant compound L-Ascorbic acid showed the highest radical scavenging activity of $90.36 \pm 1.05 \mu\text{g/ml}$. The ethanolic extracts showed the highest antioxidant activity of $71.92 \pm 1.22\%$ followed by Methanolic extracts with $70.14 \pm 0.82\%$.

The *Euphorbia platyphyllos* L. showed the radical scavenging activity of $69.76 \pm 1.48\%$ and $68.00 \pm 1.50\%$ for its ethanolic and Methanolic extracts respectively compared to the Standard L-ascorbic acid. Similarly, the antioxidant activity shown by ethanolic and Methanolic extracts of *Epimedium brevicomum* Maxim was $67.46 \pm 1.26\%$ and $66.78 \pm 0.36\%$ respectively. The *Viscum album* L. and *Psoralea corylifolia* L. showed the radical scavenging activity of 70.24 ± 1.6 and $69.76 \pm 1.48\%$ for its ethanolic and 66.13 ± 0.42 and 68.00 ± 1.50 Methanolic extracts respectively followed by *Equiseti arvense*, *Veronica officinalis*, *Fagopyrum cymosum*, *Prunella vulgaris*, *Origanum vulgare*, *Camptotheca acuminata*, *Matteuccia struthiopteris*, *Nothapodytes nimmoniana* and *Justicia procumbens* as compared to the Standard L-ascorbic acid.

This study is in accordance with the published scientific data on the chemical composition and biological activity of 10 species of *Euphrasia stricta* L.: *E. rostkoviana* Hayne, *E. brevipila*, *E. parviflora.*, *Euphorbia platyphyllos* L., *E. montana*, *E. pectinate*, *E. condensata*, *Epimedium brevicomum*, *E. stricta*, *E. salisburgensis*, *E. maximowiszii*, *Viscum album*, and *E. reuteri* (Suhinina TV and Petrichenko, 2019). The representatives of *Euphrasia* are the sources of biologically active substances, namely glycosides, organic acids, lipophylic substances, macro and microelements etc. Flavonoids, phenoloacids and iridoic glycosides, however, are the main groups.

Hypotensive, anti-inflammatory, anti-microbial, antioxidant and hepatoprotective action of *Euphrasia* representatives has been revealed experimentally.

The similar findings were observed by Ruan *et al.*, (2016) for increased concerns about the safety of synthetic antioxidants. Today's research was focused on the use of natural sources of antioxidants. The *Euphorbia platyphyllos* and *Rhazya stricta* have been an important medicinal plant in South Asia. To evaluate the antioxidant potential of these Methanolic extracts various antioxidant assays were used like DPPH, metal ion chelating assay and FRAP Assay. The Antibacterial activity and superoxide radical activity of *R. Stricta*, *Psoralea corylifolia* L and *Viscum album* were taken as parameters. The antioxidant potential in the Methanol extracts of *R. Stricta*, *Psoralea corylifolia* and *Viscum album* sheets was comparable with strong antioxidants previously exploited and largely dependent on concentration as shown in the present study.

The potential antioxidant activities of crude extracts (diethyl ether, petroleum ether, ethyl acetate, Methanol and water (infusion and decoction)) from *Euphorbia platyphyllos* L. (Euphorbiaceae) were evaluated for 1,1-diphenyl-2-picryl-hydrazyl (DPPH) scavenging, cytotoxic, DNA damaging and apoptotic activities of the extracts on MCF-7 breast cancer cells of extracts by DPPH assay, trypan blue exclusion assay, comet assay and Hoechst 33258/propidium iodide double staining, respectively. *E. platyphyllos* extracts showed significant DPPH scavenging activity, except diethyl ether and petroleum ether extracts. These results suggest that this plant has potential for being a source of anticancer agents for breast cancer treatments. These results are similar to the study conducted by (Ouml *et al*; 2013).

This study is also in accordance with the ultrasound-assisted extraction of phenolic compounds from *Epimedium brevicornu* Maxim was modeled using response surface

methodology. The optimized conditions are X_1 of 50% (v/v), X_2 of 27.5 min, and X_3 of 250 ml/g. Under these conditions, the experimental yield is $4.29 \pm 0.033\%$. The antioxidant activity was evaluated using the DPPH assay and FRAP assay (Zhao *et al.*; 2014).

The similar studies were conducted by Anandjiwala *et al.*, (2008) where the alcoholic DPPH solution reduction is because of the presence of hydrogen donor antioxidant (AH) which reacts with free radicals and convert it to non-radical DPPHH form. The ethanolic extracts of *Epimedium brevicomum* Maxim showed the maximum radical scavenging potential of $64.96 \pm 0.79\%$ followed by $62.59 \pm 0.73\%$ and $11.33 \pm 1.46\%$ for its Methanolic and aqueous extracts respectively. The lipophilic and hydrophilic compounds can also be measured for their antioxidant capacities by using this assay (Iqbal *et al.*, 2015^b). Thus, these results are in accordance with this present study.

4.2.2.3. Ferric Reducing Antioxidant Power (FRAP) Assay

In this study, the Fe^{3+} to Fe^{2+} reduction assay was used to check the reducing ability of Methanolic extracts of various medicinal plants. Here, the antioxidant activity in the samples caused change in yellow color to pale/light green or blue color in the solution. All the medicinal plant samples displayed the presence of various antioxidants, such as flavonoids and phenolic acid in considerable amounts. The electron donating capacity of a reducing agents (i.e., antioxidants) can be monitored by FRAP assay, resulting in the Fe^{3+} -ferricyanide complex to reduce to give ferrous (Fe^{2+}) ions, so creating a chromogenic complex (Kalita *et al.*, 2013). The absorbance of this resultant blue-green colored solution of samples was measured at 700 nm which was related to the Fe^{2+} amount in the mixture. Diverse abilities of the medicinal plant extracts to reduce the ferric ions (Fe^{3+}) were revealed by the present study.

Table 4.8: The Antioxidant activity of medicinal plants using FRAP Assay.

Sr. No.	Plant Sample	Free Radical Scavenging Activity at 700 nm (%)		
		Mean \pm SD (μ g/ml)		
		Ethanollic Ext.	Methanolic Ext.	Aqueous Ext.
1	<i>Euphrasiae stricta</i>	65.84 \pm 1.66	62.58 \pm 0.46	23.72 \pm 1.54
2	<i>Euphorbia platyphyllos</i> L.	65.77 \pm 1.38	64.84 \pm 0.74	12.56 \pm 0.32
3	<i>Epimedium brevicomum</i>	65.63 \pm 0.83	63.96 \pm 1.34	15.14 \pm 0.28
4	<i>Viscum album</i>	65.55 \pm 1.54	62.38 \pm 0.69	15.44 \pm 1.86
5	<i>Psoralea corylifolia</i> L.	65.46 \pm 0.88	64.92 \pm 0.64	12.86 \pm 0.44
6	<i>Equiseti arvense</i>	64.92 \pm 0.53	62.19 \pm 0.77	22.33 \pm 0.64
7	<i>Veronica officinalis</i>	64.76 \pm 1.55	61.48 \pm 0.16	13.61 \pm 0.36
8	<i>Artemisia alba</i>	64.43 \pm 1.72	63.85 \pm 0.52	11.63 \pm 0.92
9	<i>Fagopyrum cymosum</i>	64.14 \pm 1.28	62.53 \pm 1.60	20.51 \pm 1.41
10	<i>Prunella vulgaris</i>	63.88 \pm 0.54	60.68 \pm 1.86	11.28 \pm 0.58
11	<i>Origanum vulgare</i>	63.46 \pm 1.88	62.34 \pm 0.72	14.82 \pm 0.35
12	<i>Camptotheca acuminata</i>	63.72 \pm 1.22	61.56 \pm 0.15	10.00 \pm 1.42
13	<i>Matteuccia struthiopteris</i>	62.34 \pm 1.46	60.14 \pm 0.54	17.26 \pm 1.68
14	<i>Nothapodytes nimmoniana</i>	62.54 \pm 0.64	59.27 \pm 0.98	19.84 \pm 0.24
15	<i>Viscum articulatum</i>	62.73 \pm 0.82	61.46 \pm 1.34	9.58 \pm 0.45
16	<i>Justicia procumbens</i>	62.34 \pm 1.36	60.20 \pm 1.72	10.69 \pm 1.77
17	<i>Citrullus colocynthis</i> L.	62.23 \pm 0.42	61.39 \pm 0.68	18.22 \pm 1.18
18	<i>Bauhinia variegata</i>	62.94 \pm 0.83	59.72 \pm 0.80	20.87 \pm 0.33
19	<i>Cimicifuga dahurica</i>	61.07 \pm 0.38	59.66 \pm 0.26	17.55 \pm 0.68
20	<i>Psoralea corylifolia</i> L.	61.86 \pm 0.60	58.08 \pm 0.94	15.26 \pm 0.19
21	<i>Acacia catechu</i>	61.36 \pm 1.10	59.70 \pm 2.34	20.00 \pm 1.45
22	<i>Geranii robertiani</i>	60.90 \pm 0.35	59.48 \pm 0.56	9.26 \pm 1.93
23	<i>Equiseti arvense</i>	60.39 \pm 1.56	57.26 \pm 0.89	12.68 \pm 1.11
24	<i>Veronica officinalis</i>	59.76 \pm 1.65	58.18 \pm 1.36	8.22 \pm 1.04
25	<i>Artemisia alba</i>	59.62 \pm 1.30	56.63 \pm 0.42	10.68 \pm 1.46
26	<i>Rubia cordifolia</i>	59.22 \pm 1.94	57.92 \pm 1.30	9.12 \pm 1.93

27	<i>Patrinia scabiosifolia</i>	59.40 ± 1.08	58.51 ± 1.81	16.25 ± 0.57
28	<i>Salviae officinalis</i>	59.12 ± 0.88	56.76 ± 1.64	15.86 ± 0.98
29	<i>Lycopus lucidus</i>	59.02 ± 0.70	57.51 ± 0.88	21.18 ± 1.22
30	<i>Glycyrrhiza uralensis</i>	58.94 ± 0.95	56.79 ± 0.53	14.99 ± 0.65
31	<i>Satureja bachtiarica</i>	58.35 ± 1.17	55.90 ± 1.22	19.20 ± 1.06
32	<i>Bidens pilosa</i> L.	58.89 ± 1.53	57.77 ± 1.58	18.00 ± 1.37
33	<i>Fagopyrum cymosum</i>	58.07 ± 1.90	55.00 ± 1.34	20.14 ± 1.54
34	<i>Prunella vulgaris</i>	57.92 ± 1.74	55.46 ± 0.60	10.00 ± 0.48
35	<i>Rehmannia glutinosa</i>	57.27 ± 0.45	54.86 ± 0.53	13.38 ± 0.92
36	<i>Hederae folium</i>	57.30 ± 0.66	54.13 ± 0.46	14.84 ± 1.28
37	<i>Portulaca oleracea</i> L.	57.16 ± 0.92	56.94 ± 0.68	21.59 ± 0.30
38	<i>Cuscuta chinensis</i> L.	57.88 ± 2.12	54.04 ± 1.26	23.54 ± 1.94
39	<i>Asparagus cochinchinensis</i>	57.21 ± 0.65	55.77 ± 0.82	10.07 ± 0.16
40	<i>Veronicae officinalis</i>	56.79 ± 1.88	53.38 ± 1.44	14.43 ± 1.64
41	<i>Ephedra sinica</i>	56.47 ± 1.46	55.70 ± 0.86	19.16 ± 0.38
42	<i>Eclipta prostrata</i>	56.29 ± 1.67	54.56 ± 1.26	15.48 ± 0.51
43	<i>Cynarae folium</i>	56.44 ± 1.26	53.18 ± 0.74	12.10 ± 1.62
44	<i>Caesalpinia sappan</i> L.	56.39 ± 1.05	55.88 ± 1.34	18.23 ± 0.44
45	<i>Morus alba</i> L.	56.21 ± 1.76	54.62 ± 1.98	11.90 ± 1.72
46	<i>Salvia divinorum</i>	55.92 ± 1.80	53.96 ± 0.16	11.02 ± 0.38
47	<i>Rubus chingii</i>	55.29 ± 1.34	53.73 ± 1.91	20.62 ± 1.54
48	<i>Rosmarinus officinalis</i>	55.80 ± 1.98	54.26 ± 0.69	24.06 ± 1.88
49	<i>Picrorhiza scrophulariiflora</i>	55.71 ± 1.36	54.42 ± 1.50	20.64 ± 0.96
50	<i>Thymus serpyllum</i> L.	55.85 ± 0.43	52.15 ± 0.46	14.52 ± 0.60
51	<i>Rubia cordifolia</i> L.	55.53 ± 1.74	54.76 ± 1.14	19.16 ± 1.92
52	<i>Melissae officinalis</i>	54.12 ± 1.65	53.38 ± 1.74	14.94 ± 1.76
53	<i>Podophyllum hexandrum</i>	54.36 ± 0.82	53.16 ± 0.40	11.66 ± 0.98
54	<i>Lavandulae angustifolia</i> L.	54.93 ± 1.24	52.88 ± 1.59	10.33 ± 1.34
55	<i>Cassia tora</i> L.	54.44 ± 0.77	52.26 ± 0.94	18.85 ± 0.48
56	<i>Arctium lappa</i> L.	54.21 ± 1.56	51.57 ± 0.25	12.43 ± 0.55

57	<i>Saussurea lappa</i>	53.78 ± 1.63	52.00 ± 0.78	12.55 ± 1.18
58	<i>Bursera simaruba</i>	53.35 ± 1.51	51.94 ± 1.12	14.00 ± 1.31
59	<i>Viola reichenbachiana</i>	53.56 ± 1.28	52.04 ± 0.44	20.76 ± 1.86
60	<i>Zanthoxylum nitidum</i>	53.41 ± 0.83	50.72 ± 1.52	18.64 ± 1.64
61	<i>Solanum nigrum</i> L.	52.82 ± 0.96	50.46 ± 1.64	20.33 ± 0.40
62	<i>Bupleurum falcatum</i>	52.60 ± 0.52	49.58 ± 0.32	16.49 ± 0.93
63	<i>Isatis indigotica</i>	52.28 ± 0.46	51.44 ± 0.86	21.61 ± 0.79
64	<i>Epilobium parvifolium</i>	52.66 ± 1.35	48.80 ± 1.41	14.44 ± 1.96
65	<i>Punica granatum</i>	52.31 ± 0.93	49.74 ± 0.63	18.85 ± 0.38
66	<i>Angelica sylvestris</i>	52.84 ± 1.66	50.36 ± 1.22	17.56 ± 1.86
67	<i>Satureja montana</i>	52.38 ± 1.14	49.04 ± 0.54	13.00 ± 1.00
68	<i>Asperula odorata</i>	51.52 ± 1.44	48.56 ± 1.68	12.92 ± 0.54
69	<i>Gunnera perperna</i>	51.60 ± 1.96	50.64 ± 1.42	11.28 ± 1.44
70	<i>Rhus glabra</i>	51.85 ± 1.72	48.29 ± 1.96	11.64 ± 1.98
71	<i>Fritillaria thunbergii</i>	51.44 ± 0.84	49.88 ± 0.12	14.51 ± 0.62
72	<i>Melissa flava</i>	51.16 ± 0.56	48.33 ± 1.44	15.00 ± 1.18
73	<i>Glycyrrhiza glabra</i>	50.39 ± 1.88	47.96 ± 0.80	13.52 ± 0.86
74	<i>Angelica sinensis</i>	50.72 ± 1.64	48.58 ± 1.35	20.84 ± 1.54
75	<i>Rhus chinensis</i>	50.30 ± 1.46	49.06 ± 0.86	20.00 ± 1.90
76	<i>Morus nigra</i> L.	50.82 ± 0.70	47.69 ± 0.53	15.36 ± 1.48
77	<i>Sennae folium</i>	50.26 ± 1.58	48.27 ± 0.67	12.64 ± 0.76
78	<i>Centaurium erythraea</i>	50.62 ± 1.97	47.65 ± 0.54	10.33 ± 1.64
79	<i>Apium graveolens</i>	49.54 ± 0.69	46.37 ± 0.96	10.74 ± 0.41
80	<i>Ocimum basilicum</i>	49.87 ± 0.78	47.54 ± 0.45	14.22 ± 1.20
81	<i>Fragaria vesca</i>	49.40 ± 1.42	46.16 ± 1.94	11.08 ± 0.52
82	<i>Rhynchosia minima</i>	49.63 ± 0.86	48.94 ± 0.22	14.70 ± 0.82
83	<i>Achillea millefolium</i>	49.99 ± 0.24	47.53 ± 0.86	22.16 ± 1.44
84	<i>Satureja subspicata</i>	49.18 ± 0.75	48.25 ± 0.48	17.82 ± 0.78
85	<i>Phellodendron amurense</i>	49.55 ± 0.56	46.00 ± 0.52	22.91 ± 0.56
86	<i>Urticae dioica</i> L.	48.41 ± 0.64	46.38 ± 0.80	12.26 ± 0.54

87	<i>Ocimum tenuiflorum</i> L.	48.53 ± 0.31	47.87 ± 0.94	19.83 ± 0.88
88	<i>Astragalus complanatus</i>	48.34 ± 0.79	45.53 ± 0.25	15.31 ± 0.96
89	<i>Salvia miltiorrhiza</i>	47.12 ± 1.44	46.26 ± 1.88	18.42 ± 1.54
90	<i>Tragia involucrata</i> L.	47.30 ± 0.85	45.44 ± 1.36	16.40 ± 0.72
91	<i>Ailanthus altissima</i>	47.57 ± 0.33	46.55 ± 0.77	20.66 ± 0.44
92	<i>Dendrobium nobile</i>	47.22 ± 0.94	45.23 ± 1.54	10.18 ± 1.86
93	<i>Bardanae folium</i>	46.16 ± 1.88	43.86 ± 0.98	19.80 ± 0.32
94	<i>Indigofera aspalathoides</i>	46.88 ± 0.52	44.64 ± 0.57	17.23 ± 0.99
95	<i>Papever somniferum</i>	45.44 ± 1.40	42.20 ± 0.84	22.06 ± 1.64
96	<i>Coptis chinensis</i>	45.66 ± 1.16	44.34 ± 1.60	20.88 ± 1.48
97	<i>Inula Britannica</i> L.	45.92 ± 1.64	43.08 ± 1.32	15.42 ± 1.96
98	<i>Calycopteris floribunda</i>	44.28 ± 0.58	42.57 ± 0.90	19.64 ± 0.54
99	<i>Solanum torvum</i> L.	44.84 ± 1.24	41.66 ± 0.70	21.55 ± 0.92
100	<i>Scutellaria baicalensis</i>	44.52 ± 1.28	43.50 ± 1.48	14.16 ± 1.84
101	<i>Salvia fruticosa</i>	44.74 ± 1.66	41.43 ± 1.21	12.48 ± 1.40
102	<i>Sedum sarmentosum</i>	44.04 ± 0.72	42.36 ± 1.84	20.76 ± 0.68
103	<i>Vernonia cinerea</i>	43.86 ± 1.26	42.54 ± 1.52	18.23 ± 1.46
104	<i>Ocimum gratissimum</i> L.	43.32 ± 0.55	41.14 ± 0.82	21.88 ± 0.64
105	<i>Lonicera japonica</i>	43.66 ± 0.84	40.53 ± 0.38	15.34 ± 1.44
106	<i>Juniperus communis</i>	43.31 ± 0.45	42.02 ± 0.94	12.51 ± 0.75
107	<i>Cinnamomum cassia</i>	42.58 ± 1.56	40.94 ± 0.66	20.38 ± 0.68
108	<i>Withania somnifera</i>	42.86 ± 1.94	41.26 ± 0.54	18.84 ± 1.52
109	<i>Agrimonia pilosa</i>	42.40 ± 0.53	39.65 ± 0.66	16.60 ± 1.84
110	<i>Plantago lanceolata</i> L.	42.36 ± 0.24	41.44 ± 1.48	19.76 ± 0.28
111	<i>Asparagus racemosus</i>	42.29 ± 1.61	39.02 ± 1.79	17.64 ± 1.96
112	<i>Emilia sonchifolia</i>	42.54 ± 0.88	41.72 ± 1.58	22.47 ± 0.39
113	<i>Tinospora cordifolia</i>	42.14 ± 0.40	40.42 ± 1.24	15.60 ± 0.48
114	<i>Larrea tridentata</i>	41.60 ± 1.86	39.33 ± 1.96	20.18 ± 1.14
115	<i>Andrographis paniculata</i>	41.66 ± 1.42	38.36 ± 1.12	16.54 ± 0.94
116	<i>Cirsium setosum</i>	41.34 ± 2.12	40.75 ± 0.34	21.42 ± 0.83

117	<i>Gynostemma pentaphyllum</i>	40.37 ± 0.56	37.16 ± 0.72	23.58 ± 1.36
118	<i>Paris polyphilla</i>	40.60 ± 0.78	39.91 ± 0.52	15.57 ± 0.78
119	<i>Mentha piperita</i>	40.33 ± 0.54	38.42 ± 2.34	14.24 ± 0.52
120	<i>Gnetum africanum</i>	39.55 ± 1.26	37.83 ± 1.56	16.76 ± 0.84
121	<i>Croton setigerus</i> L.	39.76 ± 0.68	38.34 ± 0.44	26.12 ± 0.76
122	<i>Taraxacum mongolicum</i>	39.24 ± 1.94	36.62 ± 1.37	22.65 ± 1.53
123	<i>Tephrosia purpurea</i> L.	38.84 ± 0.42	36.28 ± 1.84	12.54 ± 0.66
124	<i>Ipomoea squamosa</i>	38.33 ± 0.28	37.76 ± 0.58	16.39 ± 0.48
125	<i>Rubus ellipticus</i>	38.52 ± 1.54	35.54 ± 1.36	21.65 ± 1.94
126	<i>Marrubium vulgare</i>	38.27 ± 1.96	34.88 ± 0.64	18.74 ± 0.72
127	<i>Gongronema latifolium</i>	38.66 ± 0.53	35.13 ± 0.80	20.34 ± 0.86
128	<i>Polygala tenuifolia</i>	37.72 ± 0.82	36.24 ± 1.48	18.16 ± 0.22
129	<i>Rheum officinale</i>	37.44 ± 0.36	35.56 ± 0.74	14.46 ± 0.65
130	<i>Vitex trifolia</i> L.	36.82 ± 1.54	34.64 ± 1.36	15.60 ± 0.34
131	<i>Artemisia absinthium</i> L.	36.60 ± 0.88	35.92 ± 0.88	14.44 ± 0.94
132	<i>Centipeda minima</i>	36.24 ± 0.65	33.06 ± 1.56	21.88 ± 0.52
133	<i>Solanum idicum</i> L.	36.08 ± 0.26	34.55 ± 0.47	21.74 ± 0.76
134	<i>Curculigo orchioides.</i>	35.94 ± 1.52	34.94 ± 1.64	15.60 ± 1.64
135	<i>Tiliae cordata</i>	35.62 ± 1.74	32.66 ± 0.95	18.44 ± 1.36
136	<i>Eucommia ulmoides</i>	35.16 ± 0.58	33.34 ± 1.88	16.26 ± 0.80
137	<i>Digitalis purpurea</i> L.	34.88 ± 1.32	32.16 ± 1.46	19.88 ± 1.70
138	<i>Nepeta cataria</i>	34.30 ± 1.47	33.44 ± 1.58	22.14 ± 1.52
139	<i>Betulae folium</i>	34.48 ± 0.64	31.85 ± 0.91	11.69 ± 0.35
140	<i>Teraxaci folium</i>	34.14 ± 0.96	32.48 ± 0.52	14.44 ± 0.84
141	<i>Rubi idaei</i>	33.94 ± 1.22	32.52 ± 0.28	11.62 ± 1.79
142	<i>Viscum coloratum</i>	33.56 ± 0.46	30.20 ± 1.74	15.44 ± 0.56
143	<i>Plantago asiatica</i> L.	33.22 ± 0.98	31.54 ± 1.52	19.70 ± 0.64
144	<i>Nepeta trachonitica</i>	31.83 ± 0.61	30.45 ± 0.69	18.32 ± 0.46
145	<i>Eclipta alba</i> L.	31.42 ± 0.86	27.67 ± 0.33	12.56 ± 0.71
146	<i>Hedyotis diffusa</i>	30.51 ± 1.68	25.82 ± 1.56	14.60 ± 1.52

147	<i>Pygeum africanum</i>	30.67 ± 1.74	28.54 ± 1.88	16.28 ± 1.24
148	<i>Satureja hortensis</i>	30.55 ± 1.34	25.78 ± 1.39	9.51 ± 1.88
149	<i>Ipomoea aquatica</i>	28.44 ± 0.25	26.62 ± 0.54	12.74 ± 0.63
150	<i>Houttuynia cordata</i> L.	28.16 ± 0.66	23.46 ± 0.82	12.60 ± 0.96
151	<i>Dianthus superbus</i> L.	27.93 ± 0.52	24.88 ± 0.56	13.44 ± 0.54
152	<i>Justicia adhatoda</i> L.	24.86 ± 1.14	22.36 ± 0.28	12.65 ± 1.43
153	<i>Chellidonium majus</i> L.	24.56 ± 0.48	19.18 ± 1.74	11.33 ± 0.92
154	<i>Galium verum</i> L.	23.64 ± 0.96	21.54 ± 1.88	10.49 ± 0.36
155	<i>Sanguisorba officinalis</i> L.	23.59 ± 0.61	18.28 ± 0.49	9.52 ± 0.48
156	<i>Smilax glabra</i> L.	22.86 ± 0.38	20.94 ± 0.72	9.66 ± 0.56
157	<i>Melia azedarach</i> L.	21.54 ± 1.64	15.20 ± 1.86	11.55 ± 1.88
158	<i>Trichosanthes kirilowii</i>	20.43 ± 1.92	17.48 ± 1.42	10.26 ± 0.34
159	<i>Trifolium repense</i> L.	17.86 ± 1.24	15.26 ± 1.96	8.74 ± 1.22
160	<i>Actaea racemosa</i> L.	15.16 ± 0.44	11.44 ± 0.78	8.33 ± 0.46
161	<i>Sophora tonkinensis</i>	8.93 ± 0.96	4.56 ± 1.33	0.74 ± 0.38
162	<i>Uncaria macrophylla</i>	8.84 ± 0.65	5.83 ± 0.94	1.88 ± 1.52
163	<i>Fraxini folium</i>	8.71 ± 1.86	6.51 ± 1.22	1.26 ± 0.66
164	<i>Trollius europaeus</i>	8.55 ± 0.34	4.68 ± 0.88	0.78 ± 0.42
165	<i>Polygoni avicularis</i>	8.14 ± 1.22	4.16 ± 1.36	1.30 ± 0.84
166	<i>Dipsacus japonicus</i>	7.88 ± 0.39	4.02 ± 0.48	0.22 ± 0.68
167	<i>Tripterygium wilfordii</i>	7.76 ± 0.58	5.28 ± 1.72	1.44 ± 1.35
168	<i>Primula officinalis</i>	7.58 ± 0.42	4.52 ± 0.34	0.16 ± 0.74
169	<i>Salicis cortex</i>	7.33 ± 0.94	5.33 ± 0.62	1.04 ± 1.44
170	<i>Dysoxylum binectariferum</i>	6.96 ± 0.53	4.49 ± 0.86	0.36 ± 0.50
171	<i>Tilia cordata</i>	6.84 ± 1.48	4.96 ± 0.47	0.88 ± 1.43
172	<i>Echinochloa crus-galli</i>	6.69 ± 1.82	4.34 ± 1.25	1.24 ± 1.76
173	<i>Pulsatilla chinensis</i>	6.55 ± 0.64	5.22 ± 0.68	1.47 ± 1.32
174	<i>Stemona sessilifolia</i>	6.46 ± 1.58	4.84 ± 1.56	0.89 ± 1.64
175	<i>Iris potaninii</i>	6.32 ± 1.24	4.52 ± 1.62	1.16 ± 0.48
176	<i>Uvae ursi</i>	6.28 ± 1.84	5.41 ± 1.33	0.34 ± 0.26

177	<i>Pteris multifida</i>	6.08 ± 1.33	4.39 ± 0.84	0.98 ± 1.54
178	<i>Lauri folium</i>	5.92 ± 1.76	4.52 ± 0.23	1.46 ± 0.62
179	<i>Crataegus monogyna</i>	5.80 ± 1.38	3.84 ± 1.56	1.14 ± 1.86
180	<i>Xanthium sibiricum</i>	5.76 ± 0.64	4.58 ± 0.42	0.34 ± 0.77
181	<i>Teucree Montana</i>	5.58 ± 0.88	5.32 ± 1.64	1.08 ± 0.92
182	<i>Sambucus nigra L.</i>	5.51 ± 0.62	4.46 ± 0.28	0.44 ± 0.64
183	<i>Erythroxyllum pervillei</i>	5.42 ± 0.76	3.24 ± 0.16	0.86 ± 0.59
184	<i>Ophiopogon japonicus</i>	5.30 ± 1.58	3.66 ± 1.72	0.04 ± 1.42
185	<i>Indigofera tinctoria</i>	5.14 ± 0.46	4.30 ± 0.54	1.32 ± 0.68
186	<i>Dioscorea opposita</i>	4.89 ± 0.28	4.22 ± 0.36	0.14 ± 0.54
187	<i>Drynaria fortune</i>	4.76 ± 1.94	4.84 ± 1.48	0.47 ± 1.12
188	<i>Hyperii Herba</i>	4.68 ± 0.42	3.76 ± 0.35	0.63 ± 0.76
189	<i>Canabis sativa L.</i>	4.56 ± 1.34	4.38 ± 1.42	0.24 ± 1.00
190	<i>Cichorium intybus</i>	4.44 ± 1.76	3.16 ± 0.64	0.38 ± 0.46
191	<i>Verbenae officinalis</i>	4.33 ± 1.52	3.62 ± 1.18	1.14 ± 1.92
192	<i>Polygonum hydropiper</i>	4.16 ± 1.40	1.32 ± 1.96	0.02 ± 0.26
193	<i>Azadirachta indica</i>	4.04 ± 1.81	3.02 ± 0.84	0.20 ± 1.54
194	<i>Hyoscyamus niger L.</i>	3.99 ± 0.58	3.46 ± 1.58	0.36 ± 0.70
195	<i>Pestemon deustus</i>	3.86 ± 1.64	2.24 ± 1.44	0.64 ± 0.68
196	<i>Cleistanthus collinus</i>	3.63 ± 0.52	3.12 ± 0.52	0.52 ± 0.34
197	<i>Diphylleia grayi</i>	3.56 ± 1.24	2.30 ± 0.74	0.41 ± 0.22
198	<i>Doronicum pardalianches L.</i>	3.46 ± 0.59	3.15 ± 0.62	0.32 ± 1.64
199	<i>Farfarae folium</i>	3.40 ± 1.62	3.54 ± 1.96	0.44 ± 1.18
200	<i>Rhodiola sacra</i>	3.28 ± 1.84	3.22 ± 0.57	0.24 ± 0.94
	Ascorbic Acid (Standard)	85.48 ± 1.56		

The ferric reducing capacities of various tested medicinal plants for ethanolic, Methanolic and aqueous extracts observed are shown in the Table 4.8. The standard L-ascorbic acid showed the 85.48 ± 1.56 % at 500 µg/ml concentration. The highest activity of

65.77 ± 1.38 % was shown by ethanolic extracts of *E. stricta*, followed by 64.84 ± 0.74% and 12.56 ± 0.32% with Methanol and aqueous extracts at (500 µg/ml), respectively. The *E. platyphyllos* L. ethanolic extracts showed the percent reducing power of 64.42 ± 0.88%, followed by 62.92 ± 0.64% and 12.56 ± 0.32% for Methanol and aqueous extracts.

Similarly, the antioxidant activity shown by ethanolic and Methanolic extracts of *Epimedium brevicomum* Maxim was 67.46 ± 1.26% and 66.78 ± 0.36% respectively. The *Viscum album* L. and *Psoralea corylifolia* L. showed the radical scavenging activity of 65.55 ± 1.54 and 62.38 ± 0.69 for its ethanolic and 65.46 ± 0.88 and 64.92 ± 0.64 Methanolic extracts respectively followed by *Equiseti arvense*, *Veronica officinalis*, *Fagopyrum cymosum*, *Prunella vulgaris*, *Origanum vulgare*, *Camptotheca acuminata*, *Matteuccia struthiopteris*, *Nothapodytes nimmoniana* and *Justicia procumbens* as compared to the Standard L-ascorbic acid.

Thus, the sequence for the reducing power based on these experimental results can be organized as: L-ascorbic acid > ethanol extract > Methanol extract > Water extract as shown in table 4.8. Similar findings were observed by Fernando and Soysa, (2014), that the reductones present in these medicinal plants were responsible for the reducing properties of the extracts and the reaction may be dependent for breaking the free radical chain by providing a hydrogen atom or may be by reacting with the certain compounds of peroxides to avoid the peroxide development.

This study is also in accordance with the published scientific data on the chemical composition and biological activity of 10 species of *Euphrasia stricta* L., *E. rostkoviana*, *E. brevipila*, *E. parviflora.*, *E. montana.*, *E. pectinata*, *E. condensata.*, *E. stricta*, *E. salisburgensis*, *Euphorbia platyphyllos*, *E. maximowiszii*, and *E. reuteri*. Flavonoids, phenolic acids and iridoic glycosides, however, are the main groups. Hypotensive, anti-

inflammatory, anti-microbial, antioxidant and hepatoprotective action of *Euphrasia* representatives has been revealed experimentally (Suhinina TV, Petrichenko, 2019).

The present study is similar to the study of Ouml *et al*; (2013), where potential antioxidant activities of crude extracts (diethyl ether, petroleum ether, ethyl acetate, Methanol and water (infusion and decoction)) from *Euphorbia platyphyllos* L. These results suggest that this plant has potential for being a source of anticancer agents for breast cancer treatments. Further studies are required for the isolation and identification of individual phenolic compounds in the extracts.

The similar studies were conducted for the ultrasound-assisted extraction of phenolic compounds from *Epimedium brevicornu* Maxim was modeled using response surface methodology. It was indicated that the phenolic compounds from *Epimedium brevicornu* Maxim possess significant antioxidant activity. HPLC analysis reveals that the main phenolic compound in the extract product was identified as gallic acid, catechin (Cianidanol), p-hydroxybenzoic acid, vanillic acid, caffeic acid, ferulaic acid, rutin, benzoic acid, and quercetin (Zhao *et al*; 2014).

This study is also in accordance with the Ruan *et al.*, (2016) where antioxidant potential of *Rhazya stricta*, an important medicinal plant in South Asia was evaluated. To evaluate the antioxidant potential of these Methanol extracts various antioxidant assays were used like DPPH, metal ion chelating assay, FRAP Assay. The antioxidant potential in the Methanol extracts of *R. Stricta* sheets was comparable with strong antioxidants previously exploited and largely dependent on concentration.

The similar findings were also observed for antioxidant properties in *Lonicera japonica* by Lee *et al.*, (2019). Total phenol, phenolic acids (chlorogenic acid, caffeine acid,

decalfinic acid 4.5 and 3.5 dicafilquinic) and flavonoids (quercetin, rutin and luteonin) increased significantly after heat treatment. Antioxidant activity such as scavenging activity of DPPH radical scavenging ABTS, FRAP and reduce energy, improved LJ roots after heating. Antioxidant activity was positively associated with total phenol content, caffeine, total flavonoids, quercetin and chlorogenic acid. Thus, these results are in accordance with this present study.

The dry extracts of mulberry leaves (*Rubus idaeus* L.) were investigated for potential antioxidant activity and phenolic content. Spectrometry and HPLCUV / HPLC-MS methods for the determination of polyphenols and for measuring antioxidant potentials various assay like ferric reducing ability (FRAP), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS• +), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and (ORAC) oxygen radical absorbance capacity methods were used. So, the hepatic associated disorders and oxidative stress can be treated by these extracts because of higher phenolic content. The similar findings were also observed here Costea *et al.*, (2014).

4.2.2.4. ABTS free radical scavenging assay:

In this assay the conversions for the ABTS⁺ (2,2-azinobis-(ethyl-benzothiazoline-6-sulphonic acid) radical cation inhibition or hunting capacities of each plant samples in various solvent extracts were investigated in comparison with the Ascorbic Acid and Trolox as standard. The ABTS free radicals were generated by mixing the ABTS in potassium persulphate. The measurement was taken by spectrophotometer at 734 nm after 30 min incubation under dark conditions. (Deepa *et al.*, 2013). The higher ABTS radical scavenging potential was shown by the tested plant extracts using different solvents as shown in Table 4.9.

Table 4.9. The Antioxidant activity of medicinal plants using ABTS Assay

Sr. No.	Plant Sample	Free Radical Scavenging Activity at 734 nm (%)		
		Mean \pm SD (μ g/ml)		
		Ethanollic Ext.	Methanolic Ext.	Aqueous Ext.
1	<i>Euphrasiae stricta</i>	68.44 \pm 1.38	66.33 \pm 0.62	26.18 \pm 0.48
2	<i>Euphorbia platyphyllos</i> L.	67.88 \pm 0.74	65.48 \pm 1.40	14.37 \pm 0.26
3	<i>Epimedium brevicomum</i>	67.56 \pm 0.36	66.72 \pm 1.16	11.84 \pm 0.90
4	<i>Viscum album</i>	67.32 \pm 1.50	63.34 \pm 0.78	21.36 \pm 0.39
5	<i>Psoralea corylifolia</i> L.	65.89 \pm 1.36	63.48 \pm 0.94	13.75 \pm 1.16
6	<i>Equiseti arvense</i>	65.79 \pm 1.68	64.84 \pm 0.24	22.66 \pm 0.58
7	<i>Veronica officinalis</i>	65.65 \pm 0.56	63.26 \pm 1.74	15.44 \pm 1.02
8	<i>Artemisia alba</i>	65.47 \pm 1.84	62.78 \pm 0.63	12.55 \pm 1.47
9	<i>Fagopyrum cymosum</i>	65.32 \pm 0.26	64.32 \pm 0.21	22.06 \pm 0.72
10	<i>Prunella vulgaris</i>	64.96 \pm 0.79	62.59 \pm 0.73	11.33 \pm 1.46
11	<i>Origanum vulgare</i>	64.27 \pm 1.65	61.18 \pm 0.86	19.51 \pm 0.67
12	<i>Camptotheca acuminata</i>	64.23 \pm 1.82	63.55 \pm 0.50	11.66 \pm 0.84
13	<i>Matteuccia struthiopteris</i>	64.51 \pm 1.46	62.32 \pm 1.06	20.52 \pm 1.49
14	<i>Nothapodytes nimmoniana</i>	63.30 \pm 0.23	60.68 \pm 1.26	20.2 \pm 0.52
15	<i>Viscum articulatum</i>	63.86 \pm 1.18	62.44 \pm 0.92	13.99 \pm 0.84
16	<i>Justicia procumbens</i>	63.88 \pm 1.22	61.15 \pm 0.53	11.40 \pm 1.46
17	<i>Citrullus colocynthis</i> L.	62.34 \pm 1.06	60.54 \pm 0.36	17.86 \pm 1.73
18	<i>Bauhinia variegata</i>	62.97 \pm 0.74	59.57 \pm 0.88	9.64 \pm 0.41
19	<i>Cimicifuga dahurica</i>	62.23 \pm 0.62	61.56 \pm 1.95	19.18 \pm 0.44
20	<i>Psoralea corylifolia</i> L.	62.94 \pm 1.12	60.55 \pm 1.72	19.38 \pm 1.68
21	<i>Acacia catechu</i>	62.77 \pm 0.69	61.85 \pm 0.69	19.62 \pm 1.18
22	<i>Geranii robertiani</i>	62.34 \pm 0.92	59.20 \pm 0.29	11.16 \pm 0.32
23	<i>Equiseti arvense</i>	61.07 \pm 0.41	59.66 \pm 0.76	12.54 \pm 0.96
24	<i>Veronica officinalis</i>	61.33 \pm 0.66	58.88 \pm 0.42	11.86 \pm 0.29
25	<i>Artemisia alba</i>	61.54 \pm 1.03	59.80 \pm 1.38	13.00 \pm 1.06
26	<i>Rubia cordifolia</i>	60.23 \pm 0.75	59.49 \pm 0.81	10.66 \pm 1.48

27	<i>Patrinia scabiosifolia</i>	60.99 ± 1.62	57.26 ± 0.44	10.28 ± 1.10
28	<i>Salviae officinalis</i>	59.36 ± 1.50	58.85 ± 1.26	16.00 ± 0.94
29	<i>Lycopus lucidus</i>	59.22 ± 1.47	56.36 ± 0.60	24.56 ± 1.69
30	<i>Glycyrrhiza uralensis</i>	59.08 ± 1.24	57.81 ± 1.62	15.66 ± 1.78
31	<i>Satureja bachtiarica</i>	59.65 ± 1.52	58.41 ± 1.73	21.52 ± 0.92
32	<i>Bidens pilosa</i> L.	59.22 ± 0.28	56.69 ± 1.94	24.66 ± 0.55
33	<i>Fagopyrum cymosum</i>	59.70 ± 0.47	57.20 ± 0.28	20.85 ± 0.33
34	<i>Prunella vulgaris</i>	58.14 ± 0.29	56.81 ± 0.54	11.39 ± 0.66
35	<i>Rehmannia glutinosa</i>	58.62 ± 1.80	55.45 ± 1.36	16.00 ± 1.18
36	<i>Hederae folium</i>	58.92 ± 1.54	57.38 ± 1.42	15.04 ± 1.90
37	<i>Portulaca oleracea</i> L.	58.15 ± 1.40	55.00 ± 1.74	20.44 ± 1.22
38	<i>Cuscuta chinensis</i> L.	57.33 ± 1.22	55.66 ± 0.24	10.96 ± 0.46
39	<i>Asparagus cochinchinensis</i>	57.44 ± 0.89	54.50 ± 0.68	12.28 ± 0.98
40	<i>Veronicae officinalis</i>	57.40 ± 0.22	54.19 ± 0.86	28.44 ± 0.35
41	<i>Ephedra sinica</i>	57.26 ± 0.82	56.92 ± 0.28	22.59 ± 0.72
42	<i>Eclipta prostrata</i>	57.12 ± 2.30	54.22 ± 1.94	15.36 ± 1.21
43	<i>Cynarae folium</i>	57.44 ± 0.66	55.86 ± 0.42	15.04 ± 0.85
44	<i>Caesalpinia sappan</i> L.	56.12 ± 1.86	53.40 ± 1.86	17.33 ± 1.56
45	<i>Morus alba</i> L.	56.70 ± 1.54	55.18 ± 0.34	12.46 ± 0.67
46	<i>Salvia divinorum</i>	56.32 ± 1.65	54.96 ± 1.56	13.35 ± 0.16
47	<i>Rubus chingii</i>	56.48 ± 1.26	53.38 ± 0.92	21.08 ± 1.63
48	<i>Rosmarinus officinalis</i>	56.63 ± 2.00	55.82 ± 1.48	22.33 ± 0.81
49	<i>Picrorhiza scrophulariiflora</i>	56.20 ± 1.68	54.14 ± 1.06	20.72 ± 1.34
50	<i>Thymus serpyllum</i> L.	55.44 ± 1.22	53.64 ± 0.63	15.42 ± 0.76
51	<i>Rubia cordifolia</i> L.	55.39 ± 1.70	53.35 ± 1.84	18.67 ± 1.54
52	<i>Melissae officinalis</i>	55.22 ± 1.58	54.46 ± 0.96	16.55 ± 1.24
53	<i>Podophyllum hexandrum</i>	55.33 ± 1.84	54.84 ± 1.30	13.24 ± 0.85
54	<i>Lavandulae angustifolia</i> L.	55.96 ± 0.51	52.25 ± 0.54	11.06 ± 0.58
55	<i>Cassia tora</i> L.	55.65 ± 1.32	54.47 ± 1.62	19.60 ± 1.46
56	<i>Arctium lappa</i> L.	54.17 ± 1.85	53.71 ± 1.22	14.42 ± 1.82

57	<i>Saussurea lappa</i>	54.96 ± 0.56	53.44 ± 0.38	15.26 ± 0.69
58	<i>Bursera simaruba</i>	54.40 ± 1.82	52.50 ± 1.53	15.38 ± 1.74
59	<i>Viola reichenbachiana</i>	54.31 ± 0.73	52.46 ± 0.94	22.62 ± 0.99
60	<i>Zanthoxylum nitidum</i>	54.56 ± 1.35	51.72 ± 0.52	19.02 ± 0.54
61	<i>Solanum nigrum</i> L.	53.88 ± 1.46	52.20 ± 0.76	23.50 ± 1.34
62	<i>Bupleurum falcatum</i>	53.45 ± 1.54	51.84 ± 1.34	18.05 ± 1.95
63	<i>Isatis indigotica</i>	53.18 ± 1.89	52.48 ± 0.46	23.66 ± 1.44
64	<i>Epilobium parvifolium</i>	53.71 ± 0.18	50.02 ± 1.82	19.64 ± 1.66
65	<i>Punica granatum</i>	52.22 ± 0.76	50.33 ± 1.54	20.22 ± 0.78
66	<i>Angelica sylvestris</i>	52.57 ± 0.50	49.28 ± 0.72	15.40 ± 0.51
67	<i>Satureja montana</i>	52.48 ± 0.94	51.62 ± 0.68	12.71 ± 0.65
68	<i>Asperula odorata</i>	52.96 ± 1.66	48.70 ± 1.21	15.06 ± 1.84
69	<i>Gunnera perpensa</i>	52.45 ± 0.82	49.44 ± 0.58	17.45 ± 0.22
70	<i>Rhus glabra</i>	52.72 ± 1.06	50.86 ± 1.36	12.58 ± 1.76
71	<i>Fritillaria thunbergii</i>	52.13 ± 1.78	49.52 ± 0.84	14.20 ± 1.92
72	<i>Melissa flava</i>	51.80 ± 1.34	48.75 ± 1.28	18.51 ± 0.41
73	<i>Glycyrrhiza glabra</i>	51.10 ± 1.96	50.33 ± 1.59	13.88 ± 1.34
74	<i>Angelica sinensis</i>	51.42 ± 1.54	48.19 ± 1.16	21.93 ± 1.42
75	<i>Rhus chinensis</i>	51.84 ± 0.28	49.58 ± 0.94	19.64 ± 0.24
76	<i>Morus nigra</i> L.	51.26 ± 0.86	48.33 ± 1.76	17.95 ± 1.70
77	<i>Sennae folium</i>	50.74 ± 1.48	47.86 ± 0.81	18.23 ± 0.56
78	<i>Centaurium erythraea</i>	50.61 ± 1.74	48.08 ± 1.45	11.66 ± 1.98
79	<i>Apium graveolens</i>	50.30 ± 1.17	49.54 ± 1.33	13.09 ± 1.36
80	<i>Ocimum basilicum</i>	50.15 ± 0.80	47.49 ± 0.66	14.96 ± 1.52
81	<i>Fragaria vesca</i>	50.59 ± 0.76	48.27 ± 1.18	12.82 ± 0.66
82	<i>Rhynchosia minima</i>	50.82 ± 1.27	48.55 ± 0.58	13.33 ± 0.20
83	<i>Achillea millefolium</i>	49.55 ± 0.68	46.77 ± 0.26	22.94 ± 0.82
84	<i>Satureja subspicata</i>	49.37 ± 0.54	47.44 ± 0.95	18.08 ± 1.44
85	<i>Phellodendron amurense</i>	49.20 ± 1.62	46.36 ± 1.54	24.66 ± 0.98
86	<i>Urticae dioica</i> L.	49.44 ± 0.86	48.00 ± 0.42	13.81 ± 0.65

87	<i>Ocimum tenuiflorum</i> L.	49.29 ± 0.74	47.43 ± 0.76	22.16 ± 1.58
88	<i>Astragalus complanatus</i>	49.18 ± 0.35	48.55 ± 0.48	16.34 ± 0.92
89	<i>Salvia miltiorrhiza</i>	49.65 ± 0.58	46.99 ± 0.52	21.25 ± 1.66
90	<i>Tragia involucrata</i> L.	48.92 ± 0.84	46.38 ± 0.84	22.66 ± 0.34
91	<i>Ailanthus altissima</i>	48.13 ± 0.61	47.72 ± 0.58	20.12 ± 0.86
92	<i>Dendrobium nobile</i>	48.66 ± 0.50	45.83 ± 0.25	13.64 ± 0.46
93	<i>Bardanae folium</i>	47.22 ± 1.78	46.50 ± 1.14	20.86 ± 1.18
94	<i>Indigofera aspalathoides</i>	47.84 ± 0.35	45.36 ± 1.96	21.40 ± 0.74
95	<i>Papever somniferum</i>	47.56 ± 0.62	46.55 ± 0.74	25.66 ± 0.92
96	<i>Coptis chinensis</i>	47.60 ± 0.94	45.48 ± 1.56	26.14 ± 1.36
97	<i>Inula Britannica</i> L.	46.16 ± 1.86	43.42 ± 0.40	18.24 ± 0.50
98	<i>Calycopteris floribunda</i>	46.48 ± 0.32	44.64 ± 0.35	24.33 ± 0.69
99	<i>Solanum torvum</i> L.	45.54 ± 1.76	42.10 ± 0.56	22.08 ± 1.22
100	<i>Scutellaria baicalensis</i>	45.36 ± 1.88	44.66 ± 1.20	15.84 ± 1.76
101	<i>Salvia fruticosa</i>	45.22 ± 1.64	43.30 ± 1.77	13.72 ± 1.96
102	<i>Sedum sarmentosum</i>	44.18 ± 0.34	42.67 ± 0.45	21.56 ± 0.44
103	<i>Vernonia cinerea</i>	44.51 ± 1.56	41.88 ± 0.28	22.84 ± 0.62
104	<i>Ocimum gratissimum</i> L.	44.39 ± 1.82	43.46 ± 1.44	21.62 ± 1.33
105	<i>Lonicera japonica</i>	44.20 ± 1.68	41.62 ± 1.69	15.26 ± 1.54
106	<i>Juniperus communis</i>	44.04 ± 0.71	42.94 ± 1.82	13.74 ± 0.94
107	<i>Cinnamomum cassia</i>	43.46 ± 1.29	42.54 ± 1.64	19.06 ± 1.16
108	<i>Withania somnifera</i>	43.86 ± 0.46	41.66 ± 0.34	20.58 ± 0.82
109	<i>Agrimonia pilosa</i>	43.65 ± 0.55	40.33 ± 0.88	18.47 ± 1.66
110	<i>Plantago lanceolata</i> L.	43.53 ± 0.34	42.16 ± 0.54	21.08 ± 0.48
111	<i>Asparagus racemosus</i>	42.32 ± 1.58	40.44 ± 1.21	22.36 ± 0.77
112	<i>Emilia sonchifolia</i>	42.54 ± 1.26	41.68 ± 0.44	20.54 ± 1.85
113	<i>Tinospora cordifolia</i>	42.82 ± 0.38	39.54 ± 0.86	17.70 ± 1.96
114	<i>Larrea tridentata</i>	42.79 ± 1.41	39.06 ± 1.65	17.32 ± 1.34
115	<i>Andrographis paniculata</i>	42.51 ± 0.84	41.62 ± 1.46	24.17 ± 0.56
116	<i>Cirsium setosum</i>	42.08 ± 1.40	40.88 ± 1.26	21.76 ± 0.84

117	<i>Gynostemma pentaphyllum</i>	41.76 ± 0.66	39.24 ± 0.64	18.39 ± 0.62
118	<i>Paris polyphylla</i>	41.62 ± 1.50	39.60 ± 1.38	17.80 ± 1.47
119	<i>Mentha piperita</i>	41.74 ± 1.92	38.42 ± 0.46	15.24 ± 1.66
120	<i>Gnetum africanum</i>	41.39 ± 2.32	40.56 ± 0.74	13.46 ± 0.83
121	<i>Croton setigerus</i> L.	40.72 ± 0.54	37.24 ± 0.55	21.52 ± 1.36
122	<i>Taraxacum mongolicum</i>	40.61 ± 0.48	39.92 ± 0.76	23.34 ± 0.68
123	<i>Tephrosia purpurea</i> L.	40.22 ± 0.55	38.51 ± 2.42	14.06 ± 1.82
124	<i>Ipomoea squamosa</i>	39.96 ± 0.34	37.82 ± 1.26	18.44 ± 0.24
125	<i>Rubus ellipticus</i>	39.54 ± 0.42	38.46 ± 0.60	22.72 ± 0.56
126	<i>Marrubium vulgare</i>	39.16 ± 1.94	36.80 ± 1.59	18.60 ± 1.33
127	<i>Gongronema latifolium</i>	38.66 ± 0.62	36.16 ± 1.62	21.24 ± 0.94
128	<i>Polygala tenuifolia</i>	38.62 ± 0.44	37.54 ± 0.34	27.69 ± 0.78
129	<i>Rheum officinale</i>	38.55 ± 1.58	35.08 ± 1.50	16.30 ± 1.44
130	<i>Vitex trifolia</i> L.	38.24 ± 1.92	34.44 ± 0.76	15.64 ± 0.86
131	<i>Artemisia absinthium</i> L.	38.76 ± 0.54	35.76 ± 0.50	13.34 ± 0.61
132	<i>Centipeda minima</i>	37.38 ± 0.32	36.48 ± 1.28	24.18 ± 0.54
133	<i>Solanum idicum</i> L.	37.42 ± 0.74	35.56 ± 0.42	20.50 ± 0.66
134	<i>Curculigo orchioides</i> .	36.24 ± 1.56	34.16 ± 1.36	17.82 ± 0.38
135	<i>Tiliae cordata</i>	36.46 ± 0.88	35.98 ± 0.58	16.20 ± 0.24
136	<i>Eucommia ulmoides</i>	36.60 ± 0.52	33.06 ± 1.46	15.98 ± 0.56
137	<i>Digitalis purpurea</i> L.	36.08 ± 0.24	34.55 ± 0.63	21.74 ± 0.92
138	<i>Nepeta cataria</i>	35.72 ± 1.96	34.61 ± 1.57	15.50 ± 1.88
139	<i>Betulae folium</i>	35.64 ± 1.72	32.33 ± 0.96	12.94 ± 1.18
140	<i>Teraxaci folium</i>	35.16 ± 0.56	33.66 ± 1.38	14.82 ± 0.55
141	<i>Rubi idaei</i>	34.80 ± 1.24	32.24 ± 1.82	12.28 ± 1.76
142	<i>Viscum coloratum</i>	34.66 ± 1.56	33.96 ± 1.54	15.92 ± 1.62
143	<i>Plantago asiatica</i> L.	34.42 ± 0.68	31.65 ± 0.85	20.34 ± 0.94
144	<i>Nepeta trachonitica</i>	34.14 ± 0.82	32.48 ± 0.58	22.16 ± 0.86
145	<i>Eclipta alba</i> L.	33.86 ± 1.26	29.34 ± 0.84	13.83 ± 1.58
146	<i>Hedyotis diffusa</i>	33.54 ± 0.48	30.16 ± 1.66	16.04 ± 0.74

147	<i>Pygeum africanum</i>	33.28 ± 0.92	31.52 ± 1.50	18.44 ± 0.66
148	<i>Satureja hortensis</i>	31.54 ± 0.56	26.34 ± 0.19	11.62 ± 0.36
149	<i>Ipomoea aquatica</i>	30.32 ± 0.18	24.86 ± 0.53	14.56 ± 0.72
150	<i>Houttuynia cordata</i> L.	30.62 ± 1.82	26.08 ± 1.94	12.44 ± 1.26
151	<i>Dianthus superbus</i> L.	30.33 ± 1.23	24.56 ± 1.71	10.76 ± 1.47
152	<i>Justicia adhatoda</i> L.	27.54 ± 1.44	23.74 ± 1.29	14.28 ± 1.84
153	<i>Chellidonium majus</i> L.	26.76 ± 0.85	21.62 ± 0.86	10.46 ± 0.68
154	<i>Galium verum</i> L.	26.24 ± 0.94	24.16 ± 1.58	12.34 ± 0.26
155	<i>Sanguisorba officinalis</i> L.	24.58 ± 0.26	22.55 ± 0.94	11.12 ± 0.54
156	<i>Smilax glabra</i> L.	24.42 ± 0.68	19.36 ± 0.38	13.26 ± 0.92
157	<i>Melia azedarach</i> L.	22.38 ± 1.86	20.64 ± 1.84	11.94 ± 1.66
158	<i>Trichosanthes kirilowii</i>	20.96 ± 1.54	17.44 ± 1.62	12.55 ± 1.74
159	<i>Trifolium repense</i> L.	19.16 ± 1.66	17.92 ± 0.46	10.64 ± 1.58
160	<i>Actaea racemosa</i> L.	17.74 ± 0.42	13.26 ± 0.34	10.31 ± 0.86
161	<i>Sophora tonkinensis</i>	10.48 ± 0.54	5.42 ± 1.14	0.96 ± 0.22
162	<i>Uncaria macrophylla</i>	9.55 ± 1.76	6.64 ± 1.86	1.12 ± 1.96
163	<i>Fraxini folium</i>	9.33 ± 1.84	7.25 ± 1.69	1.90 ± 1.54
164	<i>Trollius europaeus</i>	9.26 ± 0.68	5.44 ± 0.58	1.24 ± 0.62
165	<i>Polygoni avicularis</i>	9.14 ± 1.33	4.36 ± 1.94	0.62 ± 0.48
166	<i>Dipsacus japonicus</i>	8.66 ± 0.94	5.54 ± 0.45	1.11 ± 0.40
167	<i>Tripterygium wilfordii</i>	8.54 ± 0.46	6.33 ± 0.72	1.63 ± 0.82
168	<i>Primula officinalis</i>	8.44 ± 0.62	4.18 ± 0.38	0.56 ± 0.26
169	<i>Salicis cortex</i>	7.96 ± 0.74	5.94 ± 0.46	0.74 ± 0.88
170	<i>Dysoxylum binectariferum</i>	7.82 ± 0.34	5.24 ± 0.47	0.48 ± 0.64
171	<i>Tilia cordata</i>	7.78 ± 1.80	5.88 ± 0.24	1.80 ± 1.96
172	<i>Echinochloa crus-galli</i>	7.67 ± 1.44	4.54 ± 1.68	0.44 ± 1.54
173	<i>Pulsatilla chinensis</i>	7.56 ± 0.92	5.62 ± 0.56	0.16 ± 1.42
174	<i>Stemona sessilifolia</i>	7.48 ± 0.36	5.35 ± 1.88	1.54 ± 1.28
175	<i>Iris potaninii</i>	7.36 ± 1.24	5.14 ± 1.94	1.36 ± 0.63
176	<i>Uvae ursi</i>	7.28 ± 1.12	4.43 ± 1.50	0.92 ± 0.46

177	<i>Pteris multifida</i>	7.15 ± 1.88	4.58 ± 0.86	1.26 ± 1.52
178	<i>Lauri folium</i>	6.90 ± 1.46	4.31 ± 0.44	1.32 ± 0.84
179	<i>Crataegus monogyna</i>	6.84 ± 1.64	4.74 ± 0.90	1.48 ± 0.24
180	<i>Xanthium sibiricum</i>	6.56 ± 0.72	4.16 ± 0.24	0.62 ± 1.56
181	<i>Teucrui Montana</i>	6.44 ± 0.36	5.34 ± 0.68	1.24 ± 0.74
182	<i>Sambucus nigra L.</i>	6.38 ± 0.84	4.55 ± 1.48	0.56 ± 0.18
183	<i>Erythroxylum pervillei</i>	6.24 ± 0.92	3.94 ± 0.82	0.42 ± 0.96
184	<i>Ophiopogon japonicus</i>	6.04 ± 1.68	3.35 ± 1.54	1.64 ± 1.22
185	<i>Indigofera tinctoria</i>	5.93 ± 0.55	4.47 ± 0.21	0.94 ± 0.58
186	<i>Dioscorea opposita</i>	5.76 ± 0.48	4.53 ± 0.62	0.72 ± 0.52
187	<i>Drynaria fortune</i>	5.64 ± 1.96	4.42 ± 1.74	0.55 ± 1.44
188	<i>Hyperii Herba</i>	5.52 ± 0.38	3.57 ± 0.56	1.38 ± 1.62
189	<i>Canabis sativa L.</i>	5.47 ± 1.86	4.53 ± 1.68	0.60 ± 1.08
190	<i>Cichorium intybus</i>	5.33 ± 1.16	3.26 ± 1.42	0.44 ± 0.53
191	<i>Verbenae officinalis</i>	5.25 ± 1.94	3.58 ± 1.70	1.26 ± 1.64
192	<i>Polygonum hydropiper</i>	5.14 ± 1.32	2.46 ± 1.52	0.52 ± 1.46
193	<i>Azadirachta indica</i>	4.90 ± 1.71	3.24 ± 0.45	0.49 ± 1.88
194	<i>Hyoscyamus niger L.</i>	4.88 ± 0.48	3.54 ± 1.34	0.18 ± 0.76
195	<i>Pestemon deustus</i>	4.74 ± 1.82	2.40 ± 1.22	0.34 ± 0.42
196	<i>Cleistanthus collinus</i>	4.66 ± 0.34	3.36 ± 0.64	0.56 ± 0.24
197	<i>Diphylleia grayi</i>	4.58 ± 1.65	2.58 ± 0.86	0.73 ± 0.15
198	<i>Doronicum pardalianches</i>	4.46 ± 0.40	3.47 ± 0.74	0.59 ± 1.44
199	<i>Farfarae folium</i>	4.34 ± 1.69	3.66 ± 2.06	0.46 ± 1.38
200	<i>Rhodiola sacra</i>	4.12 ± 1.42	4.18 ± 0.68	0.38 ± 0.76
	Ascorbic Acid	88.64 ± 1.22		

The highest ABTS radical scavenging ability was shown by the standard Ascorbic acid (88.64 ± 1.22%) followed by the scavenging activity for ethanolic extracts of *Euphrasiae stricta* with 85.43±0.13%. The Methanolic extracts of *E. stricta* showed the

radical scavenging activity of ($65.48 \pm 1.40\%$) followed by aqueous extracts ($14.37 \pm 0.26\%$) at 500 $\mu\text{g/ml}$ concentration. The ethanolic extracts of *Euphorbia platyphyllos* L. showed the maximum radical scavenging potential of $65.54 \pm 1.36\%$ followed by $63.42 \pm 0.94\%$ and $13.75 \pm 1.16\%$ for its Methanolic and aqueous extracts respectively, at same concentration as shown in table 4.9.

Similarly, the *Viscum album* L. and *Psoralea corylifolia* L. showed the radical scavenging activity of 67.32 ± 1.50 and 65.89 ± 1.36 for its ethanolic and 63.34 ± 0.78 and 63.48 ± 0.94 Methanolic extracts respectively followed by *Equiseti arvense*, *Veronica officinalis*, *Fagopyrum cymosum*, *Prunella vulgaris*, *Origanum vulgare*, *Camptotheca acuminata*, *Matteuccia struthiopteris*, *Nothapodytes nimmoniana* and *Justicia procumbens* as compared to the Standard L-ascorbic acid.

The present study is in accordance with study conducted by (Ilyasov *et al.*, 2020) to 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS^{•+}) radical cation-based assays are among the most abundant antioxidant capacity assays, together with the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-based assays according to the Scopus citation rates. The main objective of this review was to elucidate the reaction pathways that underlie the ABTS/potassium persulfate decolorization assay of antioxidant capacity.

The potential antioxidant activities of crude extracts (diethyl ether, petroleum ether, ethyl acetate, Methanol and water (infusion and decoction)) from *Euphorbia platyphyllos* L. The results of this study suggested a dose-response relationship for all extract samples. Thus, these results are in accordance with this present study (Ouml *et al.*; 2013).

This study is also in accordance with the ultrasound-assisted extraction of phenolic compounds from *Epimedium brevicornu* Maxim was modeled using response surface methodology (Zhao *et al.*; 2014). A Central Composite Design (CCD) was employed to

optimize three extraction variables. The HPLC analysis reveals that the main phenolic compound in the extract product was identified as gallic acid, catechin (Cianidanol), p-hydroxybenzoic acid, vanillic acid, caffeic acid, ferulaic acid, rutin, benzoic acid, and quercetin.

The similar studies were also conducted where four Iranian herbs were investigated to evaluate their antioxidant potentials, total phenolic and flavonoid contents by Ghasemi *et al.*, (2014). The TFC (Total flavonoids content) observed were from 10.12 to 22.2 EQ /g extract, was larger in the dragon head. Experimental results suggested that the dragonhead and thyme extracts were high in antioxidant capacity and so can be used as an important dietary source of phenolic compounds.

The similar findings were also observed for phytochemical detection of *Gunnera perpensa* L. was conducted by Simelane *et al.*, (2013) and it was observed that the extracts are rich in steroids, flavonoids, alkaloids, tanins, saponin and glycosides. The *G. perpensa* Methanolic extracts showed strong inhibition in DPPH (2,2-dyiphenyl-1-picrylhydrazyl hydride) and 3-ethylbenzothiazine-6-sulfonate (ABTS) assays but a weak inhibition (<50%) was noticed in superoxide, nitric oxide and hydroxyl radical scavenging assays. The cytotoxicity of the extract (LC 50) to the saline shrimp larvae was 137.62 mg/ml. It was noticed that folk medicine is clearly affected by antioxidant activity in *G. perpensa*.

This study is also in accordance with the findings of Xiao *et al.*, (2016) where the total alkaloids, peiminine, peimine and peimisine from the *Fitillaria thunbergii* bulb were extracted using the Supercritical fluid extraction (SFE) method. When compared to equivalent ascorbic acid (EAA) /100 g, the antioxidant potential was measured as EC₅₀ value for the extracts and it was 5.5 mg /ml in DPPH, for ABTS was 0.3 mg /ml and for FRAP the value was EC₅₀ 118.2 mg /ml.

Chapter 5.

5. Discussion:

5.1. Allelopathic Activity of Medicinal Plants

The detailed allelopathic results of all selected plants (200) were demonstrated using Dishpack and Sandwich methods as shown in Table 4.1 and Table 4.2. The results revealed that the volatile compounds of different plants had exhibited inhibitory or stimulatory effect on the germination and growth of the hypocotyl and radicle of the lettuce in a different manner. The seedling growth of lettuce was completely inhibited by one specie, 5 species reflected strong inhibition, 8 species indicated medium inhibition, 18 species revealed least inhibitory effect and 112 species showed insignificant allelopathic effects. Contemporary, 64 species least promoted the growth while 15 species presented strong stimulatory allelopathic effect on lettuce seeds. The complete inhibitory effect was noticed by *Rheum officinale* L. while strong inhibitory effect was observed by *Cassia tora* L., *Ophiopogon japonicas* L., *Picrorhiza scrophulariiflora* and *Trifolium repense* L. followed by medium inhibitory effect from *Artemisia alba*, *Thymus vulgaris*, *Mentha piperita*, *Hibisci sabdariffae*, *Dioscorea opposita*, *Veronicae officinalis*, *Origanum vulgare* L. and *Salicis cortex*.

However, *Trichosanthes kirilowii*, *Rosmarinus officinalis*, *Tiliae officinalis*, *Morus nigra* L., *Sedum sarmentosum*, *Croton setigerus* L. *Scutellaria baicalensis*, *Viscum coloratum*, *Geocrypta galii*, *Geocrypta galii*, *Smilax glabra*, *Rubus chingii*, *Inula Britannica* L., *Calycopteris floribunda*, *Angelica sylvestris* and *Nepeta cataria* exhibited strong stimulatory allelopathic effect on lettuce radicle growth as compared to control. It is notable to mention that *Trichosanthes kirilowii* indicated an exceptional stimulatory allelopathic effect on radicle growth of lettuce. The similar results were shown by the aqueous extracts of

Picrorhiza kurroa, *Ocimum sanctum*, *Asperagus racemosus* and *Valeriana wallichii* (medicinal plants), which decreased the seed germination of oilseed crops due to a strong inhibitory effect of allelochemicals (Rawat *et al.*, 2016). The release of allelochemicals follows numerous mechanisms, including leaching from aerial parts, decomposition of plant biomass into soil, exudation from roots and volatilization (Latif *et al.*, 2017).

The inhibitory effects of aquatic extracts of freeze-dried clover shoots were also evaluated by Macfarlane *et al.*, (2019) on germination and anomalies in the seeds of 10 grass species from pastures. The equivalent of 800 kg/ha of dry matter content of white clover (DM) is almost complete without the germination of white clover, cayenne, crow feet and perennials. The addition of PVPP was able to eliminate up to 75% of the observed inhibition. The chromatography investigation involved phenol as an active alkaline chemical.

The similar results were also observed in the investigations for the allelopathic effects of the shrub *Artemisia alba-alba* on spatial structure (in terms of species richness and seed density) on soil bank of a semi-arid ecosystem in NE Spain. Specifically, the richness and density of the seed bank in three micro sites were evaluated: under the basement of individual *A. herba-alba* under the *Salsola vermiculata* L., canopy, and bare soil. It was concluded that the presence of allelochemicals can effectively reduce the appearance of seed bank seedlings, leading to a decrease in the wealth of plant species and near density although the presence of allelopathic plants does not cause significant changes in the structure of the seed bank with respect to non-allelopathic shrubs (Antonio *et al.*, 2017).

Similarly, this study is also supported by Fujii *et al.*, (2003), the seedling growth of lettuce was also inhibited due to strong allelopathic effects of medicinal plants, including the families Myrtaceae, Apocynaceae, Guttiferae, Annonaceae, Leguminosae, Rubiaceae and Euphorbiaceae. Moreover, the phytotoxic effects of several medicinal plant species on

germination and seedling growth of other plant species have been reported in previous studies. The factor utilization of allelopathic effect can control weed management and hamper transformation from adverse effects to beneficial effects (Fujii *et al.*, 2003; Han *et al* 2008; Islam and Kato-Noguchi 2013; Sodaeizadeh *et al.*, 2009 and Appiah *et al.*, 2015).

The similar results were found for the Allelochemicals of *Ophiopogon japonicus* K. were also studied by Dongzhi *et al.*, (2014) and the analysis revealed the presence of at least six allelopathic chemicals, such as vanillic acid, syringic acid, syringaldehyde, salicylic acid, hydro-hydroxybenzoic acid and synaptic acid were found in this plant. The chemical detected at the lowest concentration was vanillic acid (1.60µg/g). The inhibitory effects on germination and growth of both barnyard grass (*Echinochloa crusgalli* L.) and Monochoria (*Monochoria vaginalis*) were shown by Salicylic acid. This compound can play a major role in allelopathic research.

The similar studies were conducted for allelopathic properties of extracts from roots, bark and leaves of *Azadiracta indica* L. were also observed. In vitro studies were carried out, where bhendi seeds were treated with extracts of bark of neem. The morphological parameters, synthetic dyes and biochemical content were analyzed on the seventh day. It was observed that the rate of bhendi germination was decreased in all extracts of *Azadiracta indica* L. A decrease in the biodegradable chemicals contents was also observed. The control treatment (distilled water) showed the highest rate of germination. The root extracts showed the highest rate of inhibition followed by bark and leaf extract. These results are also in accordance with the present study by Vaithyanathan *et al.*, (2014)

This study is also in accordance with the chemical composition of essential oils extracted from natural (*Thymus capitatus*, TC) and *Thymus vulgaris* (TV) was studied by Ashraf *et al.*, (2014) to assess the potential for interaction between the properties of the oil

and the allelopathic potential using the biological growth of some types of test (*Lepidium sativum* L., *Citrullus colocynthis* L. and *Trigonella foenum-graecum* L. and TC and TV were obtained with a yield of about 2.3 and 1.2% (T / s) respectively in dry weight, and the compounds were represented in the percentages of about 94.26 and 79.75% respectively, the growing wild species *C. colocynthis* was more resistant to the effect of thyme oil followed by *L. sativum* and *T. foenum*.

The allelopathic effects of the water extracts of mint leaves (*Mentha piperita* L.) were also investigated on seed germination and selected physiological processes of common sunflower seedlings (*Helianthus annuus* L.) by applying in different concentrations (1, 3, 5, 10 and 15%). The highest increase in photochemical efficiency of the second photovoltaic system was detected in *H. annuus* L. tested with mint extract by 15% with respect to low concentration of extracts and with control. Photochemical and non- photochemical extinction and biotic index II decreased with increased concentrations of allelopathic sample doses in mint extracts. These results are also in accordance with this study by Edyta *et al.*, (2015).

The similar studies for allelopathic materials isolated from the water Methanol extracts of *Hibiscus sabdariffa* L. were analyzed by Suwitchayanon *et al.*, (2015) using the color column and HPLC reverse phase methods. The growth of hypocotyls and radicles were bound by Methyl oxydroxycitrate at a concentration greater than 10 mm. To inhibit the 50% growth of hypocotyls and watercress roots the concentrations needed were 20.3 and 14.4 mm, respectively. The inhibitory activity of trimethyl allo-hydroxycitrate specified that this substance may be efficiently used as an allelopathic substance for *H. sabdariffa*.

The Park *et al.*, (2010) studied the physical and chemical properties of the fermented solution of (*Dioscorea opposita*) fermented by *Lactobacillus bulgaricus* were evaluated, to note the protective effect of fermentation for *Dioscorea opposita*. The results showed that

fermented potato powder by *L. bulgaricus* showed a protective effect on stomach lesions in mice. Therefore, it can be included as an additional ingredient in yogurt production.

The results were found similar for the allelopathic properties of three types of invasive plants found in Europe were also observed, *Impatiens glandulifera*, *Solidago gigantea* and *Erigeron annuus* were observed to verify the inhibition of germination of local species through alopecia rather than a community of nearby local plants. Seed germination and seeds planted by the seed bank were monitored for 9 weeks. Thus, these findings suggested that allelochemicals may be released by these invasive species that inhibit germination; they do so to a similar extent to those in the original plant community by Carvalho and Barata (2017).

The allelopathic activity of *O. vulgare* ssp. *vulgare* cold water extracts were evaluated by Dragoeva *et al.*, (2014), a wild plant cultivated in northeastern Bulgaria under laboratory conditions. The root lengthening test and *Allium cepa* test were used to evaluate the allelopathic effect. The length of the *Triticum estivum* L. root (P-0.001) was significantly reduced under Oregano extracts of (17.5 g/L, 52.5 g/L). The weed management programs can be studied as a possibility for future use of this herb.

The Mardani *et al.*, (2016) observed the similar findings to investigate the allelopathic potential of Caucasian plant species to select the strongest allelopathic species for future studies. Dehydrated leaves of 178 species of plants collected from the Siberian state of Teberda were analyzed using Sandwich method to determine allelopathic activity, using *Lactuca sativa* (lettuce) as a test plant. The results of this study will determine the toxic chemical products of new plants useful in medical and/or industrial applications.

The similar studies for Potential allelopathic effects of four essential oils (EO) were observed by Taban *et al.*, (2013) extracted from *Satureja* spp. e.g., *S. bachtiarica*, *S.*

khuzestanica, *S. rechingeri* and *S. spicigera* against rye and tomatoes (*Secale cereale*) and (*Lycopersicon esculentum*). A significant disincentive effect against rye and tomato seeds were observed in *S. khuzestanica* and *S. rechingeri*, respectively, at 400 µl, while the lowest inhibitory effect was shown by the *S. bachtiarica* amongst the tested plants. The main components of OE were carpaciol and thymol. So, it was shown by these results that the Eos of the tested Satureja species possess allelopathic effects for tomato and rye plants and can be used as natural herbicides in future agriculture.

The same Allelopathic effects were found similar results for the of concentrations (0.1% and 0.2%) of water extracts of *Satureja montana* were conducted by Sucur *et al.*, (2013), using lipid peroxidation and antioxidant activity (GPX, PPX, SOD and CAT) in pepper leaves, roots and black buds examined after treatments with 24, 72 and 120 hours. Our results showed that a higher concentration of *S. montana* water extract was caused by fat peroxidation in dark black roots. It was noted that the water extracted at a 0.2% concentration displayed a toxic effect of 68.33% after 96 hours.

The similar studies for allelopathic effects of water extracts of wetland seeds of four species (*S. lancifolia*, *Polygonum hydropiper*, *Sagittaria trifolia* and *Monochoria korsakowii*) were studied by Wang *et al.*, (2009) on seeds germination and seedlings growth of a type of crop (*Brassica rapa* spp. *Pekinensis*) and two types of wild wetlands (*rufipogon* and *M. korsakowii*) using various laboratory tests for biological testing. All seed extracts from the cover caused a major initial delay in germination of *B. rapa* spp. *Pekinensis* and *O. rufipogon*. These results also indicated that the allelopathy effect of seeds from seeds can have significant impacts on the organization of plant communities.

The Ren *et al.*, (2017) conducted the similar studies for the evaluated the similar cytotoxic effects of *Glycyrrhiza uralensis* a traditional Chinese medicine and industrial

agriculture cultivated extensively in northern China. Extract from the granular viscosity. The (HPLC) and its concentrations on dry media were 3.32, 0.09 and 13.5 μ g/g, respectively. These alkaline chemicals, which show significant activity in plant toxins and self-toxicity, can play an important role in the failure to replant *Glycyrrhiza uralensis*.

The allelopathic potentials of isolated strains of *R. solanacearum* from tomatoes were also studied by Daniel *et al.*, (2018) from the Masino regions with the biological diversity of infected tomato plants and *Ralstonia solanacearum* both chemically and morphologically. The strains were thus determined by a randomized test in a wide range of hosts. The *R. solanacearum* infected tomato plants in the Masino region were tender 3 biovar 1 and 3 biovar 3. All isolates were pathogenic in tomato plants, isolation, and characterization of *Ralstonia Solanacearum* strains in tomato disease were observed from Maseno, Kenya. These results also indicated the same allelopathic effect as the present study.

The similar studies for phytotoxic properties of buckwheat were analyzed, to improve soil and control pests and weeds. Applying biomass or pellets from your plants to a rice field is not harmful to rice plants. 1-2 Halon biomass significantly reduce the appearance and growth of major mountain grasses (Canada Cardo and quack grass) and weeds (barnyard grass and Monochoria). Many allelochemicals chemicals (alkaloids, phenolic compounds, long-chain fatty acids, flavonoids) have been isolated from their buds, roots and seeds, and these are responsible for allelopathic activities. Utilization of the allelopathic properties of buckwheat can help reduce the use of synthetic pesticides by Xuan and Eiji (2014).

The Purohit and Neeta (2013) studied two important medicinal plants (*Ocimum sanctum* L.) and wild teal (*Tephrosia purpurea* L. Pers) which were observed for their potential allelopathic activities, which contain various chemical compounds used for medicinal purposes. The study showed that Basil and Wild Indigo had different effects in all

pulses at different concentrations. Stimulant effect was also observed, while wild indigo had a more inhibitory effect than basil. These results also indicated the same allelopathic effect as the present study.

The similar findings were also observed for the Dhanya and Benny (2012) assessed the allelopathic properties of water extracts of *Solanum torvum* Methanol extracts from leaves in some of the selected cultured plants in selected India. The results indicated that all the cultures studied were sensitive to the water and Methanolic leaves of *S. torvum* in germination stage. Methanol extracts from plant leaves had the highest inhibitory properties on growth and germination of primary cultures compared with water extracts. The presence of *Solanum torvum* allelochemicals can seriously prevent the growth of studied cultures. These same findings for allelopathic effects in these plants were observed as the present study.

The same potential phytotoxic activities of *Cupressus lusitanica*, *Callistemon viminalis*, *Senna spectabilis*, *Tephrosia vogelii* and *Polyscias fulva* were observed by Michelinet *et al.*, (2016) polysaccharides and phytosanitary activities in some areas of tomato cultivation in Cameroon. The maximum inhibition of seed germination was observed in water extracts of *T. vogelii*, *C. lusitanica*, and *C. viminalis*, while Methanol, hexane and ethyl acetate fractions in all plants caused significant increases in seedling diameter. All treatments usually reduce root length. Extracted Methanol extracts are much higher vital indicators ($P < 0.05$).

This study is also in accordance with the Mei *et al.*, (2015) who examined the allelopathic properties of the *Solidago canadensis* weed on other indigenous plant species. Various test coefficients used were, *Medicago lupulina*, *Trifolium repens*, *Portulaca oleracea*, *Lolium perenne*, *Trifolium pratense*, *Suaeda glauca*, *Plantago virginica*, *Ageratum*

conyzoides, *Kummerowia stipulacea*, *Festuca arundinacea* and *Amaranthus spinosus* were examined with various concentrations of *S. canadensis* root and rhizome extracts. The results showed that the inhibitory effect of allelopathy in the root and rhizome extracts with increased concentration and the extracts of rhizome showed a greater effect than the root extracts.

The similar allelopathic potentials of *Paspalum commersonii* weed were also observed and identification of their allelopathic materials was also carried out. The hydrolysis of Methanol was shown by *P. Commersonii* as a growth inhibitor based on the concentration of watercress, clover, canola, lettuce, farm grass, festuca foxue, Italian ryegrass and Timothy. So, it was suggested by these results that both substances possess a strong allelopathic potential in *P. commersonii*. These results also indicated the same allelopathic effect as the present study (Zaman *et al.*, 2018).

The allelopathic extract of basil (*Ocimum basilicum*) and part of the chlorophyll content of Abutilon (*Abutilon theophrasti*), Goosefoot (*Chenopodium album*) and centaury (*Centaurea depressa*) were analyzed by Fanaei *et al.*, (2013). The treatments represent four concentrations of aqueous extract of sweet basil (control, 25, 50 and 100 g) and three concentrations of basil essence (0, 50 and 100%). There were no statistically significant differences between 50 and 100% control and treatment with respect to Goosefoot content of chlorophyll.

The similar findings were also observed by Bajalan *et al.*, (2019) observed water extracts from aerial parts of *Salvia officinalis* L., to test the potential inhibitory effects on barley seed germination (*Hordeum vulgare*) and experimentally by portulaca (*Portulaca oleracea*) under 5 treatments and 4 replicates. Experimental treatments included *S. officinalis* L. (6, 12, 25 and 50%) and distilled water (control). The results showed a strong effect on the

S. officinalis L. extract on the germination of barley seeds and oleracea. The statistical comparison indicates the percentage of decrease in germination in the treatment of water extracts compared to control.

The Pula *et al.*, (2016) find similar phytotoxic effects of aqueous extracts from shoots *G. aparine* L. were identified at various concentrations of 1, 3 and 5% in the growth of *Zea mays* L. in two types: "Lokata" F2 F1 and F2 "Wilga". The seeds were placed in Petri dishes (100 units) and spread *G. aparine*, distilled water (control) was irrigated. Seed germination capacity was higher at 1% concentration for Wilga F1, F2 and lower for Lokata. Bio-analysis of seedling root effect showed a stimulating inhibitory extract of 1%, 3% and 5%. The water content in the 'Wilga' and 'F1 Lokata' F2 seedlings increased significantly from each extract, compared to the control.

This study is also in accordance with the allelopathic effect of *Teucrium chamaedrys* L. medicinal plant isolated extracts were evaluated by Antonio *et al.*, (2019) to test the potential inhibitory effect of neo-clerodane diterpenes on seed germination and seedling growth of four Mediterranean species co-existence (*Dictylis Hispanica*, *Petrorragia filotina*, *Timothy subulatum*, *Petrorragia saxifraga* and *Avena fatua*). All the structures were clarified based on their spectral properties. Biomass data analyzed by the main component analysis, have more negative effects on weeds of species that coexist. In addition, *D. hispanica*, *P. velutina* and *P. subulatum* showed catalytic or inhibitory effects depending on the type and concentration of the metabolite used in the test.

The Khan *et al.*, (2014) observed the similar phytotoxic activities from the Methanolic extracts of 13 traditionally used medicinal plants. The tested plants include *Viburnum cotinifolium*, *Euphorbia hirta*, *Caryopteris grata*, *Vitex negundo*, *Woodicornia fruticosa*, *Armala peganum*, *Brossonnetia papyrifus*, *Chenopodium ambrosoides*, *Taraxacum*

officinale, *Adhatoda vasica*, *Urtica dioica* and *Urtica dusica*. The low concentration (1 mg/ml) affects growth rather than visualizing the allelopathic effects for *P. harmala*. So, it was suggested that the radish seeds toxic activity of plants in selected medicinal plants was observed to be dose dependent.

The similar findings were also observed for the allelopathic potentials of *Portulaca oleracea* plant were studied by Silva *et al.*, (2017) found abundantly and widespread weed throughout the World. However, relatively little is known about their allelopathic properties in relation to their susceptibility to external organic chemicals. A review of *P. oleracea* was also presented as a source and destination for allopathic compounds, including laboratory studies, field vessels and future research areas.

The allelopathic studies of organic mulch extracts were observed in this study such as Pino and Pine straw etc. The previous research on allelopathic compounds in commonly used compost materials was the purpose of this review and also the identification of potential new mulch material that could be used in the field of landscape based on the characteristics of allelopathy. The current areas that need further research are identified (Saha *et al.*, 2018).

The Ibrahim *et al.*, (2018) find similar results for the strong allelopathic activities of *Polygonum aviculare* were reported v. *Cynodon dactylon* (L.) using various types of tests. In addition, various phenolic compounds appear to be important alkaline chemicals in this activity were also observed in this study. The other potential inhibitors of *P. aviculate* were also isolated and soil under *Polygonum* supports, none of which occurred in soil under *C. dactylon* support. It was observed that the additional inhibitors are long chain fatty acids containing 14 to 22 carbon atoms when revealed by GC-MS analysis. *P. aviculare* possessed nine fatty acids while 7 were found in soil residue under *P. aviculare*. The inhibition was observed in the growth of weed seedlings by both sodium salts of specific fatty acids.

The similar findings were also observed for the allelopathic effects of *Tinospora cordifolia* weed were investigated by Raouf and Siddiqui (2016) on the germination of weed seeds and the growth of grass seedlings (*Chenopodium murale* L., *Cassia tora* L., *Chenopodium album* L. and *Cassia sophera* L.). The effect on germination and growth of test plant seedlings in laboratory conditions were determined by using water extracts of *Tinospora* leaves and stems were used at 0.5, 1.0, 2.0 and 4.0% concentrations. The maximum inhibition was observed in water extract of the leaves, while less inhibition effect in stem on weeds. The aquatic extracts of different parts of *T. cordifolia* do not show significant changes with changing pH with increasing concentrations.

The Skulmanet *al.*, (2019) also observed similar findings for the potential allelopathic properties of Japanese honeysuckle in relation to pine regeneration and growth were investigated. The root potential of the Japanese shoots and the Japanese moss seed was tested against pine seedlings with short blade when honeysuckle seedlings and short Japanese pine seedlings are found using the same irrigation tank, a substantial effect on the growth of any species of pine is obtained: gas chromatography analysis. So, it was shown by the results that at least a partial role in Japanese honeysuckle interference was played by allelopathy with loblolly and shortleaf pine.

The *Eclipta alba* L. plants were studied by Aasifa and Badruzzaman (2014) to assess the allelopathic abilities of three selected Aligarh area weeds *Cassia tora* L., *Amaranthus spinosus* L. and *Cassia sophera* L. The evaluation of aqueous leaf leachate and organic fractions of donor plant on biochemical activities (carbohydrate content, chlorophyll content and protein content) were investigated for their potential allelopathic effects. The maximum toxicity in weeds was shown by the leachate compared to the organic fractions. The allelochemicals present in *E. alba* leaves were responsible for this reduction in growth.

The same findings were also carried out for the allelopathic effects of using leaf extracts of invasive plant *Solidago canadensis* were observed by Wang *et al.*, (2014) on growth and germination of local species *Lactuca sativa* observed with five different acid deposits with different ratios of SO_4^{2-} to NO_3^- ratios 5:1, sulfuric-rich acid, (1:0, sulfuric acid, 1:5, nitric-rich acid and 0:1, nitric acid). The higher allelopathic effects on germination rate, strength index and *L. sativa* germination index were shown by *S. canadensis* leaf extracts.

The Reza *et al.*, (2015) observed the similar findings for the effects of allelopathy were studied in various water extracts and essential oils of medicinal plants mentioned regarding control treatments. The measured values were germination percentage, germination rate, root length, root, firing length, fresh and dry weight and bud weight. The results showed that the milk oil at a concentration of 600 mg/L had a more significant effect on germination rate and the rate of wild mustard germination. Our results also indicated that the water extract of these plants had a stimulating effect on the development of weed seeds, while some essential oil treatments had a disincentive effect on the development of these seeds.

The similar antibacterial and allelopathic activities from Methanolic extracts of rhizomes of *Iris pseudopumila* were evaluated by Rigano *et al.*, (2016). The antibacterial effects were observed in the tested extracts. The *Raphanus sativus* seed germination was used to test the allelopathic effect *Iris pseudopumila*. The results suggested that a decrease in the percentage of seed germination and root and epicotyl growth was caused by the allelopathic effects of *I. pseudopumila*.

The allelopathic potentials from leaf extracts of (156 species) 160 medicinal plant samples from 134 genera and 74 families were observed by Shinwari (2017) using the Sandwich method. In the bioassay, test plant material used was Lettuce (*Lactuca sativa* L.) because of its germination reliability. The maximum growth stimulation or minimum growth

inhibition by *Ligustrum japonicum* (Oleaceae) followed by *Vitex rotundifolia* (Lamiaceae) and *Alpina intermedia* (Zingiberaceae) was also shown by present study. These findings can be used as standard information for further research on the explanation of allelochemicals responsible for allelopathy in nature.

The similar studies were also carried out for the lemon balm (*Melissa officinalis* L.) plant shoots extracts (acetone, *n*-hexane and water-soluble fractions) were investigated by Noguchi (2021). It was observed that the aqueous extract of acetone buds inhibited germination and growth root and inhibits the growth of cock's lettuce (*Lactuca sativa* L.), watercress, *Lepidium sativum* L., *Digitaria sanguinalis* L., fleol (*Phleum pratense* L.), *Amaranthus caudatus* L. and ryegrass (*Lolium multiflorum* Lam.). These speed-based responses from test stations to fractions indicate that each part may contain allelochemicals, but the highest potential was observed in the water-soluble extracts. These results indicated the same allelopathic effect as in the present study.

The Shilling *et al.*, (2019) conducted the similar findings for the allelopathic effects of celery crops on commercially grown lettuce *Lactuca sativa* L. were observed when it was established instantly after the elimination of the celery crop. Experiments were carried out in the greenhouse to assess the possible divine celery effects in the appearance and growth of lettuce. The growth of lettuce exposed to celery remains was increased by addition of activated carbon to the medium. Celery waste has the ability to allelopathic to develop lettuce seedlings. The type and concentration of celery tissue, soil type, celery nursery residues in the soil and the addition of carbon activated by the culture medium affected the extent of the observed plant toxicity.

The similar findings were also carried out for the allelopathic results of *Xanthium stramonium* L. leaves (family: Asteraceae) extracted essential oils (EO) were evaluated by Ahmed *et al.*, (2019), by hydrolysis and then analyzed by GC-MS. 43 basic compounds were identified. Seiceter benoid contained the main components (72.4%), including oxygenated cytirbines (61.78%) and oxygen (10.62%), followed by monoterpenes (25.19%). *X. strumarium* EOs showed a weak antibody, 1-dynyl-2-Bicryl hydrazylo (DPPH) with IC50 321.93 L/L, which was similar to the ascorbic acid reference. However, EOs has shown significant potential of allelopathic in relation to germination and growth of harmful grass in a manner dependent on concentration. Therefore, further studies are recommended to characterize the *X. strumarium* EOs as a biochemical herbicide against weeds.

The allelopathic activity of edible medicinal plant species *Euphrasia rostkoviana* was studied by Rafaela and Luis (2020) in the Himalayan regions. The *E. rostkoviana* leaves are still poorly studied despite its chemical and biological importance. The metabolites identified in this study were responsible for the allelopathic activities of this plant. So, it was suggested by these findings that extracts may be of great value for their use in pharmaceutical and food additives because of their antimicrobial and allelopathic activities. However, studies should be conducted in vivo to ensure their safety. These results indicated relatively more allelopathic potentials as in present study.

5.2. Antioxidant Activity of Medicinal Plants

The radical scavenging activity of ethanolic, Methanolic and aqueous extract of various medicinal plant extracts was examined in this research study. The standard antioxidant compound L-Ascorbic acid showed the highest radical scavenging activity of

90.36 ± 1.05 µg/ml. The ethanolic extracts showed the highest antioxidant activity of 71.92 ± 1.22% followed by Methanolic extracts with 70.14 ± 0.82%.

The highest antioxidant potential was observed in the ethanolic extracts of *Euphrasiae stricta* in all tested assays followed by *Euphorbia platyphyllos* L. and *Epimedium brevicomum* Maxim respectively. The *Viscum album* L. and *Psoralea corylifolia* L. also showed strong antioxidant activity followed by *Equiseti arvense*, *Veronica officinalis*, *Fagopyrum cymosum*, *Prunella vulgaris*, *Origanum vulgare*, *Campotheca acuminata*, *Matteuccia struthiopteris*, *Nothapodytes nimmoniana* and *Justicia procumbens* respectively as compared to the Standard L-ascorbic acid.

This study is in accordance with the published scientific data on the chemical composition and biological activity of 10 species of *Euphrasia stricta* L: *E. rostkoviana*, *E. brevipila*, *E. parviflora.*, *Euphorbia platyphyllos* L., *E. montana.*, *E. pectinata*, *E. condensata.*, *Epimedium brevicomum*, *E. stricta.*, *E. salisburgensis*, *E. maximowiszii.*, *Viscum album*, and *E. reuteri*. (Suhinina TV and Petrichenko, 2019). The representatives of *Euphrasia* are the sources of biologically active substances, namely glycosides, organic acids, lipophylic substances, macro and microelements etc. Flavonoids, phenoloacids and iridoic glycosides, however, are the main groups. Hypotensive, anti-inflammatory, anti-microbial, antioxidant and hepatoprotective action of *Euphrasia* representatives has been revealed experimentally.

The *Euphrasiae stricta* plant was also investigated by Iqbal *et al.*, (2020) for its antioxidant potentials in this research. It is an important medicinal plant in South Asia. Antioxidant assay was performed in various solvent systems, i.e., water, ethyl ether, 70% ethanol and 80% Methanol. The highest total phenol content was shown by the Methanol extracts among all the extracts; so, these were used for additional research. Thus, these

results for allelopathic activity of *Euphrasiae stricta* are in accordance with this present study.

This study is also in accordance with the potential antimicrobial and antioxidant properties of various Artemisia varieties e.g., (*A. arborescens*, *Artemisia absinthium* L., *A. campestris* L., *A. scoparia* and *A. santonicum vulgaris* L.) were observed by Erel *et al.*, (2016). The analysis was achieved by natural distribution in western and southwestern using gas chromatography. The main components were identified as camphor *A. arborescens* (33.39%), *A. absinthium* (17.55%), 1,2-dehydro-acenaphthylene in *A. campestris* and *A. scoparia* (20.71% and 11.80%, respectively) and α -tujona in *A. vulgaris* and *A. santonicum* (56.13% and 39.46%, respectively). The plants have also been tested for their potential antioxidant capacities.

The similar studies were also conducted for the antioxidant potential, volatile oils and phenolic compounds of *Ailanthus altissima* Swingle papers were thoroughly tested by Albouchi *et al.*, (2014). The antioxidant, antimicrobial and plant toxicity properties from Methanolic extracts of leaves and their residues were analyzed. The DPPH and FRAP assay were used to measure the antioxidant potentials of extracts. So, a significant support was provided by this study for high antioxidants and cytotoxic activities of specie and so can be efficiently used as a natural herbicides and antioxidants source in the food and pharmaceutical industries.

The similar studies were also conducted for the antioxidant properties of *Arctium lappa* Linn was studied by Duh (2014) for its active oxygen removal and free radical potential. The water produced the largest amount of extract that showed the strongest antioxidant activity, among all the solvents used in extraction. So, these findings proposed

that the antioxidant activity in the burdock extract was because of suspension of free radical reactions and the extinction of reactive oxygen species in burdock extracts.

This study is also in accordance with the previous study where the Sixteen plants selected in Yemen were studied by Ramzi *et al.*, (2014) for their potential antioxidant properties and phytotoxic activities. Two different solvents (Methanol and hot water) were used to extract the dried plant samples and to produce 34 raw extracts. The different types of compounds such as flavonoids, warbenoids and others were found in phytochemical examination that may be responsible for the antioxidant and antimicrobial activities.

The Zhang *et al.*, (2014) also observed similar findings for the antioxidant capacities and phenolic compounds identification of *Astragali complanati* was carried out using the ultrasonic extraction. The antioxidant capacity was measured by using a 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) test and the total phenolic content was estimated through Folin-Ciocalteu assay. The electron microscopy (SEM) analysis was also used to measure the effect of ultrasound on the microscopic structure of the sperm molecules of *Astragali complanati*.

The potential antioxidant properties from Methanolic extracts (80%) obtained from 118 medicinal plants were tested by Jung *et al.*, (2014) by the adsorption activity of the superoxide and DPH ionic roots. The antioxidant potential was observed using DPPH for Methanolic extracts of Gabonica sulphate (76.9%) and *Camptotheca acuminata* Dence (50.9%) with DPPH uptake by more than 50%, while *Perilla frutescens* (37.2%), *Amomum costatum* (34.9%), *Prunus ansu* (33.2%), *Mentha arvensis* (32.3%), *Serratula koreana* (32.2%), *Eriobotrya japonica* (30.5%) and *Artemisia asiatica* (30.5%) by showing more than 30% of radical scavenging activity. A strong antioxidant activity in DPPH assay was shown

by the *Eriobotrya japonica*, *Amomum costatum*, *Mentha arvensis* and *Camptotheca acuminata* in ultrastructural anesthesia.

This study is also in accordance with the potential phenolic components from root extracts of *Rheum officinale* and *Rubia cordifolia* were studied by Cai *et al.*, (2014) through the use of high-performance liquid chromatography and liquid chromatography-mass spectrometry and comparison to original standards. The enhanced method of ABTS (2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) was used to measure antioxidant activity (the antioxidant capacity of Trolox). The results showed the presence of an efficient antioxidant potential in these plants.

The similar studies were conducted for the *Paris polyphylla* leaves were studied to evaluate their potential antioxidant activity by Shen *et al.*, (2014). The extraction temperature (°C), the extraction time (H) and the ratio of water to the raw material were the three independent variables focused, which expressively affect the performance of sugars. Furthermore, strong antioxidant properties on hydroxyl, DPPH, and superoxide radicals in vitro were demonstrated by purified polysaccharides of extracts.

This study is also in accordance with the organo and thyme extracts were evaluated for their antioxidants effects in soybean oil is susceptible using thermal oxidation by Jorge *et al.*, (2015). About 3000 mg/kg of organo, thyme olese oil and their mixtures was found in Soybean oil it also contains tributyl hydroquinone (TBHQ; 50mg/kg) and soybean without oil exposed to thermal oxidation. Thus, physical, chemical, and fatty acids were evaluated. A greater protective effect was applied by organo and thyme separately, which prevented the increase in the formation of TBHQ, showing that by adding 3000 mg / kg ensures better protection against oxidative oxidation. The increased absorption of urea by adding the thyme and oregano extracts gave a greater protective effect.

The Iqbal *et al.*, 2015^b observed the ethanolic extracts of *Epimedium brevicomum* Maxim the maximum radical scavenging potential of $64.96 \pm 0.79\%$ followed by $62.59 \pm 0.73\%$ and $11.33 \pm 1.46\%$ for its Methanolic and aqueous extracts respectively. The lipophilic and hydrophilic compounds can also be measured for their antioxidant capacities by using this assay. From the results it was observed that the ethanolic extracts of all samples showed the highest radical scavenging capacities followed by the DPPH radical scavenging ability of Methanolic and aqueous extracts for all experiments samples. Thus, these results are in accordance with this present study.

The potential antioxidant effects in shoot extracts of *Asparagus cochinchinensis* (Laur.) were studied by Hasan *et al.*, (2015) in 80 mice Ming Kun which were randomly divided into four groups (20/group). The plant is characterized by strong antioxidant ability in-vivo and in-vitro and can be used to reduce the roots in the body and thus prevent aging.

The Simona *et al.*, (2015) observed the similar results for the *Melissa officinalis*, *Lavandula angustifolia*, *Agastache foeniculum* and *Nepeta cataria* were investigated, for evaluation of their different quantity of total phenolic contents ranging from TPC as (12.44–76.43 mg GAE/g dry plant) and also correlation with phenophase, harvesting hour and botanical origin. So, it was shown by the results that these plants play a vital role of biologically active phenolic compounds and can be potentially used as an efficient antioxidant source because of their phenolic acids and flavonoids content.

The similar study was also conducted by the *Prunella vulgaris* Linn (*P. vulgaris*) was investigated for its antioxidant activity of several water-soluble polysaccharides by Lee *et al.*, (2015), using the DEAE-Sepharose flow column for different rinsing water (PV-P1), 0.2 M NaCl (PV-P3) and NaCl 0.1 M (PV-P2). The all three extracts PV-P1, PV-P2 and PV-P3 against RA 264.7 in the tested concentrations no cellular toxicity was observed. So, it was

shown by these results that common *P. vulgaris* polysaccharides can be inspected as possible antioxidant source, medicine, immunoglobulins or functional foods.

The Singh *et al.*, (2015) observed similar findings for the antioxidant and anti-bacterial effects on some gram-positive and gram-negative bacterial strains using agar well diffusion method for peppermint oil and various *Mentha piperita* extracts. Distillate concentrations of essential oils were found that the microorganism's growth was inhibited and the same effects as to those in gentamicin antibiotics. These results indicated strong anti-bacterial and antioxidant activities for peppermint oil. However, for human consumption, to confirm the safety of these concentrations (MIC) further research should be undertaken.

The Akinsanmi and Ester (2015) studied the similar findings for potential of antioxidants in the water extracts of *Gongronema latifolium* ("Utazi") and *Gnetum africanum* ("Afang") to manage / prevent ROS generated diseases as a result of oxidative stress. Total phenol, total flavonoids, FRAP, Fe²⁺ and antioxidant activity were identified. The antioxidant potential was measured by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and the hydroxyl (OH^{*}) roots were identified. Antioxidants related to phenol/flavonoids. In conclusion, both the water extracts of *Gongronema latifolium* (Utazi) and *Gnetum africanum* ("Afang") are rich in phenolic compounds with antioxidant effectiveness, but Utazi had an antioxidant activity.

The similar studies were also conducted for the antioxidant properties of in vitro and in laboratory red clover extracts were observed by Khorassani *et al.*, (2015). The Red Lime is used for different extraction solvents, and various alkaline antioxidant assays have been tested. The highest total phenol and phenyl flavonoid compounds present in the Methanol extract are from in vitro plants from all the tested extracts. Antioxidant activity in the samples used in the order of live plant extracts> Callus extract> in the laboratory extract. So, it was

concluded that antioxidant activity was possessed by the laboratory extract of red clover, especially by callus tissues.

The antiviral, analgesic and antioxidant properties from leaf extracts of *Andrographis paniculata* were studied by Adedapo *et al.*, (2015) in laboratory animals. The chemical and plant analysis of dried leaves and plant powder were performed along with its mineral content determination. The first toxic toxicity tests were conducted to determine the safe dose level. The 1,1-diphenyl-2-picrylhydrazil (DPPH) assay was used to measure the antioxidant activity of *A. paniculata* extracts. The superior antioxidant activity was observed in *A. paniculata* ethanolic extract as compared to the water extracts. So, it was suggested by this study that this plant can be efficiently used for medicinal purposes.

The effects of different heating times (30-150 min) were studied at 100 ° C on the nutrients and antioxidants of *Lonicera japonica* properties by Laurian *et al.*, (2016) similar results were also found. Total phenol, phenolic acids (chlorogenic acid, caffeine acid, decalfinic acid 4.5 and 3.5 dicafilquinic) and flavonoids (rutin, quercetin and lutulin) increased significantly after heat treatment. Antioxidant activity, such as DPPH root uptake activity, root ABTS uptake, FRAPS and energy reduction, from LJ, was improved after heating. Antioxidant activity is positively associated with total phenolic content, total flavonoids, chlorogenic acid, caffeine and cercetin.

The Wierdak and Grazyna (2016) observed similar findings for the phytochemical evaluation of lavender, buds and flowers was carried out. A high positive correlation between aromatic oil content and antioxidant activity (AA) ($R = 0.9688$), total phenolic acids and AA ($R = 0.9303$) was found. The predominant compounds in the oil obtained from the papers were epi- α -cadinol (17.8%), Cryptone (10.4%), 1,8-cineol (7.3%) and elephant carotid oxide (7.2%). Of the flowers: acetate linalyl (22.3-32.1%) and linalool (23.9 - 29.9%).

The Ozarowski *et al.*, (2016) conducted similar studies where the Methanolic extracts of *Chelidonium majus* L. seedlings were studied for their potential total content of flavonoids and alkalis using spectral methods. Quantification of calcinedin, cipticine, schinarin and berberin was done by HPLCUV. The results indicated that the changes observed in antioxidant activity in the extract may be because of celydonin and capitecin, in particular, because these alkaloids were found at the maximum concentration in aqueous extracts of this plant.

This study is also in accordance with the Ruan *et al.*, (2016) where antioxidant potential of *Rhazya stricta*, an important medicinal plant in South Asia was evaluated. To evaluate the antioxidant potential of these Methanol extracts various antioxidant assays were used like DPPH, metal ion chelating assay, FRAP Assay. Antibacterial activity and superoxide radical anions were taken as parameters. The antioxidant potential in the Methanol extracts of *R. Stricta* sheets was comparable with strong antioxidants previously exploited and largely dependent on concentration.

The similar findings were observed by Ruan *et al.*, (2016) for increased concerns about the safety of synthetic antioxidants. Today's research is focused on the use of natural sources of antioxidants. The *Euphorbia platyphyllos* and *Rhazya stricta* have been an important medicinal plant in South Asia. The Antibacterial activity and superoxide radical activity of *R. Stricta*, *Psoralea corylifolia* L and *Viscum album* were taken as parameters. The antioxidant potential in the Methanol extracts of *R. Stricta*, *Psoralea corylifolia* and *Viscum album* sheets was comparable with strong antioxidants previously exploited and largely dependent on concentration as shown in the present study.

The (Hsu, 2016) observed similar antioxidant activity various extracts of dried *Polygonum aviculare* L. was investigated by using various assays by FRAP assay, lipid

peroxidation and analysis of DNA-induced cleavage sequences. The IC_{50} value was measured by the results for different extracts which were 50 μ g/ml, 0.9 μ g/ml and 15 μ g/ml for the DPPH antioxidant or radical scavenging assay, H_2O_2 superoxide radical assay and for lipid-peroxidation assay, respectively. Moreover, these extracts also showed a protective effect in hydroxyl radical-induced DNA strand assays. The value of TPC and TFC observed were 677.4 \pm 52.7 μ g/g and 122.7 \pm 14 μ g/g for these extracts. So, the significant antioxidant effects were shown by these findings of *Polygonum aviculare* L. extract.

This study is also in accordance with the potential antioxidants of *Origanum vulgare* L. are the most preferred spice plant tested by Azizi *et al.*, (2016), which were observed to vary greatly between different species, local varieties and additives. The genetic variation in the ability of antioxidants to adhere to oregano National Gene-bank German was calculated by determining the oxygen root absorption capacity (ORAC), rosemary acid content, total phenolic content (TPC) and the essential oil content. The ORAC and TPC correlation was discreetly close to ($r = 0.60$, $n = 352$). ORAC was not associated with rosemary acid content or primary oil content. The antioxidant potentials diversity of Origen found here allowed the better utilization of the plant in pharmaceutical applications and breeding of *Origanum vulgare* genotypes.

The similar studies were conducted where potential antioxidants of *Origanum vulgare* L. were observed to vary greatly between different species, local varieties and additives. The ORAC and TPC correlation was discreetly close to ($r = 0.60$, $n = 352$). ORAC was not associated with rosemary acid content or primary oil content. Total phenol content (TPC) and rosemary acid content ($r = 0.39$, $n = 352$) were moderately associated with each other and also with the main oil content ($r = 0.25$, $n = 352$). The antioxidant potentials diversity of

Origen found here allowed the better utilization of the plant in pharmaceutical applications and breeding of *Origanum vulgare* genotypes (Azizi *et al.*, 2016).

The Phenol concentrations and complete flavonoids were studied in *Marrubium vulgare* L. by Bouterfas *et al.*, (2016). The extracts showed a value between 39.7 and 161 mg of G /g and 26.4 and 67.4 mg of catechin /gm parallels respectively. The results showed that organic solvents used influence the antioxidant activity of highly extracted flavonoids ($P < 0.001$) during the DPPH assay. The presence of certain flavans and flavanols were observed during phytochemical examination. The results indicate a strong antioxidant activity from *M. vulgare* extracts, which can be applied in the study of the characteristics of the food and pharmaceutical industries.

This study is also in accordance with the findings of Xiao *et al.*, (2016) where the total alkaloids, peiminine, peimine and peimisine from the *Fitillaria thunbergii* bulb were extracted using the Supercritical fluid extraction (SFE) method. The (DPPH-RSA) DPPH radical Scavenging activity assay, (ABTS-RSA) ABTS radical absorption activity and (FRAP) ferric reducing capacity assay was used to investigate the antioxidant capacity of *F. thunbergii* extracts. The maximum yield was 3.8 mg / g of total alkaloids, showed 1.3 mg/g of peimine, 1.3 mg/g for each peiminine and 0.5 mg / g of peimisine. When compared to equivalent ascorbic acid (EAA) /100 g, the antioxidant potential was measured as EC_{50} value for the extracts and it was 5.5 mg /ml in DPPH, for ABTS was 0.3 mg /ml and for FRAP the value was EC_{50} 118.2 mg /ml.

The antioxidant activity various extracts of dried *Polygonum aviculare* L. was investigated by Hsu *et al.*, (2016) by using various assays by removing radical phosphate roots to remove roots, lipid peroxidation and analysis of DNA-induced cleavage sequences. The IC_{50} value was measured by the results for different extracts which were 50 μ g /ml, 0.8

$\mu\text{g/ml}$ and $15 \mu\text{g/ml}$ for the free radical scavenging activity assay, superoxide radical assay and for lipid-peroxidation assay, respectively. So, the significant antioxidant effects were shown by these findings of *Polygonum aviculare* L. extract.

This study is also in accordance with the Chen *et al.*, (2016) where the extracts from the old, cultivated land plants contain phytochemicals that provide a series of pharmacological activities of antimicrobials, antioxidants, anti-inflammatory, anti-plasma, anti-inflammatory and nerve-prevention. At the same time, further pharmacological studies are needed to illustrate *H. diffuse* characteristics in vivo. The aim of this review is to provide updated and complete information on phytochemicals, pharmacology, quality control and pharmaceutical properties of *H. Diffused* for clinical use and further development.

The ethanolic extracts from *C. sabdariffa* and *C. Caudatus* were evaluated the free radical scavenger activity in the saccharomyces yeast model system by Pattnaik *et al.*, (2017). The DPPH assay was used and *C. sabdariffa* and *C. Caudatus* showed a huge scavenging potential with 184.88 and 305.39 $\mu\text{g/ml}$ IC_{50} value respectively, at a 500 $\mu\text{g/ml}$ concentration. The significant results for hydroxyl roots and total oxidative activity were also observed in ethanolic extract of *H. sabdariffa* and *C. caudatus*. The positive control used was Ascorbic acid. So, it was suggested by these findings that *H. sabdariffa* and *C. caudatus* can be used as potent antioxidants in their applications in future treatments.

This study is also in accordance with the antioxidant activity studies of *Saussurea lappa* L. by Chang *et al.*, (2017) for its antioxidant effectiveness. The chromatic method of Folin-Ciocalteu and aluminum nitrates was used to determine the total contents of phenol and flavonoids. The phenolic content and the total fracture of the flavonoid soluble in n-butanol CB lappa Clarke S., 44.43 g of GAE / g extract were 92.15 g of the quercetin extract (QE) / gm, respectively, higher than that of another fracture solvent Soluble in *S. lappa* CB Clarke

(1000 ppm) soluble in butanol, had the strongest inhibitory potential in the 2,2-diphenyl-1-picryl hydrazyl (DPPH) and reduced capacity at 92.98% and 0.38, separately. Therefore, it was concluded that the antioxidant stress can be prevented by using the *S. lappa*.

The similar studies were also conducted for the Antioxidants and antibacterial properties of various extracts of *Achillea millefolium* seeds, silica plankton and plants were evaluated by Sarker *et al.*, (2017). DCM extracts of the three strains were active against *Staphylococcus aureus* (MRSA) and Citrobacter resistance to *Methundylene freundii* (MIC = 6.25×10^{-1} mg/ml). While extracts of *A. millefolium* MeOH and *P. pratense* were active against *C. freundii*, *P. pratense* was also active against MRSA. A significant antioxidant values of (IC₅₀ = 145 gm/ml) were detected.

The Masoko and Eloff (2017) also observed similar findings for the dry leaves of the species of *Terminalia combretum* (Combretaceae) for its antioxidant potential from different extracts with acetone, dichloro-Methanol and hexane. The antioxidant activity was shown by all types of Terminalia extract from acetone and Methanol. The total antioxidant activity in the extracts from Combretum and Terminalia was successfully assessed using DPPH and TLC in this study.

The antioxidant properties of the high-density *Podandilo hexandrum* extracts were studied for similar results by Chawla *et al.*, (2017). The spectrophotometer was used to measure the Total polyphenol content (mg% quercetin). The European Commission offered supreme protection of plasmid DNA (pBR322) in a plasmid relaxation test (68.09% retention form of SC). In addition, the maximum metal removal activity (41.59%) of the metal was demonstrated and was evaluated by the 2,2'-bisperidol tests, followed by EA (32.25%), which showed the extreme potential antioxidants (unit value less of absorption: 0.0389 +/- 0.00717)

in the energy reduction test. These findings showed that the oxidative stress caused by radiation can be effectively controlled by hydrolysis (AE) and hydrolysis (HA) fractions.

This study is also in accordance with the previous study where an eye plant (*Euphrasia brevipila*) (Scrophulariaceae family) was studied by Petrichenko *et al.*, (2017) for its biologically active substances, are isolated by chromatography on a polyamide absorbent including polysaccharides, flavonoids and phenolic acids. The following compounds were identified: chlorogenic acids, aucubin, ferric and caffeine, epigenine, diosmetin, Cynaroside and Tiolin. The automatic lanolin oxidation model at 70°C compared with butyl acetate was used to measure the antioxidant activity of isolated biologically active substances.

The Khan *et al.*, (2017) also observed the similar findings for the Methanol extracts from *Morus alba* L. were studied for their potential antioxidant activities and phenol contents using the standard spectrophotometer method. Antioxidant potentials of extracts were determined by the total antioxidant capacity, DPPH (-2-Pycryl hydrazine 1,1-dynyl) analysis of the removal of root fractures of hydroxyl radicals, antioxidant capacity and methods of testing of lipid oxidative inhibitors. The results showed a high correlation and regression of ($p < 0.001$) between antioxidant potentials and phenolic content in the extracts, but additional research is needed for real use in both modern and traditional pharmacological systems.

The similar studies were also conducted for the antioxidant potentials of herbal extract of *Melia akilia* (YE) was evaluated by Trumbeckaite *et al.*, (2017) through the use of various biological and chemical assays. Quantitative and qualitative analysis of some important phenolic phenotypes was performed by HPLC. It was shown by HPLC-DPPH online analysis that YE have important antibiotic activity due to the phenolic compound's presence acting as active components. So, it was shown by these results that YE fluorimetric

concentrations had no effect on the 3-state respiration rate resulting in H₂O₂ production reduction in mitochondria.

The Khodabande *et al.*, (2017) also observed the similar findings where the *Chelidonium majus*, Papaveraceae family plant was observed for its potential antioxidant properties during the different phenological stages using FRAP and the 1,1-diphenyl-2-picrylhydrazyl (DPPH) antioxidant assay. The total phenols, flavonoids, anthocyanins and carotenoids were studied as the factors affecting antioxidant activities. The maximum DPPH anti-radical assay activity observed was 408/88 ± 24/83 g/g DW during growth stage and FRAP reached its peak of (1.75 ± 0.04 mg / g FW) during the fruiting phase. However, in the vegetative phase the highest value of carotenoids (2.083 mg/g DW) and proteins (0.27 ± 0.034 mg/g DW).

This study is also in accordance with the ethanol extracts of *Psoralea corylifolia* seeds was studied for detection of their phytochemical properties observed by Nabi and Mukta, 2017. To determine the antioxidant activity 1,1-diphenyl-2-picrylhydrazyl (DPPH) (DPPH) assay and superoxide radical assay were used. The stronger antioxidant activity was shown by the extracts with a lower value of IC₅₀ for DPPH and the elimination of superoxide. The value of IC₅₀ for DPPH and removal of phosphorus oxide was 166.61 mg/ml and 177.69 mg/ml, respectively. The strongest antioxidant activity of ethanolic extract was observed to be due to presence of flavonoids and phenols.

The similar studies were conducted by Zhao *et al.*, (2018) where three different types of buckwheat Spp. like *Fagopyrum cymosum*, *Fagopyrum tataricum* and *Fagopyrum esculentum* were tested for their antioxidant and antimicrobial potentials of volatile oils (VOs) extracts from their flowers. A remarkable antioxidant capacity of IC₅₀= 353.15 mg /ml, 264.92 gm /ml and 210.63 gm /ml from the 1,1-diphenyl 2-picryl hydrazil (DPPH)

measured as 174.13 g/ml, 243.16 gm/ml and 216.11 mg/ml, respectively was also shown by the VOs extracts from *F. cymosum*, *F. esculentum* and *F. tataricum* flowers, when β -carotene-linoleic bleaching method was applied. Thus, the finding showed that the buckwheat flowers Vos extracts can be effectively used as natural antioxidants and antimicrobial agents.

The Zeliha *et al.*, (2018) also conducted the similar studies for the *in-vivo* antioxidant properties for ethanolic extracts of some plants containing alantoin were studied. Samples were tested containing the anthuladas. *Plantago lansulata*, *Plantago major*, *Rubinia pseudoacacia*, *Platanus orientalis* and *Isculus hypocastanum* were verified at various concentrations. The *R. pseudoacacia* plant showed the lowest antioxidant activity. In conclusion, the antioxidant properties were possessed by allantoin with a positive effect on total antioxidant capacities.

The anti-cancer polysaccharides (CPS) of *Cuscuta chinensis* seeds were studied in ECPS and CPS activity in B16F10 skin cancer cells. Antioxidant activity was assessed in vitro through its ability to reduce iron and activities to eliminate free radicals DPPH. The anti-cancer activity by regulating the expression of tyrosinase was demonstrated by ECPS, where the TRP-1 and MITF without cytotoxic effects on B16F10 skin cancer cells. So, it was suggested that ECPS has the antioxidant potential and can be efficiently used in cancer treatment (Liu *et al.*, 2018).

This study is also in accordance with the lipid oxidation and antioxidant activity of the *Rheum officinale* extract was studied by Chang and Choon (2018) to assess its potential as a new treatment of natural antioxidant novel and functional component in dietary and pharmacological formulations. The results of the analysis of the active substances of thio-bituric acid showed that the rheumatoid extract of rheumatism significantly delayed lipid

oxidation. It was suggested by the results that *R. officinale* extract has an excellent natural antioxidant property.

The antioxidant activity of *S. glabra* phenol (PEESG) extracts in 70% ethanol were observed by Lu *et al.*, (2018) in HP-20 color column analysis. Its antioxidant and anti-inflammatory activities were assessed by antibacterial analysis, which reduces the determination of the strength and analysis of RAW264.7 cells caused by lipid sugars (LPS), respectively. These conclusions strappingly recommended the potential of PEESG as a natural antioxidant and anti-inflammatory source.

The free radical scavenging potentials of *Emilia Sanchevolia* were tested for n-hexane extracts of the entire plant by Sophia *et al.*, (2018) using different assays in the laboratory, such as the DPPH antioxidant assay, for feroxide root activity and hydrogen peroxide removal activity with the values of 180 ug/ml, 160 and 160 IC₅₀, respectively. The antioxidant activity may be because of the presence of terpenoids found in *E. sanchevolia* n-hexane extracts which were analyzed through High-grade chromatography (HPTLC) method. Therefore, *E. sancheviola* n-hexane extracts can be used as a dynamic lively source of natural antioxidants.

The similar studies were also conducted for the study is also in accordance with the antioxidant and antimicrobial activities of various solvents extracted from *Vernonia cinerea* were observed by Sonibare *et al.*, (2018). The antioxidant properties of the *V. cinerea* fractions were assessed by identification of antibacterial activity, total content of measured flavonoids and phenol contents tested 2,2-diphenyl-1-bicrylhydrazyl (DPPH), aluminum chloride and feline-cyclato, respectively. The results indicated that *V. cinerea* fractures have antioxidant and anti-microbial activity, which justifies the common use of plants to treat various diseases in traditional medicine.

The flavonoids were extracted from the Methanolic extracts of *L. (heartsease)* by Vukics *et al.*, (2018) and similar results were found by using column chromatography Sephadex LH-20. Nuclear fuillant in (6-C-8-C-glucosyl-ramnosilapigenin) was used for the identification of the main components of this plant. The in vitro 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay and Trolox (TEAC) capacity assay were used to investigate the antioxidant potentials of various flavonoids. The highest donor capacity was found on the electron for the main component of flavonoids (rutin), while less of the component-rich flavonoids showed higher donor activity in hydrogen.

The similar findings were also observed for the *Nepta cataria* extracts and its essential oils were studied by Adiguzel *et al.*, (2019) because of its laboratory potential of antimicrobial and antioxidant activities. The antioxidant activity of these samples was also measured using DPPH assay by exposing to a selection of beta-carotene / linoleic acid assays. Both aromatic oil extracts performed a weak bonding activity to inhibit linoleic acid oxidation and 27.0% respectively, 16.4%, in the latter case. The antioxidant capacity of BHT was determined in parallel experiments in both systems.

The similar findings were also observed for antioxidant properties in *Lonicera japonica* Thunb by Lee *et al.*, (2019). Total phenol, phenolic acids (chlorogenic acid, caffeine acid, decalfinic acid 4.5 and 3.5 dicafilquinic) and flavonoids (quercetin, rutin and luteonin) increased significantly after heat treatment. Antioxidant activity such as scavenging activity of DPPH radical scavenging ABTS, FRAP and reduce energy, improved LJ roots after heating. Antioxidant activity was positively associated with total phenol content, caffeine, total flavonoids, quercetin and chlorogenic acid. Thus, these results are in accordance with this present study.

The Sekiou *et al.*, 2019 studied the *Artemisia alba* herba (AHA) for its potential antioxidant activity of its extracts by using the self-protective effect of powder against oxidation induced by alkanes in diabetic mice. The four groups were designed for mice random division: the first group receiving control of a saline solution of 9%, the second group 150 mg of alloxan was used to treat with administered peritoneal. These results indicated that AHA improves oxidative damages, hyperlipidemia and hyperglycemia in alloxan-induced diabetes in mice.

The Hevesi *et al.*, (2019) also observed similar findings for all *Epilobium* species contain a high percentage of polyphenols and macrocyclic tannins, primarily oenothain B responsible for antioxidant potentials. Meritetin, kercetin, kimpferol and in the samples many glycosides were higher, but with distinct combination and proportion. The high antioxidant activity was shown by *Epilobium* extracts with high radical-scavenger activity, with the Trolox and ascorbic acid. The highest antioxidant capacity of (EC_{50}) = 1.71 +/-0.05 $\mu\text{g/ml}$) was shown by *Epilobium parviflora* extract among all the examined species.

The Lee *et al.*, (2019) conducted similar studies for the Antioxidant properties in *Lonicera japonica* The Total phenol, phenolic acids (chlorogenic acid, caffeine acid, decalfinic acid 4,5 and 3.5 dicafilquinic) and flavonoids (quercetin, rutin and luteonin) increased significantly after heat treatment. Antioxidant activity such as scavenging activity of DPPH radical scavenging ABTS, FRAP and reduce energy, improved LJ roots after heating. Antioxidant activity was positively associated with total phenol content, caffeine, total flavonoids, quercetin and chlorogenic acid.

The *Epimedium brevicomum* was also evaluated by Mihailovic *et al.*, (2019) similar findings for its total phenol content, antioxidant capacity of flavonoids, free radicals and potential effects of high blood pressure were studied for the water extract. The total phenol

contents in TPC were measured as 2008.33 ± 10.6 mg / L from GAE, and the predominant phenolic compounds were rosmarinic and caffeic acids. Detection of antioxidant/iron strength reduction was carried out along with the analysis of antioxidant capacity for strong antioxidant properties of TE. The absorption activity of nitrogen oxide in vitro was 1 mg /L TE 63.43% showing an IC_{50} value of 122.36 μ g/ml. It was noticed that in all experimental mice, after treatment with TE the heart index was the same. Thus, these results are in accordance with this present study.

The similar findings were also observed for the anti-radical properties of *Taraxacum officinale* leaves extracts were investigated by Michal *et al.*, (2019) compared to extracts of flowers. Flavonoids (mainly derived from luteolin) and phenolic acids, predominant between the specified polyphenols. The non-flammability is an advantage over traditional organic solvents. In addition, the low concentrations of surface-active materials can also be used.

The similar findings were also observed by Jia *et al.*, (2019) to assess its potential antioxidant activity and anticorrosive fractions of purified polysaccharides. The results showed that the optimum extraction was as follows: ultrasonic exposure time, 21 minutes; water / material ratio, 46 ml/g; ultrasonic temperature 63 °C extraction, in these conditions the maximum efficiency was 16.95% PRM -0.08%. Moreover, abundant main monosaccharides of the purified fractions were also found. PRM3 and PRM5 display absorb the large DPPH radical and reduce the energy in the laboratory activities. So, a strong inhibition activity on MCF-7 cell growth in vitro was shown by the PRM3 in the plant extracts.

The similar study was also observed for the plant extracts of *Actaea racemosa* L. and *Actaea cordifolia* were analyzed by Szymczak *et al.*, (2019) for isoflavones, phenolic acids, caffeinated acid (CA), folic acid and isofirolic acid (IFA), both free and mixed with Actaea,

were performed using HPLC-PAD. The top CA content was found in *A. racemosa*, while *Actaea cordifolia* contains a large amount of Haifa. Isoflavones were not found in the plants that were examined. The antioxidant activity test showed free high-volume removal extracts obtained from different parts of plant roots.

The antioxidant potential was observed in *Eclipta prostrata* L. traditionally used by Gani and Nalini, (2019) as well as used in the treatment of liver diseases and is also used as a tonic of the liver. The herb contains many phytochemicals that are characterized by their distinctive properties as antioxidant, anti-viral, anti-bacterial and anti-bacterial. The study confirmed that the extract of *E. prostrata* L has a good antioxidant property, which was assessed using the methods of 2-diphenyl-1-picrylhydrazyl (DPPH).

The similar studies were also conducted for the aqueous extracts of *Lavandula angustifolia*, *Foeniculum Agastache*, *Melissa officinalis* and *Nepeta cataria* were identified by Duda *et al.*, (2019) for its potential antioxidant properties and polyphenol content. The potential use of these plants as value-added products because of their content of phenolic acids and flavonoids with antioxidant activity were highlighted by biomarkers of biologically active compounds.

The similar findings were also observed for the selected poppy seed material (*Papaver somniferum* L.) was studied by Kroslak *et al.*, (2019) to evaluate metabolites, antioxidant and protein-in-vitro activities were analyzed in seed extracts of fifteen genetic genotypes. Significant variation in all parameters was detected within a range of poppy genotypes analyzed. The main genotype expresses the highest antioxidant activity determined by the four methodological methods (DPPH, ABTS, FRAP, RP). Poppy seeds are a valuable source of natural compounds that can be useful in conditions associated with oxidative stress or increased protein activity.

The similar studies were also conducted for the *Rosmarinus officinalis* L. was studied for the chemical composition of isolated essential oils by Raskovic *et al.*, (2019) using spectroscopy and gas chromatography. The oil was identified in this study, the chief components were 1.9 sinol (42.77%), camphor (13.53%) and α -benin (12.51%). So, these findings suggested that essential oil of rosemary, besides exposing the activity of free radical removal determined by the DPPH test, also halves its hepatic effects by activating physiological defense mechanisms.

Antioxidant activity and the protective effect of asymlotic roots of racemosus against apoptosis induced by Lipofectamine were studied by Lalana *et al.*, (2020). Five parts of subsequent extraction, ranging from non-polar solvents to polar solvents, were obtained. It was found that the antioxidant activity measured using the DPPH method in terms of the average active concentration (EC₅₀) for the water fracture was 600 mg / ml for ascorbic acid / ml 1.5. It was suggested that root extracts of asparagus from sweat inhibited the effectiveness of apoptosis induced by fat due to its protective effect and may be a viable alternative to gene release.

This study is also in accordance with the *Poppy somniferum* was investigated by Sharopov *et al.*, (2020) for the formation of alkaloids and biological activity of several parts of plants (stems, leaves, flowers, roots, capsules and seeds) in a variety of *P. somniferum* decorations. Antioxidant values were considered as IC₅₀ values ranging from DPPH= 35.1 to 157.6 μ g/ml for roots and for root removal of ABTS⁺= 138.5-306.3 mg/ml. The range for Ferric values in FRAP for the antioxidant capacity was 59.75 to 1348.71 mm. A significant cellular toxicity against most cancer cell lines was shown by the Methanol extract.

The aqueous extracts obtained from *Thymus serpyllum* L. were evaluated by Stanojevic *et al.*, (2020), due to the antioxidant potentials, total phenol and flavonoids

contents, free-radical scavenger activity and the potential effect on high blood pressure of the Lamiaceae herb (currently present in the Mediterranean diet), contains a family in the high-pressure automatic rats (SHR) and in Wistar normotensive mice. After treatment with TE in all experimental mice, the heart index was unchanged. No significant activity in the uptake of nitric oxide in vivo was shown by the dose given by TE. SO, it was suggested that the hypertension in a pilot model of primary hypertension may be protected by using TE.

The similar studies were also conducted for the antioxidant potentials of Methanolic extracts of oregano and sage samples were tested Pizzale *et al.*, (2020). The *Origanum onites* and *O. indercedens* species samples were oregano samples, whereas *Salvia officinalis* and *Salvia fruticosa* species samples were called sage samples. The crocin test had a higher antioxidant activity evaluated for samples of *S. fruticosa*. The higher antioxidant activities were shown by sage as compared to the oregano samples.

The potential antioxidant properties of *Polygonum cuspidatum* were tested by Hsu *et al.*, (2020) for in this study. The dried *P. cuspidatum* roots were extracted with ethanol and the extract was dried. The free radical survey showed the results IC₅₀ value, 110 mg/ml of extracts, 3.2 µg / ml in the phosphate biodegradation test and in the lipid peroxide test 8µg/ml, respectively. The extract possessed 641.1 ± 42.6 mg/g and 62.3 ± 6.0 mg/g of total phenol and flavonoid content. The higher antioxidant properties were suggested in *P. cuspidatum* extracts.

The present study is in accordance with study conducted by (Ilyasov *et al.*, 2020) to 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS') radical cation-based assays are among the most abundant antioxidant capacity assays, together with the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-based assays according to the Scopus citation rates. The main

objective of this review was to elucidate the reaction pathways that underlie the ABTS/potassium persulfate decolorization assay of antioxidant capacity. ABTS-based assays can still be recommended with certain reservations, particularly for tracking changes in the same antioxidant system during storage and processing.

The similar studies were also conducted for the evaluation of the antioxidant effect of *Solanum nigrum* L. (SN) leaves was done by Campisi *et al.*, (2021), mainly used to prepare soup in different parts of the world. The inhibition of excessive glutamate poisoning and increasing uptake of glutamate was observed in extracts, which indicated significant antioxidant properties by preventing the radical induced cell damage.

This study is also in accordance with the Plant extracts from *Scutellaria baicalensis* were evaluated by Wozniak *et al.*, (2021) of the antioxidant potentials of the four chief flavones: wogonin, pycaline, glucuronides - baicalin and wogonoside. Three in vitro assays were used, the elimination of free radical radicals using 1,1-diphenyl-2-picrylhydrazyl (DPPH), which reduces the ions of transition ions by testing phospholipidene and inhibition of peroxide caused by the hydroxyl radical linoleic acid test. The oxidation of linoleic acid can be protected by Wogonin. The wogonosida did not have any antioxidant activity whereas the Baikalin showed less potent antioxidant properties.

The similar studies were also conducted for the potential antioxidant activity and the total content of specific phytochemical and phenolic chemicals (alkaloids and andrographolides) from *Andrographis paniculata* were tested by Kurzawa *et al.*, (2021). The observed total phenolics were (GAE 175.13-1723.79mg/100g) and the content of andrographolides were measured (19.44-85.13mg/g) which were found in correlation in the samples studied with an antioxidant activity determined by CUPRAC, FRAP and DPPH ($r > 0.95$, $p < 0.05$ level).

The antioxidant activities of aqueous root extracts of *P. multifida* Poiret (EMPA) were studied by Kuang-ping *et al.*, (2021). The high antioxidant activity was shown by AEPM with conjugated din method. The EMPA showed high removal effects at 20 mg/ml, with α - β -diphenyl picrylhydrazyl, hydroxyl, iron radical (81.5, 80.2 and 85.3, respectively) and reduced capacity (Abs 700 nm=1.03). It was observed that various antioxidant components were found in AEPM naturally, resulting in higher antioxidant activities in this plant.

The Fathi *et al.*, (2021) observed the similar findings for the antioxidant properties of the essential oils of *Satureja hortensis* L. (SHEO) were observed using oxidation of safflower oil. Various assays like total phenol content (TPC), DPPH, ABTS +, FTC, carotene whitening was used to evaluate the antioxidant activities of these extracts. So, it was observed that this plant is an efficient source of antioxidants and can be effectively used in oxidative stress related disorders.

Thus, these all-previous studies also confirm that these tested medicinal plants are very rich in their antioxidant potentials and phytotoxic properties, also very rich in Total phenols and Flavonoids contents and so can be effectively used in further studies to develop efficient protocols in medicinal and pharmaceutical products to treat different human lethal diseases.

CHAPTER 6

6. CONCLUSIONS AND RECOMMENDATIONS

In this research the composition and antioxidant potentials of about 200 medicinal plants were analyzed by using various assays. The medicinal plants investigated in this experiment along with their used parts and traditional medicinal uses. The free radical scavenging potential observed in these medicinal plant samples was because of the presence of some natural source such as phenol, flavonoid or tannin contents. In this experiment the free radical scavenging activity (RSA) of various medicinal plant samples was measured using different extracts depending upon the ability to eliminate the free radicals using synthetic DPPH. The reactivity of different compounds with the stable free radicals was because of the odd number of electrons present in them. The results showed that ethanolic extracts of different medicinal plant samples have the higher level of free radical scavenging or antioxidant activity, followed by Methanolic extracts comparing to the $IC_{50} = 37.337 \mu\text{g/ml}$ value of the ascorbic acid (standard). The highest absorbance was observed in the *Euphrasiae stricta* ($IC_{50} = 38.972 \mu\text{g/ml}$), *Euphorbia platyphyllos* L. ($IC_{50} = 40.817 \mu\text{g/ml}$) and *Epimedium brevicomum* Maxim ($IC_{50} = 46.265 \mu\text{g/ml}$), medicinal plants for both of their ethanolic and Methanolic extracts.

The total antioxidant potential of these medicinal plants was because of high amount of polyphenol and other phytochemical components found in them. These finding also indicated that all tested medicinal plants samples likely to possess significant level of free radical scavenging activity although comparatively less than standard ascorbic acid. So, this research suggested that all medicinal plants and particularly *Euphrasiae stricta*, *Euphorbia*

platyphyllos L. and *Epimedium brevicomum* Maxim possess a significant antioxidant potential and can be efficiently applied as an important antioxidant source for the treatment and inhibition of widely spreading oxidative stress related degenerative diseases like cancer, cardiovascular & inflammatory joint disorders, atherosclerosis, dementia, diabetes, asthma and eyes related degenerative diseases etc.

This study showed that ethanolic extracts of *Euphrasiae stricta* plant possessed the highest radical scavenging potential followed by the *Euphorbia platyphyllos* L. and *Epimedium brevicomum* Maxim, so resulting in significant antioxidant potential for these traditionally used medicinal plants as compared to the highest antioxidant activity of standard ascorbic acid in all three assays (DPPH, ABTS and FRAP). Different levels of scavenging activity were focused for all extracts in all used assays like DPPH, ABTS and FRAP assays. It was also observed that the electron donating and/or free radical scavenging properties were the responsible for the possible antioxidant potentials of these plant extracts which has been always concentration dependent. The phenol and flavonoid contents from these plants were observed as a potential source of natural antioxidants which can be efficiently used in the inhibition of oxidative stress associated diseases. This research may also lead to additional investigation of other specific compounds in various medicinal plants and their antioxidant potentials in vivo using various antioxidant assays. Thus, from these findings it was concluded that the *E. stricta*, *E. platyphyllos*. and *E. brevicomum* medicinal plants are important source of natural antioxidant like phenols, flavonoids and tannins etc. and can be efficiently used in treatment of various oxidative stress related diseases, most importantly cardiovascular disorders and cancer.

CHAPTER 7

7. REFERENCES

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