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Determination of Phytochemicals and Pharmacological Activities of *Viola canescens*, *Equisetum debile* and *Zanthoxylum armatum*

by

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Thesis is dedicated to Allah Almighty, Hazrat Muhammad (SAW) and to my great husband, my beloved family, adorable friends, teachers and all those students and staff members who have supports me since the beginning of this thesis.

CERTIFICATE OF APPROVAL

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Abstract

Nature offers a treasure trove of potential therapeutic agents, exemplified by *Viola canescens*, *Zanthoxylum armatum*, and *Equisetum debile*. These traditionally used medicinal plants hold immense promise for human health due to their diverse bioactive compounds. Research is increasingly revealing the scientific basis for their historical applications in treating a wide range of ailments, including headaches, fevers, asthma, migraines, stomach problems, respiratory issues, kidney disorders and cardiovascular diseases. The presence of various active compounds within these plants contributes to their broad biological properties, making them prime candidates for further scientific exploration and potential drug discovery efforts.

Different bioassays were performed to analyse the pharmacological efficiencies and phytochemical properties of these plants. In phytochemical analysis I determine total phenolic and flavonoid content in *Viola canescens*, *Xylum armatum* and *Equisetum debile*. It was observed that all three plant extracts contain phenols and flavonoids in them but *Viola canescens* has highest content of these phytochemicals with 19.68± 0.27mg GAE/g flavonoid and 111.18 ± 2.06 mg GAE/g phenolic content.While in pharmacological activities like (antibacterial and antifungal), anticoagulant and antioxidant result was quite different. It was observed that all of these extracts *Viola canescens*, *Xylum armatum* and *Equisetum debile* showed no significant antibacterial activity against *Staphylococcus aureus* and *Klebsiella pneumoniae*. In antifungal activity, no zone was observed against Aspergillus niger. The highest antioxidant capacity measured by using DPPH free radical scavenging assay at 1,5 and 50 μg/mL concentrations. It was observed *Zanthoxylum armatum* has highest antioxidant capacity4.35± 0.04 followed by Viola canescens 5.03 ± 0.14. It means both have high antioxidant property due to high phenolic and flavonoid content. Antioxidant capacity increases with the increase of concentration. Forth pharmacological activity performed in this study was anticoagulant activity. *Viola canescens* shows the best clotting time nearly equal to positive control while the samples *Xylum armatum* and *Equisetum debile*

appear to clot much faster than the positive control, indicating potentially abnormal clotting activity. FTIR spectroscopy is a powerful and versatile tool that has revolutionized the field of chemical analysis. Its ability to provide rapid, accurate, and reliable results has made it an indispensable asset for scientists, researchers, and industries alike.

Various plant species possess recognized antioxidant properties, my study positions *Viola canescens* as a particularly promising candidate for natural bioactive molecule discovery. This research highlights *V. canescens*' potential to not only contribute to human health through the development of therapeutic drugs but also to promote biovalorization and environmental well-being. Biovalorization refers to converting underutilized resources into valuable products, and sustainable utilization of *V. canescens* could provide a valuable source for drug development without harming the environment. Further research on *V. canescens*' specific bioactive compounds and their mechanisms of action is crucial to unlocking its full therapeutic potential within this framework of environmental responsibility

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Abbreviations

ED	Equisetum debile
MP	Medicinal plants
TFC	Total Flavonoid content
TM	Traditional medicines
TPL	Total Phenolic content
VC	Viola canescens
ZA	Zanthxylum armatum

Chapter 1

Introduction

1.1 Background

A wide range of plants with therapeutic qualities are referred to as medicinal plants. Compounds that can be utilized to create drugs are abundant in these plants [1]. Several varieties of seeds, roots, leaves, fruits, skins, flowers, and even the entire plant are among the parts of medicinal plants that can be utilized. The majority of medicinal plants' active ingredients are employed as medical agents because they have either direct or indirect therapeutic effects. Certain chemicals, known as active compounds, are created and stored in the bodies of these plants and have physiological effects on living things [2]

Plants are important, as we all know. The kingdom of plants is a treasure trove of possible medications, and the value of medicinal plants has gained attention in recent years. Plant-based medications are widely accessible, reasonably priced, effective, safe, and seldom cause adverse effects. The most obvious alternative for analyzing the current quest for therapeutically effective novel medications, such as anticancer drugs [3], antibacterial drugs [4], and antihepatotoxic chemicals, is to look at the plants that have been selected for medical usage over thousands of years.

Nature is usually a brilliant indicator of the common occurrence of coexistence. The foundation for treating human illnesses is natural items derived from plants,

animals, and minerals[5]. There is currently a demand for medicinal herbs, and there is a gradual increase in their acceptance. Plants are vital because they sustain ecosystems by delivering necessary services. In any case, herbals, particularly therapeutic herbs, have consistently served as a general indicator of the health of the ecosystem[6]

Without a doubt, people have thought about medicinal herbs since ancient times. One could argue that before recorded history began, early humans became somewhat aware of the characteristics of the plants they encountered and used for food, clothing, shelter, and fuel. China, Greece, Egypt, and India are among the nations where the oldest sciences have incorporated the study of medicinal plants. Plants were widely employed as medicines, cleansers, and fragrant agents in ancient Persia[7].

The World Health Organization (WHO) states that the greatest place to find a wide range of medications is from medicinal plants. Approximately 80% of people in affluent nations take traditional medicines, which are made of substances derived from medicinal plants. [8]. Certain organic components found in medicinal plants have specific physiological effects on humans. These compounds are known as bioactive substances and include tannins, alkaloids, carbohydrates, terpenoids, steroids, and flavonoids [9, 10].

Living things produce these substances through their primary or more accurately secondary metabolism. Secondary metabolites are incredibly diverse substances with unknown functions in terms of chemistry and taxonomy. They are extensively employed in numerous fields, including scientific research, veterinary medicine, agriculture, and human therapy [11].It has been demonstrated that a wide variety of phytochemicals from various chemical classes have inhibitory effects on microbes of all kinds in vitro [12].

Phytomedicines have included plant-based ingredients since the beginning of time. Barks, leaves, flowers, roots, fruits, and seeds can all be used to make this [13]. Understanding the chemical components of plants is important since it will help with the production of complicated chemicals [14–16].Humans mostly rely on unprocessed plant materials to supply their medical demands and treat illnesses [17].

1.2 History of Medicinal plants

It's really tough to pinpoint the precise moment to use plants as a medication. There is proof that plants were initially grown for their medicinal properties some 60,000 years ago [18]. Nearly 5000 years ago in Egypt, China, and India, and not less than 2500 years ago in Greece and Central Asia, are the earliest known records on medicinal herbs [19]. People have used natural remedies to treat their own illnesses since ancient times. Similar to how using animals was once instinctual, using plants was also instinctual[20].

First people to use plants as medicine were the Egyptians and the Chinese, who did so for about 27 centuries BC [21]. Certain medicinal plants had therapeutic qualities that the ancient Greeks were well aware of, and Hippocrates, the father of Greek medicine, and his student Aristotle employed these plants to cure illnesses.

Following that, Greek scientist Theophrastus established the School of Medicinal Plants. Then, in the form of a series of scientific research on medicinal plants, Pedanius Dioscorides (who lived in the first century A.D.) published an encyclopedia called De Materia Medica to describe 600 therapeutic medicinal plants (he practiced medicine and surgery from 75 to 45 BC) [22–24].

1.3 Use of Medicinal Plants in World

In Asia, Africa, and Latin America, many traditional drug kinds are frequently utilized to treat fundamental medical requirements. This, sometimes known as supplementary or alternative medicine, is being used more and more in developed nations. Complementary and alternative medicine (CAM) is the term used by the National Institutes of Health (NIH) in the United States to describe health systems, practices, and products that are currently not regarded as a part of mainstream medicine. Traditional Chinese medicine (TCM) is currently the most widely used traditional medical system worldwide, followed by Indian medicine. While "Asian medicine" frequently encompasses TCM, India (Ayurveda), and

Tibetan medicine, in Western countries "Oriental medicine" refers to Chinese, Japanese, and Korean remedies chosen by immigrants from Korea [25].

1.4 Medicinal Plants in Pakistan

Pakistan is a country with an area of 80,943 km, located between $60^o55'$ to $75^o30'$ E longitude and $23^o45'$ to $36^o50'$ N latitude [26]. It boasts a varied climate with height variations of 0 to 8611 m and an abundant variety of medicinal plants. 6000 species of higher plants are found in Pakistan, of which 600–700 are used for health purposes [27].

Of these 6000 species, 3000 have been found to be from Northern areas, of which 124 have therapeutic significance [28, 29]. 4940 flowering plants are native to Pakistan. Regretfully, just 10% of Pakistan's plant species have been found to provide therapeutic benefits [30].

Traditional medicine is practiced widely in Pakistan and is now unquestionably ingrained in the country's cultural legacy [31]. More than 84% of Pakistan's population addressed the majority of health-related problems using traditional indigenous methods in the early 1950s, but now days, this practice is only used in the nation's more isolated regions [32, 33].

The similar percentage (84%) of Pakistani people were said to rely on traditional medicinal plants in 1958 by Hocking to treat various illnesses. According to reports, 63% of Pakistani citizens, particularly those living in countryside, used herbal remedies that were provided by traditional doctors in 1983 [34].

1.5 Reported Biological Activities

It has been observed that compounds obtained from medicinal plants display a range of biological activities, including antioxidant, antibacterial, and anti-inflammatory properties [35]. Antimicrobial chemicals derived from medicinal herbs have the potential to impede the spread of bacteria, fungus, viruses, and

protozoa through distinct processes compared to currently available antimicrobials. Antimicrobial chemicals derived from medicinal herbs have the potential to impede the spread of bacteria, fungus, viruses, and protozoa through distinct processes compared to currently available antimicrobials. This makes them potentially valuable in the clinical treatment of resistant microbial strains [36].

Numerous substances, including *spermidine, rutin, quercetin, tocopherol* and *carotenoids* have antibacterial, antioxidative, anti-inflammatory, and antiviral properties, were shown to be present in phytochemical studies [37].

Foods and medicinal plants are rich sources of natural antioxidants. Numerous biological effects, such as anti-inflammatory, anti-aging, anti-atherosclerosis, and anti-cancer properties, are exhibited by these naturally occurring antioxidants, particularly those found in phenols and flavonoids.

To investigate prospective sources of antioxidants and encourage their use in functional meals, medications, and food additives, it is essential to extract and evaluate antioxidants from food and medicinal plants in an efficient and correct manner[38].

1.6 Phytochemicals

Carotenoids, polyphenols, isoprenoids, phytosterols, saponins, dietary fibers, and certain *polysaccharides* are examples of phytochemicals, which are plant-based bioactive compounds produced by plants for their security.

Phytochemicals can be obtained from a variety of sources, including whole grains, fruits, vegetables, nuts, and herbs, and over a thousand of them have been discovered to date[39, 40].

Essential phytochemicals, a range of primary and secondary plant metabolites with known biological activities and effects such as anti-hyperglycemic, anti-inflammatory, anti-diabetic, and anti-microbial properties, are abundant in medicinal plants and herbs[41, 42]

Classification	Main groups of compounds	Biological function
NSA (Non-starch poly-saccharides.)	Cellulose, hemicellulose, gums, mucilages, pectins, lignins	Water holding capacity, delay in nutrient absorption, binding toxins and bile acids
Antibacterial & Antifungal	Terpenoids, alkaloids, phenolics	Inhibitors of micro-organisms, reduce the risk of fungal infection
Antioxidants	Polyphenolic compounds, flavonoids, carotenoids, tocopherols, ascorbic acid	Oxygen free radical quenching, inhibition of lipid peroxidation
Anticancer	Carotenoids, polyphenols, curcumine, Flavonoids	Inhibitors of tumor, inhibited development of lung cancer, anti-metastatic activity
Detoxifying Agents	Reductive acids, tocopherols, phenols, indoles, aromatic isothiocyanates, coumarins, flavones, carotenoids, retinoids, cyanates, phytosterols	Inhibitors of procarcinogen activation, inducers of drug binding of carcinogens, inhibitors of tumourogenesis
Other	Alkaloids, terpenoids, volatile flavor compounds, biogenic amines	Neuropharmacological agents, anti- oxidants, cancer chemoprevention

FIGURE 1.1: Bioactive phytochemicals in medicinal plants[43]

1.7 Plants Under Study

1.7.1 *Viola canescens*

Hailing from the majestic Himalayas, *Viola canescens*, or the Himalayan White Violet, is a captivating perennial herb [44]. This treasure thrives in the cool, damp embrace of the mountains, flourishing between 1500 and 2400 meters above sea level [47]. *Viola canescens* boasts a low-growing form, characterized by long, leafy runners that gracefully sprawl instead of a central stem [45]. Adorning the plant are delicate, heart-shaped to kidney-shaped leaves, veiled in a soft layer of hairs. During spring (March-June), the crown jewel emerges - pale violet or white flowers with a short spur, adding a touch of whimsical charm to the landscape [47].

Beyond its undeniable beauty, *Viola canescens* offers a treasure trove of medicinal benefits. Traditionally used by Himalayan communities, this plant has earned a reputation for combating various ailments [46]. The aerial parts, encompassing both flowers and leaves, are particularly prized for their cough-relieving properties. Intriguing research suggests that *Viola canescens* may also hold promise in managing colds, fevers, malaria, and even jaundice [46]. Additionally, studies have

hinted at its potential as an anticancer agent, warranting further exploration in this area.

However, the growing popularity of *Viola canescens* for medicinal purposes has resulted in its excessive harvesting. This unsustainable practice has cast a shadow over its future, with some regions listing it as endangered [47]. Implementing sustainable harvesting methods and exploring cultivation techniques are critical steps towards ensuring the continued existence of this precious Himalayan gem.

1.7.2 *Equisetum debile*

Equisitem debile, a captivating plant with a branched structure, is a member of the Equisetaceae family, commonly referred to as horsetails. Previously classified as a distinct species, its taxonomic status is undergoing revision, with some recognizing it as a variety of Equisitem ramosissimum. This intriguing plant thrives in tropical Asia and parts of China, showcasing a remarkable ability to adapt to a wider range of habitats compared to its moisture-loving relatives. Its defining characteristic is the segmented, hollow, and branched green stem, reminiscent of a horse's tail – a signature feature of the Equisetaceae family [48]. Unlike typical plants, Equisitem debile lacks true leaves, instead sporting whorls of tiny, scale-like appendages at each joint on its stem [49].

Despite its seemingly simple appearance, Equisitem debile holds potential for exciting scientific exploration. Research suggests the presence of various bioactive compounds within the plant, such as silica and flavonoids. Silica, responsible for the plant's rigidity, could offer avenues for biomimetic research, a field that investigates nature's designs to create innovative materials. Flavonoids, known for their antioxidant properties and potential health benefits, warrant further investigation to understand their specific role within Equisitem debile[50].

The current ambiguity surrounding Equisitem debile's taxonomic classification presents a challenge. Further research is necessary to definitively determine its exact position within the Equisetaceae family. Additionally, the potential impact of habitat loss and climate change on the distribution and abundance of Equisitem

debile remains unexplored. Addressing these knowledge gaps is crucial for gaining a comprehensive understanding of this fascinating plant and ensuring its future survival [51].

1.7.3 *Zanthoxylum armatum*

Zanthoxylum armatum, a captivating shrub or small tree, graces the Himalayan region with its presence. This versatile plant is a member of the Rutaceae family, placing it in close kinship with citrus fruits like lemons and limes. *Zanthoxylum armatum* flourishes in the warm embrace of subtropical valleys, reaching heights of up to 3.5 meters and captivating onlookers with its dense foliage [52]. A defining characteristic of this plant is its spiky armor. The trunk, branches, and even young shoots are armed with prickles, a feature that has earned it the nickname "toothache tree" in some regions [53].

Despite its prickly exterior, *Zanthoxylum armatum* offers a treasure trove of uses. Himalayan communities have traditionally employed various parts of the plant for medicinal purposes for generations [52]. The fruits, seeds, and bark are particularly valued for their aromatic and stomachic properties, providing relief from digestive ailments [54].

Interestingly, the young twigs hold a dual purpose. Not only can they be used as natural toothbrushes, but their extract is also believed to be a remedy for toothaches, further solidifying the plant's association with the "toothache tree" moniker [53]. Additionally, the essential oil extracted from the fruits, known as sansho oil, boasts deodorant and antiseptic properties, adding to the plant's diverse applications [54].

The significance of *Zanthoxylum armatum* extends far beyond the realm of medicine. The unique aromatic qualities of its dried fruits have secured its place in the culinary world as a distinctive spice, particularly prized in Sichuan cuisine where it's known as Sichuan pepper[54].

Furthermore, the plant's attractive foliage and sturdy structure make it a desirable choice for ornamental gardens, lending a touch of exotic charm to landscapes.

However, the rising popularity of *Zanthoxylum armatum* for its various uses necessitates the implementation of sustainable practices to ensure its continued existence within the delicate Himalayan ecosystem.

1.8 Hypothesis, Aim and Objectives

1.8.1 Hypothesis

Extracts from the selected plants will exhibit antioxidant, antimicrobial and anticoagulant activity.

1.8.2 Aim

Determination of phytochemicals and pharmacological activities *Viola canescens, Equisetum debile* and *Zanthoxylum armatum.*

1.8.3 Objectives

1. To determine phytochemical analysis of *Viola canescens, Equisetum debile* and *Zanthoxylum armatum.*
2. To determine pharmacological activities of *Viola canescens, Equisetum debile* and *Zanthoxylum armatum.*

1.9 Scope of the Research

The demonstrably lower cost, reduced toxicity, and biocompatibility of herbal medicines compared to synthetic drugs highlight the promise of further exploring these plants as potential therapeutic agents.

Chapter 2

Literature Review

Herbal medicine is an integral element of Pakistani culture. People use botanical, herbal, and inorganic medicine as a backup and additional treatment for a number of ailments. Patients who are concerned about the negative effects of allopathic drugs can benefit from phytopharmaceuticals, which are an effective way of therapy. The other type consists of persons who, as the initial phase, seek to care personally by using natural remedies in accordance with their medical record, past therapy, and clinical episodes. Much of the population in Pakistan prefers herbal medication. Some herbal products are used because herbs frequently have essential components such as vitamins and minerals that promote vitality and keep the body in good health. As an outcome, herbal medicine is now available, which is cheaper and has less side effects such as fever. Numerous clinically proven plant based remedies are being offered as medical treatments, and certain herbal elements are used as allopathic dose forms [55].

The prevalence of indigenous communities relying on natural plant resources for both medicinal purposes and economic gain is more frequently observed in developing nations [56–58].. Around the globe, self treatment is very common, and herbal plants have played a vital role in various regions [59, 60]..Modern and conventional healthcare systems rely on medicinal plants due to the increased demand for therapeutic drugs from a natural source.

Thousands of plants are in use as far as medicinal purposes are concerned [61].. Poor economic conditions and lack of provision of proper medical facilities to the remote communities, especially the rural ones, have necessitated the use of local plants of herbal importance [62, 63].. Due to urbanization and modernization, herbal medicines have only been limited to rural communities [64]..Throughout history, the utilization of natural products and medicinal plants has been a crucial aspect in promoting healthcare and maintaining the overall health of various societies [65, 66].. Around 3/4 of the world's population is dependent on herbal methods derived from drugs used to cure and prevent various human ailments. The modern drug industry is also highly dependent on herbal plants; about 1/4 of the active ingredients in the drugs are sourced from herbal plants [67, 68].

Pakistan is a similar case; i.e., around 80% of the population is from a rural background and dependent on herbal medicines to treat various human diseases [69]. With the recent developments in the drug discovery industry, the use of plants with therapeutic importance has been enhanced due to their proven harmless nature with a more beneficial effect against various human diseases [70, 71]. In Pakistan, various researchers have investigated local plants with ethno-medicinal importance. In this regard, around 400 - 600 medicinal plants have been identified locally [72]..

Approximately 350 drugs have been extracted from 456 plant species with medicinal value and have been used to treat various human diseases [73]. It is highly pertinent that the areas where herbal medicine is practiced are rich in ethno-medicinal knowledge. For sustainable development, this wealth of knowledge related to therapeutic plants needs to be upheld for future generations. Moreover, this treasure of knowledge could be used to identify new herbal plants with ethnomedicinal importance against various human ailments and ultimately toward drug discovery [74, 75].

A total of 432 registered enterprises are producing medicinal plant items in the country. These represent the thriving demand for plant-based health goods, an increasing number of available medicinal plant arrangements, and an increasing number of the consumer base resulting from the growing view of plant products as safe

substitutes to standard pharmaceuticals.Traditional medicinal herbs are essential components of local medical systems in China and around the world.Traditional medicine is defined as any traditional and locally based health care method that differs from modern medical science and is mostly passed verbally by communities of various cultures.Traditional medicine is a centuries-old technique that has long served as a companion to humanity in the protection against ailments and living a healthy lifestyle. Local people have been employing the distinctive method of their traditional system of medicine for generations, and most popular are the Chinese, Indian, and African medical systems [76, 77].

For thousands of years, herbal treatments have been used in traditional medicine to treat a wide range of human illnesses. Various diseases continue to be a leading cause of death and disability around the world, and there is an increasing curiosity in identifying newly found compounds that can be utilized to combat these ailments. A number of research investigations have evaluated the efficacy and acceptability of traditional and herbal medicine mixes used to treat a variety of conditions, and the findings indicate that the side effects are low. Still, as plant-based and herbal therapies gain popularity in Western society, the benefits and potential hazards of these treatments must be evaluated [78].

The positive effects of using natural products with high bioactive compounds have sparked greater curiosity in the pharmaceutical industry. Plants include a wide range of phytochemicals, antioxidants, and bioactive compounds that can neutralize free radicals and so slow the progression of many chronic diseases linked to the effects of oxidative stress and reactive oxygen species.Consuming antioxidant-rich infusions or meals has been connected with favorable effects against cancer, cardiovascular illnesses, diabetes, and other disorders caused by aging. As a result, antioxidants defend against free radicals produced by the glycation of non-enzymatic proteins, which causes oxidative stress [79].

Plants have created a wide range of compounds via several biosynthetic processes. Many of them are regarded important for the everyday functioning and growth of the plant, such as carbohydrates, lipids, and proteins; these are known as primary metabolites. The metabolic pathways also produce secondary metabolites, which

are relatively tiny molecules. These secondary metabolites do not appear to be needed for plant growth. Well, technology has shown that secondary metabolites have significant roles in plants, such as protecting against UV rays being exposed, fighting illnesses induced by viruses, fungus, bacteria, and phytopathogens, and repelling herbivores. These secondary metabolites are extremely important in therapeutics since they are included in three major groups: polyphenols, terpenes, and alkaloids.Pharmacology and therapies originated with natural ingredients. Initially, they were utilized as therapeutic plants, but after that separate compounds or phytochemically described extracts [80].

Extraction of medicinal plants is the process of extracting active plant ingredients or secondary metabolites including alkaloids, flavonoids, terpenes, saponins, steroids, and glycosides from inert or inactive material using an appropriate solvent and standard separation procedure. Plant materials high in phenolic compounds and flavonoids were shown to have antioxidant effects.The main purpose of this research was to assess the phytochemical analysis and pharmacological activity of specific plants. Although extracts, bioactive fractions, or chemicals derived from medicinal plants are employed for a variety of applications, the procedures involved in their production are generally the same, regardless of the desired biological tests. The key stages of getting a quality bioactive molecule include plant choice, extraction methods, phytochemical analysis procedures, fractionation methods, and identification techniques. The specifics of these procedures, as well as the exact road map followed, are totally determined by the research design. Polar solvents, such as water and alcohols, are often utilized in the removal of medicinal plants. Generally, extraction processes involve Several chromatographic techniques are used to separate and purify phytochemical compounds [81].

Plants under study

1. *Viola canescens*
2. *Equisitem debile*
3. *Zanthoxylum armatum*

2.1 *Viola canescens*

FIGURE 2.1: *Viola canescene*

V. canescens belongs to the Violaceae family, a diverse group encompassing over 500 species worldwide. Recent scientific studies have highlighted the potential of Viola species as a valuable resource in ethnomedicine, particularly for treating non-communicable diseases (NCDs). These chronic health conditions, such as diabetes, asthma, lung disorders, and fatigue, are a growing global concern. Research suggests that various Viola species may possess properties that could be beneficial in managing these NCDs. *V. canescens* itself exhibits promise for its potential antimalarial, analgesic, and antispasmodic properties, warranting further exploration within the Violaceae family for its contributions to therapeutic development [82].

The Violaceae family, which includes *Viola canescens*, emerges as a treasure trove of potential therapeutic agents. Scientific research has increasingly revealed a remarkable array of bioactivities associated with various plants within this family. These include neuroprotective, immunomodulatory, anticancer, antihypertensive, antidyslipidemic, analgesic, antipyretic, diuretic, anti-inflammatory, anthelmintic, and antioxidant properties [82]. *Viola canescens* itself shows promise for its potential antimalarial, analgesic, and antispasmodic effects. This therapeutic potential is likely linked to the presence of a diverse range of bioactive compounds within Viola species. Recent decades have seen the isolation and identification of several

key groups, including flavonoids, terpenoids, and phenylpropanoids, each known for its unique pharmacological actions [83]. Further exploration of these chemical constituents and their mechanisms of action is crucial to unlocking the full potential of the Violaceae family for medicinal applications.

Despite a rich history of traditional use and emerging scientific interest, the therapeutic potential of the Viola genus remains largely unexplored. While ethnopharmacological practices and initial phytochemical studies suggest promise, comprehensive pharmacological and clinical studies are lacking [82]. This review aims to address this gap by consolidating current knowledge on the phytochemistry and pharmacological properties of Viola species. By highlighting the genus' beneficial potential and identifying knowledge gaps, this review seeks to pave the way for future research efforts in Viola pharmacology. Unveiling the mechanisms behind the traditional uses and exploring the bioactivities of various Viola species hold immense promise for the development of novel therapeutic agents.

2.1.1 Classification

Kingdom	Plantae
Family	Violaceae
Genus	Viola
Specie	*Viola canescens*

2.1.2 Description

Viola canescens, popularly known as hoary violet, is a herbaceous perennial endemic to East Asia. It has alternating, plain leaves and little purple blooms. It is found in arid, stony regions, usually in grasslands or on slopes.

2.1.3 Uses and Benefits

Viola canescens is an ornamental plant that has been taken to treat fevers, headaches, and other diseases. The blossoms and the entire plant are antitussive. They relieve coughs, colds, and asthma[82]

2.1.4 Flower, Seeds and Seedlings

Viola canescens produces little white blooms with five petals and golden anthers. The seeds are tiny, black, and rounded. The seedlings have one cotyledon and two tiny, pointy leaves.

Flowering Period and Habitat

Flowering period ranges from March to June and it produces beautiful, small pale violet to white flowers during this period. It requires shady place for its growth or it might require shady edges[83].

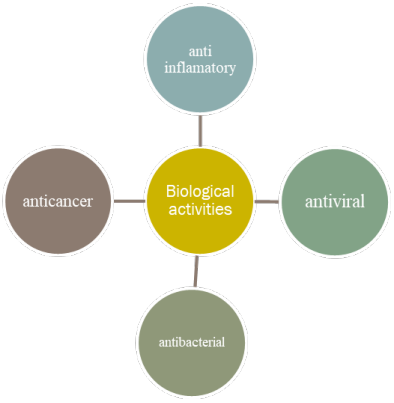

FIGURE 2.2: Biological activities of *Viola canescens*

2.1.5 Biological Activity

Viola canescens (*V. canescens*) stands out for its diverse range of potential therapeutic applications, supported by both traditional knowledge and emerging scientific research. Traditional medicine practices have long utilized *V. canescens* to treat a wide variety of ailments, including liver disorders, hypertension, eczema, malaria, rheumatism, gastric issues, diarrhea, respiratory problems, fever, epilepsy, and even cancer . Intriguingly, scientific studies have begun to shed light on the potential basis for these traditional uses. Research suggests that *V. canescens* may possess antimalarial, analgesic (pain-relieving), and antispasmodic properties, warranting further investigation to fully understand its medicinal potential [82]. This convergence of traditional wisdom and scientific exploration highlights *V. canescens* as a promising candidate for future drug discovery efforts.

2.1.6 Phytochemical Activity

Equisetum plants exhibit a remarkable array of potential therapeutic benefits due to the presence of various secondary metabolites. These specialized compounds, distinct from those directly involved in growth and primary functions, are known to possess a range of bioactivities. Research suggests that Equisetum species contain secondary metabolites with properties including anticancer, antioxidant, antibacterial, anti-inflammatory, antiviral, insecticidal, and cytotoxic effects [82]. This diverse chemical profile positions Equisetum as a genus worthy of further exploration for its potential contributions to medicine and pharmacology.

2.2 *Equisetum debile*

FIGURE 2.3: *Equisitem debile*

Equisetum, a fern ally belonging to the Equisetaceae family, stands out for its unique reproductive strategy and extensive global reach. Classified within the Equisetopsida class and Equsetales order, Equisetum species are perennial plants that reproduce via spores rather than seeds [85]. These fascinating plants boast a vast global distribution, thriving across much of the world with the exception of Australasia and Antarctica. This widespread presence highlights the genus' remarkable adaptability and potential for growth in diverse environments.

Equisetum, a genus of vascular plants with a potentially ancient lineage, has garnered significant interest for its medicinal potential. Found worldwide except for Australasia and Antarctica, Equisetum encompasses roughly 30 recognized species. These plants hold a prominent place in traditional medicine across various cultures [85, 87]. This review specifically focuses on the existing scientific literature to evaluate Equisetum species with promising pharmaceutical properties and potential therapeutic applications for kidney diseases.

Equisetum species have a long history of traditional use in various cultures, particularly as a diuretic and for treating genitourinary disorders. Our review of scientific literature revealed that traditional applications of Equisetum most commonly target these areas, including kidney disease, urethritis, kidney stones, swelling, wound healing, heart problems, urinary tract infections, and hypertension. Equisetum arvense L. emerges as the most prevalent species used medicinally within the Equisetum genus. Research, including animal studies and human trials, has provided some support for its diuretic properties. Furthermore, studies on Equisetum bogotense Kunth, both in labs and clinical settings, have shown promise for its diuretic effects [86]. These findings highlight the potential of Equisetum species for therapeutic applications, particularly in genitourinary health, and warrant further investigation to fully understand their efficacy and mechanisms of action.

The Equisetum genus presents a treasure trove of potential therapeutic applications, particularly for kidney health. While traditional medicine utilizes various Equisetum species for diverse purposes, scientific research is ongoing to explore their full potential. Studies have investigated numerous species, revealing a range of biological effects likely due to their unique chemical composition. Equisetum species are known to contain a variety of potentially active ingredients, including alkaloids, flavonoids, phenols, phytosterols, saponins, sterols, silicic acid, tannins, triterpenoids, and volatile oils [87]. However, despite this rich phytochemical profile and extensive traditional use, many Equisetum species require further rigorous scientific investigation to validate their efficacy and safety for various applications. Kidneys appear to be a particularly promising area for therapeutic development, with several Equisetum species demonstrating potential in this domain.

2.2.1 Classification

Kingdom	Plantae
Family	Equisetaceae
Genus	Equisitem
Specie	*Equisetum debile*

2.2.2 Morphology of Plant

The extract of horsetail leaves form whorls, which merge into terminal blades. The stems are typically green , and they can be distinguished as hollow, attached, and wrinkled (up to 40 ridges). The nodes may or may not have whorls of branches.

Equisetum's stem consists of two parts: a perennial underground rhizome with numerous branches and an annual aerial shoot. Branching is monopodial; shoots are divided into nodes and internodes.

2.2.3 Traditional and Local Use

Equisetum debile (*E. debile*) boasts a rich history and diverse traditional applications. Fossil records reveal Equisetum as one of the most ancient vascular plant lineages, with remarkable adaptability that allowed its spread across the northern hemisphere. This resilience is further reflected in its traditional medicinal uses among indigenous communities.

For centuries, *E. debile* decoctions have been employed by these groups to address a range of ailments, including hair loss, urinary tract infections, kidney stones, back pain, wounds, warts, bone fractures, and joint pain [87].

This long history of use suggests the potential of *E. debile* to offer therapeutic benefits, warranting further scientific exploration to validate traditional knowledge and elucidate the mechanisms of action behind these various applications.

2.2.4 Biological Activities

Equisetum debile (*E. debile*) emerges as a potentially valuable plant within the Equisetaceae family. *E. arvense* specie of this family has received significant research attention while *E. debile* remains relatively underexplored. However, initial studies on *E. debile* extracts have shown promise, demonstrating antioxidant and antibacterial properties. Furthermore, research suggests *E. debile* may offer benefits for hair health, although the specific phytochemicals responsible and their mechanisms of action, such as 5α-reductase and IL-6 inhibition, require further investigation [88].

This highlights the need for further exploration of *E. debile*'s potential therapeutic applications.

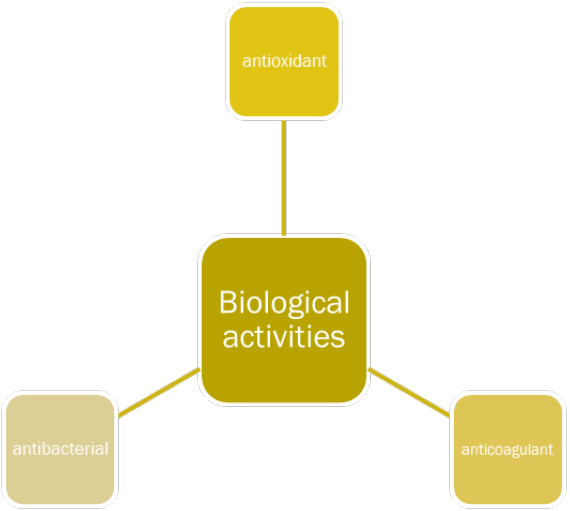

FIGURE 2.4: Biological activities of *Equisitem debile*

2.2.5 Phytochemical Activities

It have been thoroughly examined for phytochemical and pharmacological purposes, highlighting the need for more scientific research to completely comprehend their efficacy[89]

2.3 *Zanthoxylum armatum*

FIGURE 2.5: *Zanthoxylum armatum*

Zanthoxylum armatum (*Z. armatum*) is a fragrant shrub or small tree native to the Himalayan foothills, particularly in India, Nepal, and Bhutan. This subdeciduous plant, reaching heights of 6-7 meters, has been used for centuries in traditional medicine to treat a variety of ailments. In these regions, *Z. armatum* is known for its carminative and antiseptic properties, and it is commonly used to alleviate stomach issues, toothaches, chest infections, dental problems, digestive problems, and scabies.

Modern scientific research is shedding light on the potential of *Zanthoxylum armatum* beyond traditional uses. Studies have revealed a promising array of biological and pharmacological activities associated with *Z. armatum*, including antibacterial, antifungal, antiviral, anti-inflammatory, and antioxidant properties [90]. Transcriptome assembly is proving to be a valuable tool in identifying the genes responsible for these bioactivities. However, further research is necessary to fully understand the active biological components within *Z. armatum*. Additionally, optimizing plant tissue cultivation techniques is crucial for gaining a deeper understanding of the plant's biological actions and enabling efficient, large-scale propagation for commercial applications. This ongoing research holds immense promise

for unlocking the full potential of *Z. armatum* as a source of novel therapeutic agents.

2.3.1 Classification

Kingdom	Plantae
Family	Rutaceae
Genus	Zanthxylum
Specie	*Zanthxylum armatum*

2.3.2 Morphology of Plant

It is a fragrant, evergreen spiky shrub that grows to 3.5 meters in height. It is evergreen plant and leaves are oval in shape. The twigs are hairless and bear reddish brown spines. The leaflets have prickles/spines. The stamens are yellow before anthesis, while the gynoceium has 1-3 carpels. The fruit cones are purplish red and very small in size while the seeds are black in colour having strong odour.

Zanthoxylum armatum transcends its medicinal significance, offering a unique combination of culinary, therapeutic, and ornamental value. The plant's fruits and seeds find use as a spice, resembling timut pepper but with a milder intensity. Beyond its culinary application, *Z. armatum* holds a prominent place in traditional medicine across India, Nepal, and Thailand, with the bark, fruit, and seeds serving as the foundation for various treatments [91]. Additionally, the plant yields Wartara Oil, an essential oil with potential uses. *Z. armatum*'s appeal extends beyond its practical applications, with its attractive shrub-like form making it a popular choice for ornamental gardens. This multifaceted value positions *Z. armatum* as a plant with significant cultural and economic importance.

2.3.3 Traditional and Local Use

For centuries, local communities across Asia, America, and Africa have utilized Zanthoxylum species (also known as Fagara species) as a source of both food and

medicine. Traditional medicine practices in these regions particularly emphasize the use of various Zanthoxylum species for treating a range of ailments, including sickle cell anemia, trypanosomiasis, malaria, and microbial infections. This historical use highlights the potential of Zanthoxylum as a source of bioactive compounds with therapeutic applications.

Traditionally, the seeds and bark have been employed as aromatic stimulants for the digestive system, particularly to alleviate fever and indigestion. Powdered seeds mixed with warm water are a common remedy for stomach ailments. Furthermore, *Z. armatum* finds use in treating cholera, toothaches, and leech bites. Modern research is exploring the potential of *Z. armatum*'s various parts, including fruits, leaves, seeds, and stem bark, for treating a wider range of conditions like headaches, fevers, toothaches, tonsillitis, diarrhea, dysentery, and altitude sickness. The antibacterial and disinfectant properties of the fruit's essential oil suggest potential applicationsinthe pharmaceutical and flavoring industries, highlighting *Z. armatum*'s multifaceted value[92].

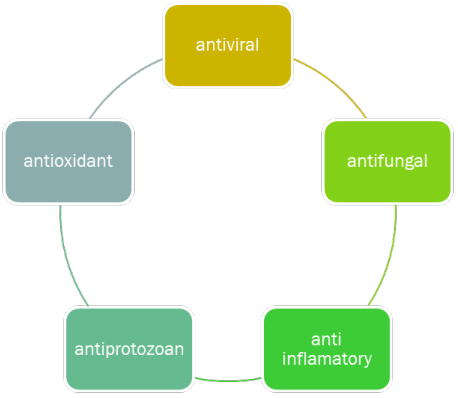

FIGURE 2.6: Biologocal activities of *Zanthoxylum armatum*

2.3.4 Biological Activity

Zanthoxylum armatum (*Z. armatum*) demonstrates a wide range of medicinal and biological effects. Studies have shown that various *Z. armatum* extracts, such as dichloromethane, acetone, water, ethanol, methanol, and petroleum ether extracts, possess properties that kill larvae (larvicidal), fight fungi (antifungal), protect the liver (hepatoprotective), dissolve keratin (keratolytic), combat viruses (antiviral),

combat single-celled parasites (antiprotozoan), kill pests and insects (pesticidal/insecticidal), kill bacteria (antibacterial), expel parasitic worms (anthelminthic), and interfere with the growth of other plants (allelopathic). Furthermore, research suggests that *Z. armatum* species might be useful in cancer treatment, with studies evaluating its effectiveness against both cancers that respond poorly to existing medications and those that respond well [93]. This diverse array of bioactivities emphasizes *Z. armatum*'s potential as a source of new therapeutic treatments.

Natural products from plants with a history of use in treating infections are being investigated for their effectiveness against drug-resistant microbes. Notably, plants in the Zanthoxylum genus have shown promising antimicrobial activity against bacteria and fungi that pose significant public health threats. This research is often driven by traditional medicinal practices, where specific Zanthoxylum species, like Z. zanthoxyloides, have been used for generations to combat infections in various regions of Asia and Africa [94]. The focus on Z. zanthoxyloides exemplifies how traditional knowledge can inform the discovery of novel antimicrobial agents.

2.3.5 Phytochemistry

The distribution of phytochemical compounds within a plant is not uniform. Studies have shown that a diverse array of secondary metabolites, including terpenoids, flavonoids, alkaloids, phenolics, lignins, coumarins, glycosides, benzoids, steroids, fatty acids, alkenoic acids, and amino acids, can be isolated from various plant parts like seeds, leaves, fruits, roots, and bark. This variation in phytochemical composition across different plant organs highlights the importance of specifying the plant part used in research and traditional medicine.

2.4 Bioassays

Biological assays, also known as bioassays, are a cornerstone technique for assessing the biological effects of various materials. These procedures employ living systems, ranging from cells and tissues to whole organisms, to gauge the impact of a test

substance. Bioassays often utilize enzyme or receptor preparations, providing a standardized foundation for comparisons.

2.4.1 Significance of Bioassays

Bioassays play a pivotal role in several crucial scientific endeavors. They are instrumental in:

- **Drug Discovery and Development:** Bioassays are employed to evaluate the potential toxicity of candidate drugs, ensuring their safety profile.
- **Environmental Monitoring:** By quantifying the impact of pollutants on living systems, bioassays serve as vital tools for environmental scientists to assess pollution levels and identify potential hazards.
- **Determination of Unknown Substance Concentrations:** Bioassays provide a means to estimate the concentration of unknown substances based on their effects on biological systems.
- **Pharmacological Potential Assessment:** Bioassays aid in uncovering the potential therapeutic effects of various substances, guiding drug discovery efforts.
- **Quantification of Pollutant Release:** Bioassays can be designed to quantify the amount of pollutants released by specific sources, enabling targeted environmental interventions[95]

2.4.2 Antibacterial Assay

Microbial pathogens, including fungi, bacteria, and some algae, pose a significant threat to plant, animal, and human health, causing a wide range of diseases. Bacterial and fungal infections remain leading causes of mortality worldwide.

The discovery of penicillin, the first clinically effective antibiotic, marked a major turning point in medicine. Subsequently, numerous antibiotics have been isolated from natural sources and synthesized for use in medical practice.

However, the battle against bacteria and fungi is far from over. A major challenge lies in the emergence of new pathogenic species and the development of antibiotic resistance among existing ones. This is an ongoing evolutionary process [96]. This study focuses on several bacterial strains selected due to their significance as human pathogens and their potential contribution to the problem of antibiotic resistance.

2.4.2.1 *Staphylococcus aureus*

Staphylococcus aureus (*S. aureus*), discovered by Sir Alexander Ogston, is a Gram-positive, spherical bacterium. Commonly referred to as "staph," it often forms grape-like clusters (hence the "staph" prefix). The "coccus" suffix denotes its spherical shape, and "aureus" likely refers to its golden color. *S. aureus* can survive in temperatures ranging from $18*o$C to $40*o$C. Alarmingly, up to 20% of the human population may carry this bacterium without experiencing any symptoms.

S. aureus is a major concern in healthcare settings, being a leading cause of hospital-acquired infections (HAIs). It ranks among the top five pathogens responsible for post-surgical and wound infections. Transmission of *S. aureus* can occur through various means, including contaminated beads or canned goods, direct contact with infected objects (food, water, inanimate surfaces), or even bites.

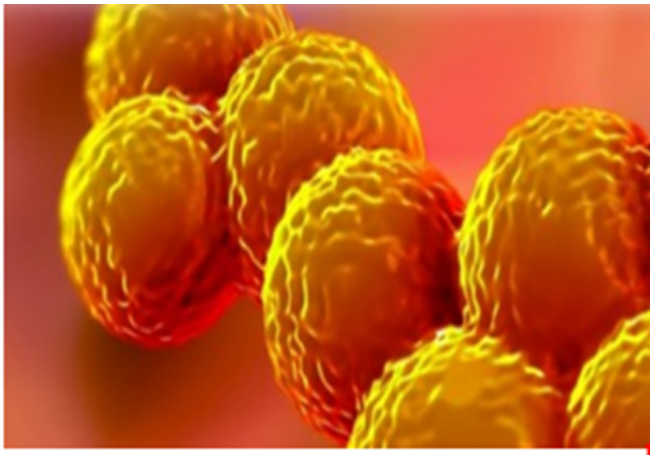

FIGURE 2.7: *S. aureus* [97]

S. aureus infections can manifest in a wide range of severities, from mild skin conditions to life-threatening illnesses. The bacterium can cause localized infections

like abscesses and boils, but it also has the potential to invade any organ system within the body.

One of the most concerning aspects of *S. aureus* is its ability to produce toxins that contribute to foodborne illness. Notably, a specific toxin produced by this bacterium is implicated in Toxic Shock Syndrome (TSS), a potentially fatal condition.

Fortunately, meticulous hand hygiene remains a cornerstone strategy for minimizing the risk of *S. aureus* infections.

2.4.2.2 *Klebsiella pneumoniae*

Klebsiella pneumoniae (*K. pneumoniae*) are Gram-negative, encapsulated, and non-motile bacteria commonly found in the human gut microbiota. While normally harmless within the intestines, they possess a concerning ability to develop antibiotic resistance.

However, *K. pneumoniae* can become opportunistic pathogens if they gain access to other body sites beyond the intestinal tract. This can occur through various means, leading to a range of infections. Some of the most common *K. pneumoniae* infections include:

- Urinary tract infections (UTIs)
- Intra-abdominal infections
- Meningitis
- Pyogenic liver abscesses
- Bloodstream infections (bacteremia)

FIGURE 2.8: *Klebsiella pneumoniae* [98]

The risk of *K. pneumoniae* infection is particularly concerning due to the emergence of antibiotic-resistant strains. These strains, sometimes referred to as "superbugs," pose a significant challenge for treatment with traditional antibiotics.

Several factors contribute to a person's susceptibility to *K. pneumoniae* infections. Individuals with weakened immune systems, due to illness or medical treatments, are more vulnerable because their body's natural defenses are compromised. Hospital settings pose a particular risk, as these environments can harbor *K. pneumoniae*, especially for patients relying on medical devices like catheters that can introduce the bacteria directly into the bloodstream. Additionally, recent use of broad-spectrum antibiotics can disrupt the natural balance of gut bacteria (microbiome). This disruption can allow *K. pneumoniae* to overgrow and become pathogenic, taking advantage of the reduced competition from other gut bacteria. These factors combined significantly increase the risk of *K. pneumoniae* infections[98].

2.4.3 Antifungal Assay

Fungal diseases in humans manifest in various forms. These can be allergic reactions triggered by fungal proteins, toxic reactions caused by toxins produced by certain fungi, and most importantly, fungal infections. Fungal infections are particularly concerning due to their ability to adapt and develop resistance to

treatment. The growing use of intensive therapies for cancer, HIV, and organ transplants creates a population of immunocompromised individuals more susceptible to these infections.

Unfortunately, the current antifungal armamentarium is limited, and the emergence of multidrug-resistant fungal strains further complicates treatment options. This limited availability of effective drugs necessitates the development of novel antifungal agents. A recent study, for instance, highlighted the alarming rise in resistance against fluconazole (a common antifungal drug) by Candida albicans, a major fungal pathogen. This emphasizes the urgent need for the discovery and development of novel antifungal drugs with the potential to combat the growing threat of resistant fungal pathogens[99].

2.4.3.1 *Aspergillus niger*

Aspergillus niger, often called black mold, is a fungus that likes to hang out in many places, from dirt and the air outside to inside our homes. Even though it has "black" in its name, it can actually be dark green or black, depending on where it's growing. This fungus is known for its dark colored spores, which are like tiny seeds that help it spread. This mold can infect a bunch of living things, including people, animals, and plants. In people, it can cause different problems depending on how it gets in and how strong their immune system is. The most common issue is aspergillosis, which happens when Aspergillus fungus starts growing in the lungs[100].

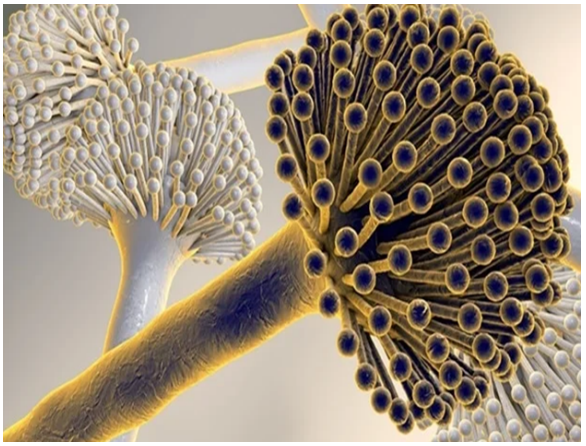

FIGURE 2.9: *Aspergillus niger* [101]

2.4.4 Antioxidant Assay

Free radical reactions are a constant presence within the human body and food systems. These reactions involve naturally occurring reactive oxygen and nitrogen species (ROS and RNS) produced during normal physiological processes.

However, an imbalance in the body's antioxidant system can lead to the over-production of these reactive species, resulting in a condition known as oxidative stress. Oxidative stress occurs when free radical formation outpaces the body's ability to neutralize them.

These unpaired electrons in free radicals cause them to react readily with various biomolecules, including proteins, lipids, and DNA. This damage can ultimately lead to cellular injury and death, potentially contributing to the development of chronic diseases like cancer and cardiovascular disorders.

In research settings, the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a widely used method to determine the primary antioxidant activity of various substances. This assay specifically measures the free radical scavenging abilities of pure antioxidant compounds, plant and fruit extracts, and food materials. The assay's effectiveness lies in its ability to gauge an antioxidant's ability to reduce the stable DPPH radical [102].

2.4.5 Anticoagulant Assay

Medications that help prevent blood clots from forming or growing. They are a crucial part of treatment for various thrombotic conditions. When your blood clumps up too much inside your veins and arteries, it can cause big problems. These clumps, called clots, can block blood flow and lead to serious issues like heart attacks, strokes, and blocked veins in the legs or lungs. There are medications called blood thinners that can help prevent these clots from forming or getting bigger[103]

Some techniques like clot-based tests, chromogenic or color assays, direct chemical measurements, and ELISAs are used for coagulation testing. From these techniques, clot-based and chromogenic assays are used mostly. These clotting assays gives a global assessment of coagulation function[104]

2.4.6 Phytochemical Analysis

in human health throughout history. Their efficacy has fueled the development of traditional medical systems like Ayurveda, which emphasizes plant-based remedies. In India, the use of crude plant extracts for treating microbial, fungal, and deficiency diseases has a long-standing tradition, and this approach is gaining global recognition.

Ayurveda's emphasis on natural remedies resonates with the growing interest in exploring plant-based solutions for certain diseases previously treated solely with conventional medications. Recognizing this potential, researchers are actively exploring the vast chemical diversity offered by plants. This pursuit is driven by the potential to discover novel drug leads from plant species with potent phytochemical components.

India, with its rich biodiversity and diverse flora, boasts a remarkable wealth of medicinal plant varieties. These resources, coupled with the country's varied topography and climatic conditions, present a unique advantage for cultivating and studying new plant species with potential therapeutic applications. Additionally, India's favorable agro-climatic conditions hold promise for the introduction and cultivation of exotic plant varieties with untapped medicinal potential [105].

The phytochemical analysis comprises of following steps [106].

- Sample preparation.
- Feature extraction
- Data Collection and Chemometrics
- Compound identification

2.4.7 Types of Phytochemicals

Plants produce a diverse range of organic chemicals beyond those essential for primary metabolic processes. These secondary metabolites, including alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumins, saponins, and glycosides, are not directly involved in basic plant growth and development. Literature analysis reveals that among these secondary metabolites, phenolics stand out as the most abundant and chemically diverse group of plant phytochemicals [107].

2.4.7.1 Phenolic

Since the late 19th century, the observation of the "French paradox" – the apparent reduced risk of cardiovascular disease despite a high saturated fat diet in France, potentially linked to red wine consumption rich in phenolics. Researchers have shown increasing interest in the potential health benefits of plant phenolics. This has spurred extensive research on the biosynthesis, bioactivities, detoxification pathways, and chemical identification of phenolic compounds in various plants [105]. Furthermore, the food industry and research have placed growing emphasis on analyzing the stability and durability of phenolic content throughout the food chain, from harvest to processing and consumption [108]. Phenolic compounds, the most abundant class of phytochemicals, are ubiquitous throughout the plant kingdom (Plantae). They are characterized by the presence of hydroxyl groups (OH) directly bonded to an aromatic hydrocarbon ring. This diverse group can be broadly categorized into three major dietary types: flavonoids, phenolic acids, and polyphenols [109]. The vast potential of phenolics has attracted significant research interest in various fields, including agronomy, pharmacology, chemistry, and medicine, with numerous studies investigating their composition and functionalities [110].

2.4.7.2 Flavonoids

Flavonoids are a diverse group of plant secondary metabolites structurally derived from flavones. They possess a characteristic chemical structure consisting

of two benzene rings linked by a propane unit. Generally water-soluble, flavonoid complexity often correlates with increased color vibrancy. Interestingly, plants primarily store flavonoids as glycosides, which can impact their bioavailability and stability [111].

In recent years, flavonoids have garnered significant interest due to their diverse pharmacological properties. Research suggests they exhibit a range of biological activities, including antimicrobial, cytotoxic (anti-cancerous), and anti-inflammatory effects [112]. This growing body of evidence highlights the potential of flavonoids for therapeutic applications.

2.5 FTIR Spectroscopy

2.5.1 Fourier Transform Infrared (FTIR)

FTIR spectroscopy operates by measuring the absorption of infrared radiation by different chemical bonds within a molecule. This absorption pattern, unique to each substance, serves as a molecular fingerprint that can be used for identification and analysis. The Fourier transform process converts the raw data into a recognizable spectrum, allowing for easy interpretation of the results.

2.5.2 Advantages of FTIR

FTIR offers several advantages over traditional infrared spectroscopy, including:

- **Speed**: FTIR can acquire spectra significantly faster, enhancing efficiency and throughput.
- **Sensitivity**: It is more sensitive, allowing for the detection of smaller amounts of analytes.
- **Versatility**: FTIR can analyze a wide range of samples, from solids and liquids to gases.

- **Accuracy**: The technique provides highly accurate and reproducible results.

2.5.3 Applications of FTIR

FTIR has found widespread applications in various fields:

- **Chemical Analysis**: Identifying unknown substances, determining purity, and quantifying components in mixtures.
- **Quality Control**: Ensuring product quality and compliance with standards.
- **Research**: Studying molecular structure, chemical reactions, and physical properties.
- **Environmental Analysis**: Detecting pollutants and monitoring environmental conditions.
- **Forensic Science**: Analyzing evidence such as fibers, paint, and drugs.

The Principle of FTIR for Polymer Identification FTIR spectroscopy operates by measuring the absorption of infrared radiation by different chemical bonds within a polymer. This absorption pattern, unique to each substance, serves as a molecular fingerprint that can be used for identification. By comparing the spectrum of an unknown polymer to the spectra of known polymers, it is possible to make a confident identification [126].

Chapter 3

Materials and methods

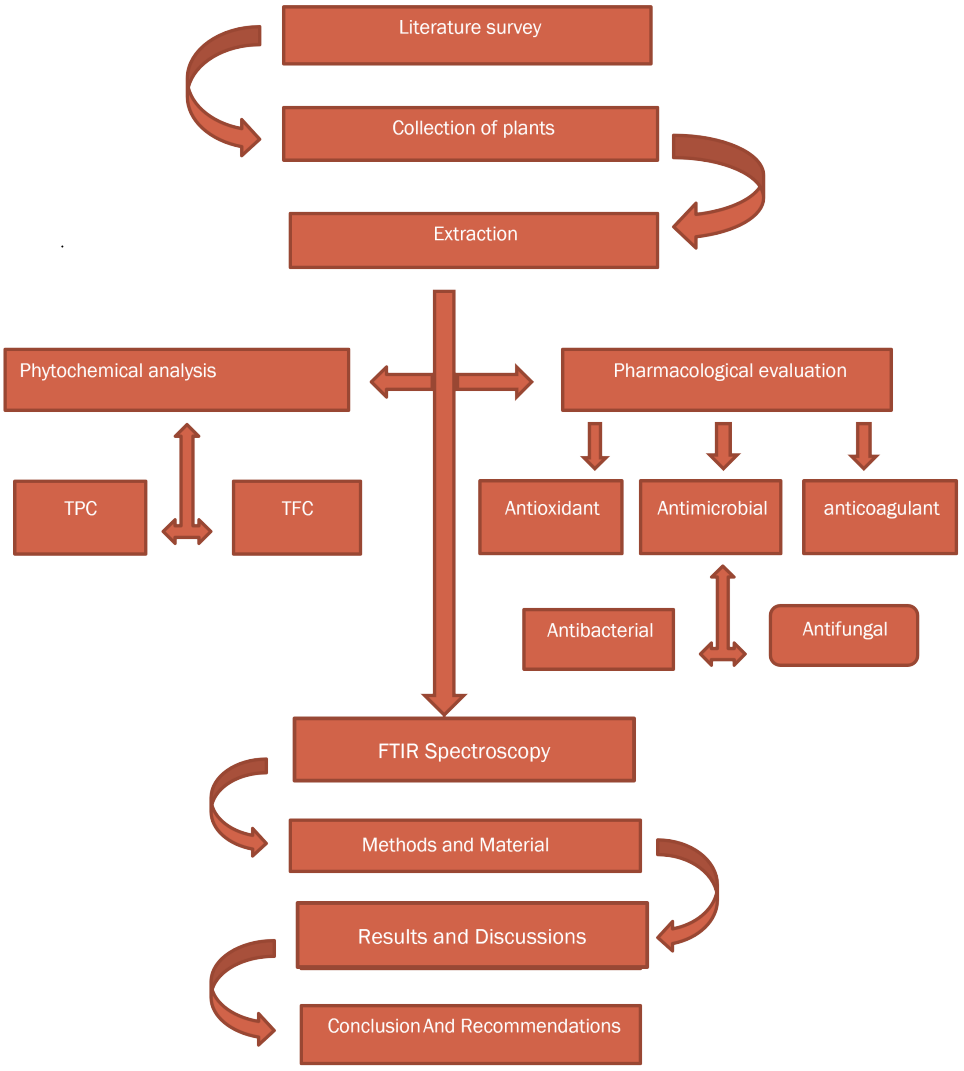

FIGURE 3.1: Overview of methodology

3.1 Chemicals

Methanol

Ethanol

Folin–Ciocalteu reagent

Distilled water

Gallic acid

NAOH

Sodium carbonate.

3.2 Equipments

Beakers, flask, ependorf, funnel, iron stand, filter paper, muslin cloth,petri dishes, forceps, measurind cylinder, a spectrophotometer, an electronic balance, blender, electronic shaker.

3.3 Collection and Identification of Plants

Fresh plant samples were collected from Dharnoian region of Kotli Sattian District Murree in mid of March and April.Plant samples were taxonomically identified and authenticated by an expert botanist from" National History Museum Islamabad".

Table 3.1: Selected plants and their accession number

Plants	**Accession number**
Viola canescens	047514
Equisitem debile	047511
Zanthoxylum armatum	047512

After confirming their identity, the museum assigned an accession number to each specimen.

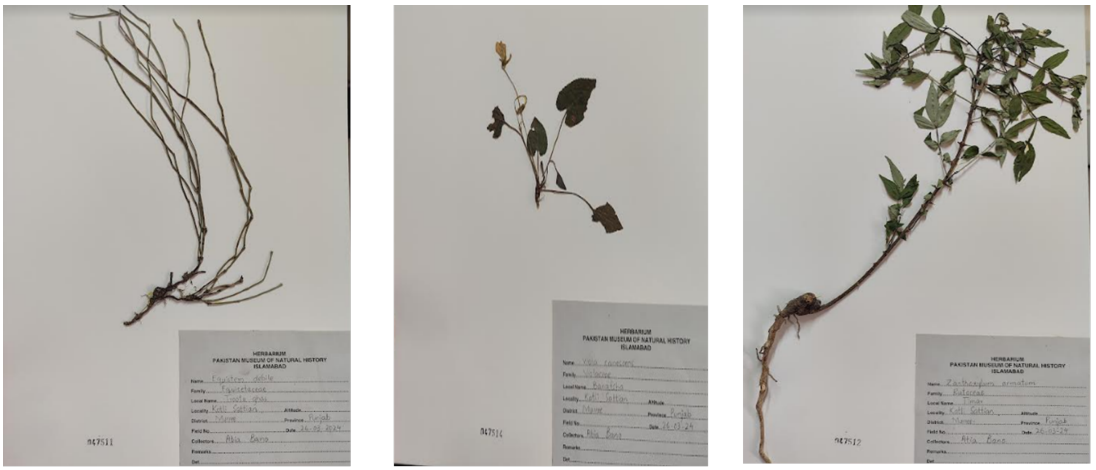

FIGURE 3.2: Authentication of *Viola canescens, Equisitem debile, Zanthoxylum armatum*

3.4 Extraction

3.4.1 Drying

The collected plant samples were cleaned to remove dirt and then dried under the sun. This drying process likely took up to five days.

3.4.2 Grinding

After drying completely, the plants were ground into a fine powder using a blender.

3.4.3 Preservation

Transfer this powder in airtight bags for further studies.

3.4.4 Extraction Method

Maceration method was employed to extract components from the plant material. Methanol and water were used as extracting solvents.

3.4.4.1 *Viola canescens* Extraction

Powdered Viola canescens sample was weighed precisely, 89.69 g of this powder is added in a flask and 200 mL methanol was added in it.

3.4.4.2 *Equisitem debile* Extraction

Similarly,Powdered Equisitem debile sample was weighed precisely, 100.87 g of this powder is added in a flask and 300mL methanol was added in it.

3.4.4.3 *Zanthoxylum armatum* Extraction

Same like above plant samples, Powdered *Zanthoxylum armatum* sample was weighed precisely, 59.25 g of this powder is added in a flask and 200mL methanol was added in it.

Flasks were sealed with stopper, afterwards this mixture was then put in electronic shaker for 72 hours at 150 rpm at room temperature to shake mixture well. To remove any solid particles, the extract was strained using a muslin cloth. Next, the resulting liquid was passed through Whatman filter paper No 1 for further purification.

Finally, the filtrate, the clear liquid after filtration, was transferred to petri dishes. Methanol evaporates after 5-6 days leaving a concentrated plant extract. These concentrated extracts were weighed for further investigations and stored at -20 degree celcius.

Following formula is used to find solvent efficiency to calculate percentage yield.

$$\%yield = \frac{weight\ of\ dry\ extract}{weight\ of\ dry\ sample} \times 100.$$

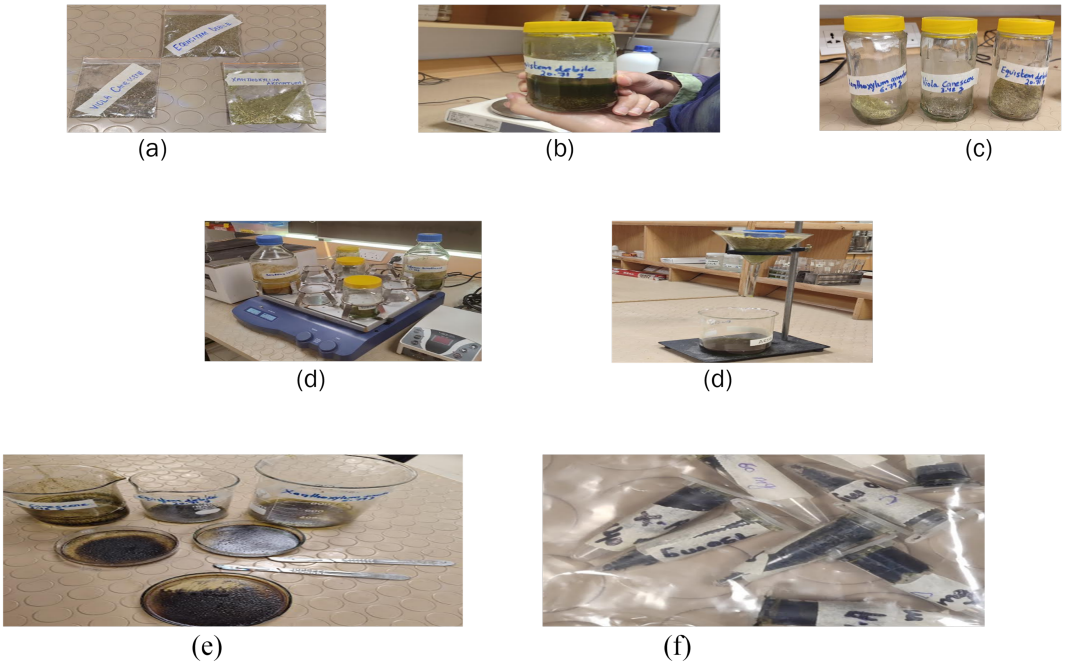

FIGURE 3.3: Extraction steps of *Viola canescens, Equisitem debile, Zanthoxylum armatum*

3.5 Phytochemical Analysis

Phytochemical analysis was performed on concentrated extracts of *Viola canescens, Equisitem debile , Zanthoxyulum armatum* to determine the total content of phenoles and flavonoids.

3.5.1 Determination of the Total Phenolic Contents

With few modifications, the Folin–Ciocalteu colorimetric procedure as reported by Singleton and Rossi [113] was utilized to calculate the amount of phenol utilized in various extracts. In short, after five minutes, aliquots of the corresponding extracts in methanol were combined with the Folin-Ciocalteu reagent, and the reaction mixture was then mixed with sodium carbonate solution. A UV–vis spectrophotometer was used to calculate the absorbance of the final reaction mixture at 760 nm after it had been incubated for two hours at room temperature.

The standard 1 mg/ml of gallic acid was used. The milligram gallic acid equivalent per gram dry weight of the corresponding formulation extract (mg GAE/g extract of the formulation) and ±SD (standard deviation) for three replicate studies were used to express the total phenolic content in the formulation [116, 117].

3.5.2 Determination of the Total Flavonoid Contents

The aluminum chloride colorimetric method [114, 115] was modified to ascertain the flavonoid concentration of the various extracts. To put it briefly, sodium nitrite was combined with aliquots of the formulation extracts in methanol. After thoroughly mixing the reaction mixture and allowing it to settle at room temperature for five minutes, the AlCl3 • 6H2O solution was added. NaOH was then added after an additional five minutes of room temperature incubation. Distilled water was added to the reaction mixture to boost its final volume.

Using a UV–vis spectrophotometer, the completed reaction mixture was allowed to settle at room temperature for 15 minutes in the dark before the absorbance at 510 nm was calculated. Milligram quercetin equivalent per gram dry weight of the corresponding composition extract (mg QE/g extract of the formulation) and ±SD for three replicates were used to express the overall flavonoid content [116, 117].

3.6 Pharmacological Evaluation of Plants

3.6.1 Apparatus and Equipment

Methanol,Nutrient agar,Distilled water,Ascorbic acid,Petri plates, test tubes, vials, micropipette, cotton plugs, cotton swabs, aluminum foil, eppendorf tubes, beakers, forceps.

Pharmacological evaluation of collected samples of *Viola canescens, Equisitem debile, Zanthoxylum armatum* undergoes different activities. These are

- DPPH radical scavenging activity
- Antibacterial activity
- Antifungal activity
- Anticoagulant activity

3.6.2 Antioxidant Activity

3.6.2.1 DPPH Radical Scavenging Activity

Antioxidant DPPH (2, 2-Diphenyl-1-picrylhydrazyl) free radical scavenging was used to measure the capacity of extracted samples of *Viola canescens, Equisetum debile* and *Zanthoxylum armatum.*

3.6.2.2 Sample Preparation

Solution was prepared and used for the analysis at (1,5,50μg/mL) concentration.

3.6.2.3 Preparation of DPPH Solution

The reagent solution was prepared by adding DPPH in ethanol.

3.6.2.4 Procedure

The total antioxidant capacity of the extracts was determined according to the method described by Prieto et al [118].

An aliquot of *Viola canescens, Equisitem debile, Zanthoxylum armatum* and the reagent (DPPH) were placed in separate tubes. There were three copies of the entire process completed. Ethanol was utilized as the negative control while and DPPH was used as a positive control. These tubes were left in a dark area for 45 minutes.

Using distilled water as a blank reference, the absorbance of the samples was measured at 517 nm after 45 minutes. Since water is utilized as a solvent in this test, water was used as a blank reference to determine whether or not water has an individual antioxidant activity. Three copies of the process are run, but only the best outcome is chosen. The activities of the samples were evaluated by comparison with a control [119]. The higher absorbance value indicated higher antioxidant activity [120].

The formula for calculating free radical scavenging is as follows.

$$\%\ Scavenging = \frac{Control\ absorbance\ -\ Extract\ samples\ absorbance\ \times\ 100}{Control\ absorbance}$$

3.6.3 Antibacterial activity

3.6.3.1 Materials and Methods:

- **Plant Extracts**: Three plant extracts were prepared: *Viola canescens, Equisetum debile* and *Zanthoxylum armatum.*

3.6.3.2 Bacterial Strains

Following bacterial strains were used to identify antibacterial activities.

- Staphylococcus aureus
- Klebsiella Pneumoniae

3.6.3.3 Composition of Muller Hinton agar

- **Beef extract (2.0 g)**: A source of essential nutrients like nitrogenous compounds, vitamins, and amino acids.
- **Acid hydrolysate of casein (17.5 g)**: Another source of nitrogen, vitamins, and amino acids for bacterial growth.

- **Starch (1.5 g)**: This component functions as a thickening agent and neutralizes any potential toxic metabolites produced by bacteria, allowing for clearer observation of growth zones during analysis.

- **Agar (17.0 g)**: This gelling agent solidifies the mixture, creating a petri dish with a solid surface for streaking and isolating bacterial cultures [121].

By streaking the bacterial inoculum (a sample containing the bacteria being tested) onto sterilized MH agar plates, researchers can achieve even distribution and facilitate observation of growth patterns [121]. This technique is crucial for various microbiological applications, including antimicrobial susceptibility testing.

3.6.3.4 Procedure

3.6.3.5 Inoculum Preparation:

- To minimize contamination, a sterilized loop or needle was used to obtain a sample of four to five well-isolated colonies of the target bacteria.

- These colonies were then suspended in 2 ml of sterile saline solution within a test tube .

- A vortex mixer or gentle swirling motion was employed to create a homogenous suspension.

- The turbidity of this suspension was adjusted to match a 0.5 McFarland standard. This standardization ensures consistency in the amount of bacteria used for testing [122]. If the suspension was too dense, it was diluted with additional sterile saline; conversely, more bacterial colonies could be added if the suspension was too clear.

3.6.3.6 Antimicrobial Activity Testing

- A sterile cotton swab was then dipped into the standardized inoculum.

- The swab was used to uniformly streak the bacterial suspension across the surface of a sterilized Petri dish, creating a confluent lawn of growth.
- Using a sterile borer or micropipette tip, wells were created within the agar layer of the Petri dish [122]. These wells serve as reservoirs for the plant extract being tested.

3.6.3.7 Triplicate Testing

The entire experimental procedure, from inoculum preparation to well creation, was repeated two additional times to ensure reproducibility of the results [122]. Performing the experiment in triplicate strengthens the validity of the findings.

3.6.3.8 Preparation of Plant Extract Solution

- A precisely weighed amount of 0.1 g of the plant extract was dissolved in 1 ml of dimethyl sulfoxide (DMSO) [122].

3.6.4 Antifungal Activity Testing

3.6.4.1 Materials and Methods

- **Plant Extracts:** Three plant extracts were prepared: Viola canescens, Equisetum debile, and Zanthoxylum armatum.
- **Fungal Strain:** Aspergillus niger was used as the test organism.
- **Media:** Sabouraud dextrose agar (SDA) was used to cultivate the fungus.

3.6.4.2 Inoculum Preparation

- A sterile loop or needle was used to collect a sample of the fungal mat and suspend it in 2 ml of sterile saline solution within a test tube.
- The suspension was homogenized using a vortex mixer to achieve a uniform consistency.

- The turbidity of the suspension was adjusted to match a 0.5 McFarland standard for standardization of the inoculum amount [123]. This involved diluting the suspension with sterile saline if it was too dense or adding more fungal cells if it was too clear.

3.6.4.3 Positive and Negative Controls

- Distilled water served as the negative control, representing the absence of any antifungal activity.
- Aspergillus niger was used as the positive control to ensure the fungal strain was viable and capable of growth under the experimental conditions.

3.6.4.4 Antifungal Assay

- A sterile cotton swab was dipped into the standardized fungal inoculum.
- The swab was used to uniformly spread the inoculum across the surface of a sterilized SDA plate, creating a confluent fungal lawn.
- Using a sterile borer or micropipette tip, wells were created within the agar to accommodate the plant extracts being tested for antifungal activity.
- Preparation of Plant Extract Solution: A precisely weighed amount of 0.02 g of each plant extract was dissolved in 1 ml of DMSO (dimethyl sulfoxide) to prepare a stock solution [122].
- Dosing and Incubation: Fifty microliters (μl) of each prepared plant extract solution were dispensed into designated wells on the SDA plates containing the fungal lawn [123]. The plates were then incubated to allow for fungal growth and interaction with the plant extracts.
- Triplicate Testing: The entire experiment, from inoculum preparation to well creation and extract application, was repeated two additional times (in triplicate) to ensure the reproducibility of the results [123].

3.6.4.5 Data Analysis

- After the incubation period, the fungal growth in each well was measured to determine the antifungal activity of the plant extracts. Percent inhibition was calculated using the following formula:

$$\%Inhibition = \frac{(Linear growth in negative control - Linear growth in sample)}{Linear growth in negative control} \times 100$$

This formula provides a quantitative measure of how much the plant extracts inhibited fungal growth compared to the negative control.

3.6.5 Anticoagulant Activity Testing

3.6.5.1 Materials and Methods

- **Plant Extracts**: The anticoagulant activity of extracts from three plants was evaluated: Viola canescens, Equisetum debile, and Zanthoxylum armatum.
- **Blood Collection**: Freshly collected whole blood was used in the anticoagulant assay. To prevent clotting before the experiment, the blood was anticoagulated with 3.2% sodium citrate.
- **Other Materials**: Additional materials included 0.025 M calcium chloride (CaCl2) solution, glass tubes, disposable petri dishes or glass slides, pipettes and tips, a stopwatch, and an incubator set at 37^{o}C [124].

3.6.5.2 Procedure

- **Blood and Extract Mixture**: The anticoagulant activity was measured by mixing equal volumes (100 μl each) of the plant extract solution and the citrated whole blood.

- **Incubation**: The mixture of plant extract and blood was incubated for 5-10 minutes at 37^{o}C to allow for potential interaction between the plant components and blood clotting factors.

- **Recalcification and Clot Formation**: After incubation, 100 μl of 0.025 M CaCl2 solution was added to 50 μl of the incubated mixture (plant extract + blood) to initiate clot formation. The time taken for a visible clot to form was recorded using a stopwatch. This clotting time is referred to as the recalcification time. A separate control sample containing only 50 μl of incubated whole blood (without plant extract) was also treated with 100 μl of 0.025 M CaCl2 solution to determine the baseline clotting time of the blood without any anticoagulant intervention [124].

3.6.5.3 Data Analysis

- The anticoagulant activity of the plant extracts was evaluated by comparing the recalcification time of the extract-blood mixture with the recalcification time of the control blood sample.

- Prolonged clotting times in the extract-blood mixture compared to the control would indicate potential anticoagulant activity of the plant extract. Conversely, clotting times similar to or shorter than the control would suggest minimal or no anticoagulant effect.

3.7 FTIR Spectroscopy

The KBr pellet method is a common technique used for preparing solid samples for FTIR analysis.

3.7.0.1 Sample Preparation

- **Sample Grinding**: The sample should be finely ground to ensure homogeneous mixing with the KBr and to minimize scattering losses. Excessive

grinding should be avoided to prevent the absorption of moisture from the air.

- **KBr Preparation**: Potassium bromide (KBr) is a hygroscopic salt that should be handled with care to avoid moisture absorption. It is typically dried in an oven before use.
- **KBr Mixture**: A small amount of the sample is mixed with KBr in a mortar and pestle. The mixture should be ground to a fine powder to ensure thorough mixing.
- **Pellet Formation**: The powdered mixture is pressed into a pellet using a hydraulic press. The pressure applied during pellet formation is critical for obtaining a transparent and homogeneous pellet [126].

3.7.0.2 Data Analysis

- Characterization of potential phytochemicals
- Qualitative phytochemical screening confirmed the presence of phenols, flavanoids,tannis, fats and oils.
- Revealed various characteristics band values with different functional groups in extract such as amines, alcohol, phenol, alkanes,esters etc

Chapter 4

Results

4.1 Collection and Identification of Plants

Accession number was given to all collected samples of plants after taxonomic identification and authentication.

4.2 Extraction Yield

By using formula I calculated yield of selected plants *Viola canescens*, *Equisitem debile*, *Zanthoxylum armatum*.

TABLE 4.1: Total calculated yield of dry samples of plant extracts

No. of plants	**Plant**	**Extraction yield %**
1	*Viola canescens*	2.62
2	*Equisitem debile*	0.86
3	*Zanthoxylum armatum*	1.25

4.2.1 Results

Extraction yield of *Viola canescens* (2.62) is higher than *Equisitem debile* (0.86) and *Zanthoxylum armatum*(1.25).

4.3 Phytochemical Analysis

4.3.1 Total Phenolic Contents

The total phenolic content determined in various extracts of the formulation are given in Table below. The phenolic content was expressed in terms of gallic acid equivalents (GAE). Each value in the table is represented as mean ± SD (n = 3)

Means that do not share a letter are significantly different at $P < 0.05$ probability level in each Column.

TABLE 4.2: Total phenolic contents of dry extracts

No of Plants	**Plants**	**Total Phenolic Contents**
		(mg GAE/g of Dry Extract)
1	*Viola canescens*	111.18± 2.06
2	*Zanthoxylum armatum*	65.31± 4.91
3	*Equistem debile*	7.17± 1.03

The highest content of total phenolic compounds was quantified in the *Viola canescens* extract with a value of 111.18 ± 2.06 mg GAE/g, followed by the *Zanthoxylum armatum* extract with a content of 65.31 ± 4.91 mg GAE/g, while Equistem debile extract showed the least content 7.17 ± 1.03 mg GAE/g. In selected plant extracts, the decreasing order of phenolic content is as follows: *Viola canescens* extract > *Zanthoxylum armatum* extract > *Equisitem debile* extract. These findings suggest that *Viola canescens* may be a richer source of antioxidant compoundcompared to *Zanthoxylum armatum* and Equistem debile. The higher content of phenolics in *Viola canescens* could be new researchgate to biosynthetic pathways.

4.3.2 Total Flavonoid Contents

The total flavonoid content determined in various extracts of the formulation are given in Table below. The flavonoid content was expressed in terms of gallic acid

equivalents (GAE). Each value in the table is represented as mean ± SD (n = 3).

Means that do not share a letter are significantly different at P & lt; 0.05 probability level in each Column.

Table 4.3: Total Flavonoid Content of dry extracts

No of Plants	**Plants**	**Total Flavonoid Contents**
		(mg GAE/g of Dry Extract)
1	*Viola canescens*	19.68± 0.27
2	*Zanthoxylum armatum*	12.8± 0.32
3	*Equistem debile*	11.08± 0.32

The highest content of total phenolic compounds was quantified in the *Viola canescens* extract with a value of 19.68± 0.27mg GAE/g, followed by the *Zanthoxylum armatum* extract with a content of12.8± 0.32 mg GAE/g, while Equistem debile extract showed the least flavonoid content 11.08± 0.32 mg GAE/g. In selected plant extracts, the decreasing order of flavonoid content is as follows:

Viola canescens extract > *Zanthoxylum armatum* extract > *Equisitem debile* extract

These findings suggest that *Viola canescens* may be a richer source of antioxidant compound compared to *Zanthoxylum armatum* and Equistem debile. The higher content of flavonoids in *Viola canescens* to explore active ingredients and bioavailable products in the food-pharm, nutraceutical or cosmeceutical industries.

4.4 Pharmacological Evaluation of Plants

4.4.1 DPPH Radical Scavenging Activity

The antioxidant ability of these plant extracts were assessed by DPPH assay. Free radical scavenging activity was exhibited by these plant extracts. Antioxidant assay was performed at different concentrations. Results of antioxidant assay of all extracts are shown in table below.

TABLE 4.4: Antioxidant effect (IC50) on DPPH radical of methanol extracts of *Zanthoxylum armatum*, Equisetum debile, and *Viola canescens*

S.No	Plants	IC50(ug/ML)
1	*Viola canescens*	5.03± 0.14
2	*Zanthoxylum armatum*	4.35± 0.04
3	*Equistem debile*	5.04± 0.28
4	Ascorbic acid	3.28 ± 0.02

The table shows the percent scavenging of DPPH radical by four different extracts at various concentrations. DPPH radical is a stable free radical molecule commonly used to evaluate antioxidant activity. The scavenging activity of an extract is measured by its ability to reduce DPPH. A lower percentage of scavenging indicates greater antioxidant activity.

In the table, the concentration of the extract is listed in μg/mL (micrograms per milliliter). For example, *Zanthoxylum armatum* extract shows the highsest scavenging percentages at 1, 5 and 50 μg/mL. As the concentration of the extract increases, the percent scavenging of DPPH radical also increases. This trend is observed in this graph.

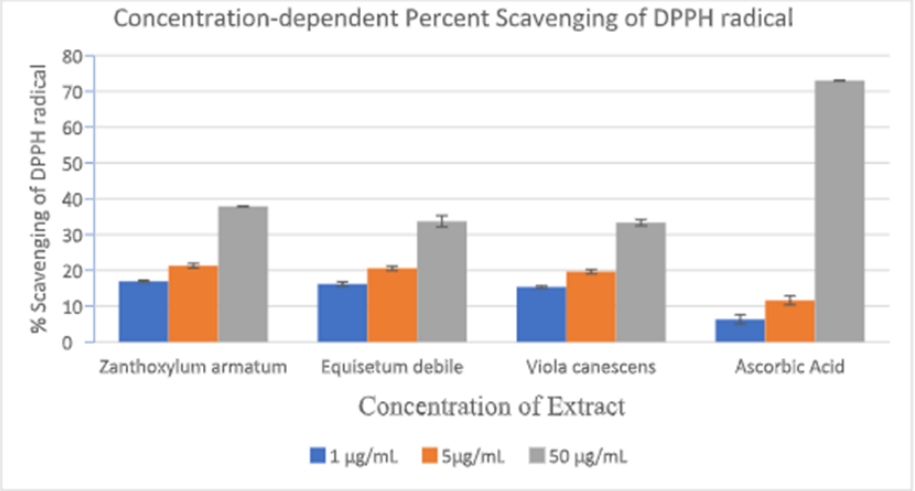

FIGURE 4.1: DPPH radical scavenging activity of methanol extracts of *Zanthoxylum armatum*, Equisetum debile, and *Viola canescens* at different concentrations. Each value represents a mean ± SD (n = 3)

4.4.2 Antibacterial Activity

Agar well diffusion method was employed to assess the antibacterial activity of extracts from *Viola canescens*, *Zanthoxylum armatum*, and Equisetum debile. This method involves evaluating the ability of the plant extracts to inhibit the growth of two bacterial strains: *Staphylococcus aureus* (Gram-positive) and *Klebsiella pneumoniae* (Gram-negative) [125].

4.4.2.1 Results

Following incubation for 24 hours, no inhibition zones were observed around the discs containing the plant extracts in the disc diffusion petri dishes. This suggests that, under the tested conditions, the plant extracts did not exhibit antibacterial activity against either S. aureus or K. pneumoniae.

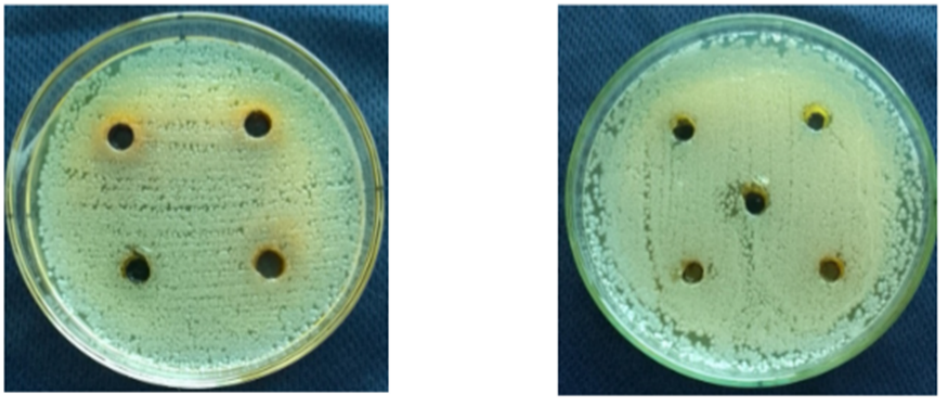

FIGURE 4.2: Antimicrobial Activity against *Klebsiella pneumoniae* (ATCC)

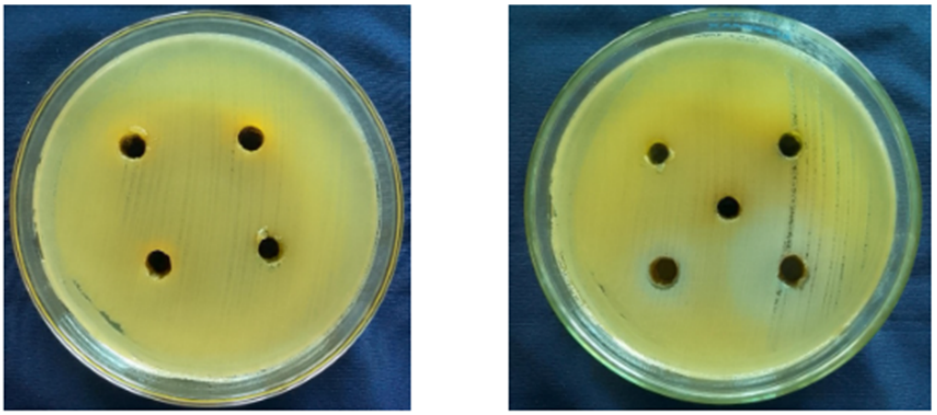

FIGURE 4.3: Antimicrobial Activity against *Staphylococcus aureus* (ATCC)

Results of antibacterial activity is shown in table below:

TABLE 4.5: Antibacterial activities of methanol extracts of *Zanthoxylum armatum*, Equisetum debile, and *Viola canescens*

S. No.	**Plants**	**Antibacterial Activity**	**Antibacterial Activity**
		(*S. aureus*)	(*K. pneumonae*)
1	*Viola canescens*	X	X
2	*Zanthoxylum armatum*	X	X
3	*Equisitem debile*	X	X

4.4.3 Antifungal Activity

The antifungal activity of *Viola canescens*, *Zanthoxylum armatum*, and Equisetum debile extracts was evaluated against *Aspergillus niger*. However, none of the extracts demonstrated inhibitory effects on the fungal growth.

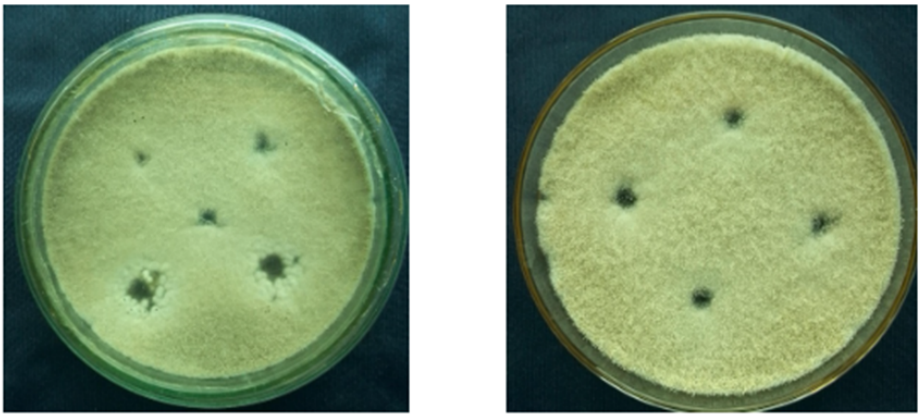

FIGURE 4.4: Antifungal activity against *Aspergillus niger*

Results shown below in table

TABLE 4.6: Antifungal activities of methanol extracts of *Zanthoxylum armatum*, Equisetum debile, and *Viola canescens*

S.NO	**Plants**	**Antifungal activity**
		(A. niger)
1	*Viola canescens*	X
2	*Zanthoxylum armatum*	X
3	*Equisitem debile*	X

4.4.4 Anticoagulant Activity

This activity summarizes the results of a blood clotting test. This test measures how long it takes blood to clot after adding a substance for that triggers clotting.

Negative Control: This sample should not clot, and the table indicates "no agglutination," which means the red blood cells haven't clumped together, as expected.

Positive Control: This sample should clot quickly, and the table shows a clotting time of 21 seconds, which serves as a reference point for the test's validity.

Viola canescens

It has a clotting time of 26 seconds. Ideally, this clotting time should be within a specific range compared to the positive control.

Xanthoxylum armatum

Its clotting time is significantly faster at 7 seconds, suggesting increased clotting activity compared to the positive control.

Equisitem debile

With a clotting time of 5 seconds, it shows the fastest clotting among all samples.

TABLE 4.7: Active clotting Time of *Viola canescens*, *Equisitem debile*,*Zanthoxylum armatum*

S.NO	Control/ Plants	Active clotting time
1	Negative control	No agglutination
2	Positive control	21 seconds
3	*Viola canescens*	26 seconds
4	*Equisitem debile*	5 seconds
5	Zanthoxylum armatum	7 seconds

In conclusion, this table suggests the test functioned properly (negative and positive controls behaved as expected). The samples X.armatum and E.debile appear to clot much faster than the positive control, indicating potentially abnormal clotting activity.

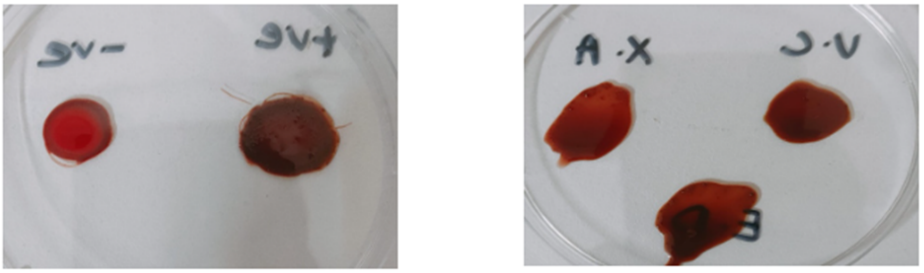

FIGURE 4.5: Anticoagulant activity of *Viola canescens*, *Equisitem debile*,*Zanthoxylum armatum*

4.5 FTIR Spectroscopy

4.5.1 FTIR Characterization of *Zanthoxylum Armatum*

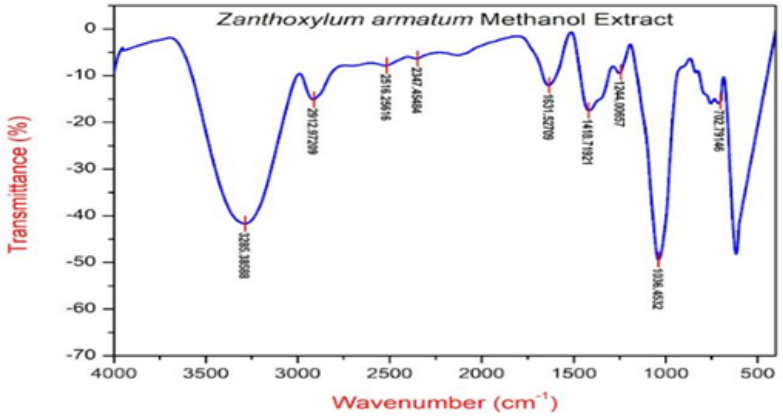

FIGURE 4.6: Characterization of *Zanthoxylum Armatum*

TABLE 4.8: FTIR Spectral Analysis of *Zanthoxylum armatum* Methanol Extract

Wavenumber (cm^{-1})	**Peak Assignment**	**Functional Group**
3382.3358	Broad band	O-H Stretching(alcohol)
2912.97209	Sharp peak	C-H stretching (alkanes)

Table 4.8 continued from previous page

Wavenumber (cm^{-1})	Peak Assignment	Functional Group
2516.25616	Broad band	O-H stretching (carboxylic acid)
2347.45484	Sharp peak	CO2 stretching (carboxylic acid)
1631.52709	Sharp peak	C=O stretching (carbonyl)
1418.71921	Sharp peak	C-H bending (alkanes)
1244.00657	Sharp peak	C-O stretching (ester,ether)
1036.4532	Sharp peak	C-O stretching (alcohol)
702.79146	Sharp peak	C-H bending (aromatic)

4.5.2 FTIR Characterization of *Viola canescens*

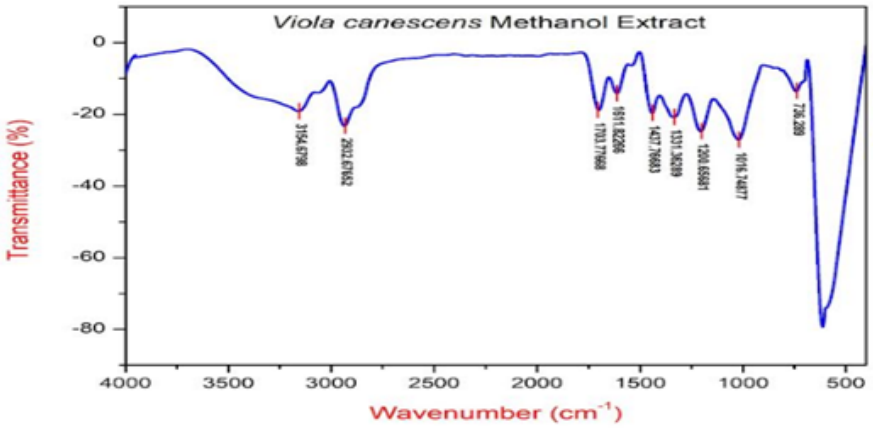

FIGURE 4.7: Characterization of *Viola canescen*

TABLE 4.9: FTIR Spectral Analysis of *Viola canescens* Methanol Extract

Wavenumber (cm^{-1})	Peak Assignment	Functional Group
3311.74153	Broad band	O-H stretching (alcohol)
2932.67652	Sharp peak	C-H stretching (alkanes)
2346.8296	Sharp peak	CO stretching (carboxylic acid)
1703.77668	Sharp peak	C=O stretching (carbonyl)
1611.82266	Sharp peak	C=C stretching (alkene)
1437.76683	Sharp peak	C-H bending (alkanes)
1331.36289	Sharp peak	C-O stretching (ester, ether)
1200.65681	Sharp peak	C-N stretching (amine)
1016.74877	Sharp peak	C-O stretching (alcohol)
736.289	Sharp peak	C-H bending (aromatic)

4.5.3 FTIR Characterization of *Equisetum debile*

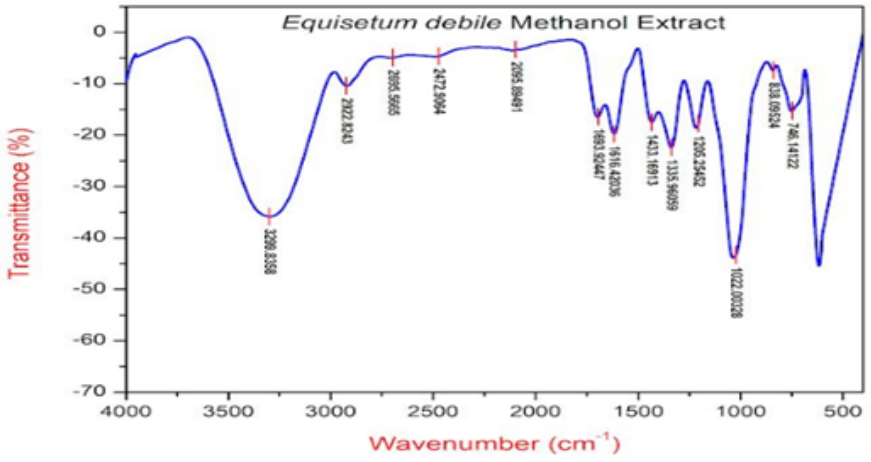

FIGURE 4.8: Characterization of *Equisetum debile*

TABLE 4.10: FTIR Spectral Analysis of *Equisetum debile* Methanol Extract

Wavenumber (cm^{-1})	**Peak Assignment**	**Functional Group**
3299.8358	Broad band	O-H stretching (alcohol)
2922.8243	Sharp peak	C-H stretching (alkanes)
2326.8338	Sharp peak	CO stretching (carboxylic acid)
2172.90649	Sharp peak	C C stretching (alkyne)
2095.89491	Sharp peak	C C stretching (alkyne)
1693.92447	Sharp peak	C=O stretching (carbonyl)
1616.42036	Sharp peak	C=C stretching (alkene)
1433.16913	Sharp peak	C-H bending (alkanes)
1335.96059	Sharp peak	C-O stretching (ester, ether)
1205.25452	Sharp peak	C-N stretching (amine)
1022.00328	Sharp peak	C-O stretching (alcohol)
838.09524	Sharp peak	C-H bending (aromatic)
746.14122	Sharp peak	C-H bending (aromatic)

4.6 Result

Revealed various characteristics band values with different functional groups in extracts of selected medicinal plants.

Chapter 5

Discussion

Nature's bounty of medicinal plants harbors a wealth of bioactive phytochemical compounds exhibiting demonstrably efficacious pharmacological properties. These naturally occurring products present a persuasive alternative to established treatment regimens. For an extended period, researchers have relentlessly pursued the discovery of novel phytomolecules with potent pharmacological activity within the plant kingdom, particularly focusing on herbs. This targeted investigation holds immense promise for tackling a diverse array of pathological conditions, encompassing cancer, cardiovascular diseases, and metabolic disorders.

This research project looked at three plants: *Viola canescens, Equisetum debile* and *Zanthoxylum armatum.* I wanted to find out what medicinal effects they have and what chemicals they contain. These plants have been studied before and shown to be helpful for treating different diseases, like diabetes, cancer, and inflammation.

In my analysis the mixture I made has high levels of phenolics and flavonoids, which are chemicals known to have health benefits. This isn't surprising because the plants used by me have been studied before and shown to be helpful for medicine. The results from this study suggest that the chemicals which were found in the plants might be the ones that have medicinal effects. These plants seem to be a good source of valuable chemicals that could be used as medicine.

In this study I identify phytochemical analysis and pharmacological activities of *Viola canescens* (banafsha), *Equisetum debile* (trotu ghas) and Z*anthxylum armatum* (timar).Phytochemical analysis shows that all of these plant extracts contain huge content of phenols and flavonoids in them. *Viola canescens* with highest amount phenolic and flavonoid content shows that this plant is highly valuable in therapeutic research. I also performed antioxidant activity with these plants. As *Zanthoxylum armatum* has huge amount of phenolic and flavonoid content so it shows more antioxidant property than other plant extracts.

On the other hand, I also perform pharmalogical activities like antibacterial, antifungal and anticoagulant. Only anticoagulant shows positive response while antibacterial and antifungal shows no result. But past studies shows that these plant extracts exhibit a strong antibacterial and antifungal property. Reason of zero results may be due to change of region from where these plants were collected.Change in soil, humidity, temperature, altitude may affect their properties whereas these plants possess strong anticoagulant property [127].

Different bioassays were performed to analyse the pharmacological efficiencies and phytochemical properties of these plants. In phytochemical analysis I determine total phenolic and flavonoid content in *Viola canescens*, *Zanthoxylum armatum* and *Equisetum debile*. It was observed that all three plant extracts contain phenols and flavonoids in them but *Viola canescens* has highest content of these phytochemicals with 19.68± 0.27mg GAE/g flavonoid and 111.18 ± 2.06 mg GAE/g phenolic content. Scientists studied a plant *V. canescens*, to see how altitude affected its levels of natural antioxidants. These antioxidants are total phenolics (TPC) and total flavonoids (TFC). Analysis revealed *Viola canescens* generally possessed the highest levels of total phenolic content (TPC) and total flavonoid content (TFC) compared to other investigated plant species. However, the precise quantities of these antioxidant compounds exhibited variation across geographical locations. Interestingly, a distinct pattern emerged regarding TPC and TFC levels. These values initially displayed a decline with increasing altitude, followed by a moderate increase at mid-elevations. Subsequently, a further decrease was observed at the highest altitudes. The researchers posit that environmental factors or the stress

response elicited by high altitudes might be responsible for this observed rise and fall in TPC and TFC.

While in pharmacological activities like (antibacterial and antifungal), anticoagulant and antioxidant result was quite different. It was observed that all of these extracts *Viola canescens*, *Zanthoxylum armatum* and *Equisetum debile* showed no significant antibacterial activity against Staphylococcus aureus and Klebsiella pneumoniae.In antifungal activity, no zone was observed against Aspergillus niger. Researchers have investigated how altitude affects the ability of Viola species to fight microbes. They measured this ability by looking at the 'zone of inhibition,' which is essentially the area around the plant extract that's free of microbes. In general, plants from higher altitudes seemed to have a larger zone of inhibition, suggesting they were more effective against microbes. However, some high-altitude plants also showed weak or no antimicrobial activity. This suggests other factors besides altitude might be influencing the plant's effectiveness, such as the specific environmental conditions where the plant grew. Overall, the observed link between altitude and the zone of inhibition suggests a potential connection, but there are exceptions [127].

The highest antioxidant capacity measured by using DPPH free radical scavenging assay at 1,5 and 50 μg/mL concentrations. It was observed *Zanthoxylum armatum* has highest antioxidant capacity4.35$\pm$ 0.04 followed by *Viola canescens* 5.03$\pm$ 0.14. It means both have high antioxidant property due to high phenolic and flavonoid content.Antioxidant capacity increases with the increase of concentration. Forth pharmacological activity performed in this study was anticoagulant activity.Viola canescens shows the best clotting time nearly equal to positive control while the samples *Zanthoxylum armatum* and *Equisitem debile* appear to clot much faster than the positive control, indicating potentially abnormal clotting activity.

Finally, regardless of the widely recognized antioxidant capacities of these plants, this research indicates Viola canescens can stand out as a comparatively more valuable plant source of natural bioactive compounds to produce medicinal products with an opportunity to not only promote human wellness but also improve bio-valorization and the natural world.

FTIR spectroscopy is a valuable asset for the identification of unknown polymers, providing a rapid and reliable method for classifying materials. However, its effectiveness is influenced by factors such as the complexity of the polymer structure and the availability of reference spectra. By combining FTIR with other analytical techniques, it is possible to achieve more accurate and comprehensive polymer identification, enhancing the overall reliability of material analysis.

Given findings of this investigation, it is reasonable to believe that the samples extracted have strong antioxidant potential; this could be due to the significant TPC and TFC levels discovered in them. Most of the bioactive chemicals found in these plants may be extracted using methanol, which is a polar solvent. As a result, these can be employed as natural sources of phenolics, flavonoids, and antioxidants to treat various disorders.

Chapter 6

Conclusion and Recommendations

The results confirmed the existence of medicinally significant components in the plants examined. Numerous proofs were accumulated in previous investigations that validated the discovered phytochemicals to be beneficial. Multiple investigations have demonstrated that the existence of these phytochemicals contributes both pharmacological and physiological properties to the plants researched for the therapy of various diseases. As a result, extracts from these plants could be considered a promising source of therapeutic medications.

Traditional medicine has high recommendations for these herbs, and additional research should be conducted to separate, purify, and define the chemicals that are responsible for their efficacy. Additional research is recommended to identify the probable path for the effects of these extracts.

The present research's results actually explain the anticoagulant properties of active bio-ingredients found in the methanolic extract of Viola canescens. The extract verifies the plant's primordial use as a curative medication for a variety of ailments. More immune-toxicological and in-vivo investigations with model animals are required to check the efficacy and dosage of the phytochemical.

The rise of antibiotic resistance poses a serious threat to global health. In this critical scenario, the exploration of antibiotic activity in herbal remedies offers

a promising new avenue for combating resistant pathogens. Medicinal plants, particularly those with minimal prior investigation, represent a vast reservoir of potentially bioactive compounds. The remarkable diversity of these phytochemicals has already shown promise as antimicrobials and modulators of bacterial resistance. Therefore, focusing research efforts on the discovery and isolation of novel bioactive molecules from these plants is crucial for the development of new therapeutic strategies to combat the growing challenge of antibiotic resistance.

While the discovery of new bioactive compounds from plants offers exciting possibilities, significant hurdles remain before their therapeutic potential can be fully realized. Extensive in vitro and in vivo testing is crucial to ensure the selection of safe and effective plant-derived antimicrobials. Furthermore, unraveling the complex interplay of compounds within and between medicinal plant extracts presents a major challenge. These interactions can be synergistic, enhancing the overall antimicrobial activity, or antagonistic, potentially negating the desired effects. Deciphering these interactions is essential for optimizing the therapeutic potential of these novel compounds.

Advancements in biotechnology hold immense promise for unlocking the full potential of medicinal plants. These advancements will enable me to delve deeper into the complex chemical composition of these plants. Sophisticated techniques for extraction, fractionation, and identification of bioactive compounds will become increasingly available. This, in turn, will allow me to explore the vast array of chemical structures and mechanisms of action present within these natural resources. By harnessing the power of biotechnology,I can usher in a new era of drug discovery, leveraging the unique therapeutic potential offered by medicinal plants.

Standardized methods for extraction and in vitro testing are crucial for a systematic and efficient exploration of bioactive compounds in medicinal plants. This standardization allows for more reproducible results across different studies, facilitating accurate interpretation and comparison of data. However, current research often focuses on isolated compounds rather than the complex mixtures present in plant extracts. The future holds promise for the development and application

of reference models specifically designed to evaluate the activity of these plant extract mixtures. By implementing these standardized approaches and reference models,l can significantly enhance the effectiveness and reliability of our search for novel therapeutic agents from medicinal plants.

High priority should be placed on elucidating the mechanisms of action, potential interactions with existing antibiotics and other medicinal compounds, and the pharmacokinetic and pharmacodynamic profiles of promising plant extracts. Understanding how these extracts exert their therapeutic effects is crucial for optimizing their use and minimizing potential side effects.

Furthermore, investigating interactions with other medications can ensure safe and synergistic treatment strategies. By characterizing the absorption, distribution, metabolism, and excretion (pharmacokinetics) and the relationship between dose and response (pharmacodynamics) of these extracts,I can pave the way for their efficient and successful clinical application. Addressing the identified challenges associated with the exploration of medicinal plants will ultimately lead to the development of more streamlined and effective methods for bringing these novel therapeutic agents to fruition.

This study has identified *Viola canescens, Zanthoxylum armatum,* and *Equisetum debile* as promising sources of bioactive constituents with a wide range of potential pharmacological effects. These findings highlight the exciting therapeutic possibilities offered by these medicinal plants. Notably, Viola canescens demonstrated significant antioxidant and anticoagulant properties, suggesting its potential applications in the fields of herbal medicine and even cancer genetics.

6.1 Recommendations

Future research holds immense promise for unlocking the full potential of these plant extracts. Nanobiotechnology offers exciting possibilities for exploring the use of these extracts in nanoformulations, potentially enhancing their therapeutic efficacy and delivery. However, continued exploration must be balanced with the sustainable use of these valuable resources.

Viola canescens, in particular, plays a crucial role in traditional healthcare systems, highlighting its medicinal importance. This widespread use, coupled with its threatened status according to the IUCN, necessitates practical conservation efforts. Implementing both ex situ and in situ conservation strategies is paramount to ensure the continued availability of these plants for future generations. Further research is warranted to elucidate the specific mechanisms underlying these activities and explore their efficacy in pre-clinical models. By delving deeper into these promising leads, I can unlock the full therapeutic potential of these plants and contribute to the advancement of natural medicine.

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