



## Anticoagulant, Antioxidant, and Antimicrobial Activity: Biological Activity and Investigation of Bioactive Natural Compounds Using FTIR and GC-MS Techniques

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### Abstract:

Patients suffering from coagulation abnormalities can benefit from the use of anticoagulants as therapies. These plants have anticoagulant characteristics, and it has been established that medicinal plants have been used throughout history as a conventional treatment for this illness. Haemorrhage or bleeding that is brought on by damage or injury to blood vessels can be stopped by the body through a homeostatic mechanism known as blood coagulation. A number of diseases, including haemophilia, which is brought on by deficiencies or absences of certain coagulation factors, have the potential to be extremely dangerous and even sometimes fatal. It is possible for abnormal blood clots and needless coagulation to obstruct normal blood flow, which in turn prevents oxygen and nutrients from reaching the tissues that are next to the affected area. As a result, the use of medicinal plants in institutions ought to be taken into consideration, particularly in situations in which patients make the decision to buy and use herbs without first consulting a medical professional. A wide variety of medicinal plants have a significant capacity for antioxidants. A wide variety of human disorders, including as cancer, cardiovascular diseases, and inflammatory diseases, can be effectively treated with antioxidants because they lessen the oxidative stress that occurs within cells. Extracts from the stems, roots, bark, leaves, fruits, and seeds of a number of significant medicinal plants are examined in this research to determine their possible antioxidant properties. However, many plant species have antioxidant potentials that are comparable to those of synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). These synthetic antioxidants are often utilised as food additives. In the fight against pathogenic microorganisms, chemicals derived from medicinal plants might offer fresh and uncomplicated techniques. The antibacterial activity of medicinal plants is the subject of this discussion, with particular emphasis placed on the challenges that are ongoing and the prospects for the future. Although medicinal plant extracts have been shown to be effective against microorganisms, there are certain inherent difficulties associated with their use.

## Introduction:

Controlling the harmful effects that microorganisms have on the environment is becoming an increasingly essential topic as an increasing number of people are concerned about the viability of human life. The human body and the settings in which it lives are home to a diverse collection of microorganisms that coexist in a state of biological equilibrium. However, the unchecked and rapid development of bacteria can result in a number of potentially hazardous issues [1]. The use of antimicrobial agents as antibiotic medications is intended to manage infections that occur within the human body; nevertheless, these agents have the potential to induce a variety of adverse consequences, particularly an increase in reactive oxygen species (ROS) within the human body [2]. Reactive oxygen species (ROS) pose a significant threat to human health and well-being, as they are involved in the development of cancer and can also raise the likelihood of adverse health effects. Different kinds of plants are included in the category of herbal materials that are utilised as medicinal plants. A significant number of these herbal compounds have therapeutic properties, including antioxidant, anticancer, anti-inflammatory, antibacterial, and antiviral properties. Moreover, these herbs have the potential to play a primary role in the production and development of pharmaceuticals. These materials have been shown to play a key role in a variety of biological applications, including the treatment of neurological diseases, the treatment of cardiovascular diseases, the treatment of cancer, and the regeneration of skin [3].

In ancient cultures in Africa, Europe, the Americas, and particularly in Asia, herbal medicines were the primary means by which medical tasks were carried out [4]. When it comes to treating infections, herbal medications are the major form of treatment in several impoverished countries. Extraction of herbal materials represents ongoing efforts to discover novel compounds that have the ability to inhibit the growth of bacteria [5]. Several studies have demonstrated that various herbal medicines are

sources of a wide variety of molecules. A significant number of these molecules include antibacterial and radical scavenger capabilities, which enable them to protect the human body from pathogens and also to provide protection against cellular oxidation reactions [6, 7]. Therefore, these materials are important in the process of synthesising various kinds of herbal medicine due to the fact that they have the power to inhibit the growth of microorganisms, viruses, and antioxidants [8, 9]. Due to the fact that these various compounds have the ability to control and inhibit pathogens while causing minimal damage to cells, they are currently being investigated as potential materials for research on new antimicrobial medicines.

Antibiotics and other chemical antimicrobial agents, such as metal oxide and metal nanoparticles, have the ability to limit the growth of bacteria; nevertheless, they can increase the production of reactive oxygen species (ROS) and have other adverse consequences. On the other hand, herbal medicines including clove, portulaca, tribulus, eryngium, cinnamon, turmeric, ginger, thyme, pennyroyal, mint, fennel, chamomile, burdock, eucalyptus, primrose, lemon balm, mallows, and garlic have the ability to eradicate germs by virtue of their ability to serve as a free radical scavenger [10]. There are a variety of different types of bacteria that these herbal medicines are effective against, and the antibacterial activity of these herbal medicines varies depending on the extract and essential oil that they include. In this light, it was exhibited [11] that the minimum inhibitory concentration (MIC) of these herbal medicines such as the essential oil of thyme towards various bacteria is in the range of 50–400 ppm for *E. coli*, *S. aureus*, *P. mirabilis*, *S. typhimurium*, *P. vulgaris*, *Y. enterocolitica*, *S. marcescens*, *B. licheniformis*, *P. putida*, *S. flava*, *P. fluorescens*, *L. innocua*, *Micrococcus spp.*, and *B. thuringiensis* [12]. In spite of this, there are other studies that have analysed the advantages and disadvantages of nanofibrous dressings. These dressings have a large surface area and the ability to release

antibiotics, herbal medicine, and antibacterial agents locally into the wound in order to treat infection. By preventing the production of reactive oxygen species (ROS), herbal medications have the potential to operate as direct antioxidants. As a result, they can stop the pathways that lead to the intentional death of cells. As a result, the defence mechanism is activated prior to the apoptotic pathway being triggered by glutamate [13]. This is because the concentration of reactive oxygen species (ROS) is lower. The powerful antioxidant and antibacterial effects of these herbal remedies are frequently demonstrated by the presence of phenolic components in the extract or essential oil of these medications. Therefore, herbal medicines are frequently favoured over synthetic antimicrobial agents due to the fact that they are less toxic and do not cause any adverse effects. This is because these herbal remedies have a strong antibacterial and antioxidant capacity, which means that they have the potential to be effective for the healing of any kind of wound [14]. In addition, herbal medicines have a great potential for antibacterial activity and are more affordable. In order to determine whether or not herbal medication is beneficial to human health, the bioavailability of the medicine is the most important factor to consider. In recent years, the concept that natural medicines are safer than prescribed prescriptions has gained traction, which has directly contributed to a significant increase in the number of applications for phytopharmaceuticals [15].

#### **Anticoagulant Activity:**

It is common practice to refer to the coagulation process as a "waterfall" or "cascade." These terms are used to describe the sequence of events that occur in the formation of a blood clot after an injury. These events are triggered by the activation of a cascade of proteins that are associated with clotting. Over the course of the coagulation cascade, a series of enzymatic reactions take place. Each of the molecules that are involved in the coagulation process is referred to as a "factor." It is usual practice to identify these coagulation factors by employing a predetermined Roman

numeral that is selected in accordance with the order in which they were discovered, and by utilising a lowercase "a" to signify the active form [16]. During these reactions, a zymogen, which is a precursor to an enzyme that is not yet active, and its glycoprotein cofactor are transformed into active components. These components subsequently catalyse the succeeding reaction in the cascade. An active enzyme will cleave a part of the subsequent inactive protein in the cascade, so activating it. This process will ultimately result in the synthesis of fibrin that is cross-linked.

By making it more difficult for the blood to coagulate, anticoagulants prevent the creation of blood clots or the expansion of existing ones, and they also make it more likely that clots will vanish. When an injury occurs to a blood artery or tissue within the body, and bleeding ensues as a result, the body begins a process of clot creation at the site of the damage in order to assist in stopping the bleeding; this mechanism is known as hemostasis [17, 18]. Platelets will clump together and become activated at the location of the damage as this process is still in progress. Additionally, the activation of coagulation factors, such as fibrinogen, occurs concurrently with the beginning of the coagulation cascade. Thrombin is responsible for the transformation of fibrinogen into insoluble fibrin strands that cross each other. This results in the formation of a fibrin network that is adhered to the primary site of the lesion.

#### **Antioxidant Activity:**

One definition of antioxidant activity is the property of a certain substance that, when present in low quantities, has the ability to inhibit or reduce the oxidation of the substrate it is interacting with. An antioxidant is a substance that takes action against free radicals, which are known to have a detrimental impact on biological systems, by neutralising them by electron donation or by destroying processes [19]. This inhibition or neutralisation can be assessed in vitro using chemical methods (such as ABTS, DPPH, FRAP, ORAC, and CUPRAC) as well as biochemical methods (such as the oxidation of low-density lipoprotein (LDL) assay and the

thiobarbituric acid reactive substances (TBARS) assay). Despite this, the first ones are favoured above the second ones because of the ease of use, the quickness, and the less expensive nature of the first ones. Phenolic compounds, flavonoids [20, 21], anthocyanins, and polysaccharides [23] are some examples of phytoconstituents and metabolites that have been proven to possess antioxidant capabilities due to their potential to scavenge free radicals. As a result of the fact that many of these compounds are frequently found in natural sources, the investigation of their antioxidant qualities is an essential component of virtually every study that is conducted on biological activities.

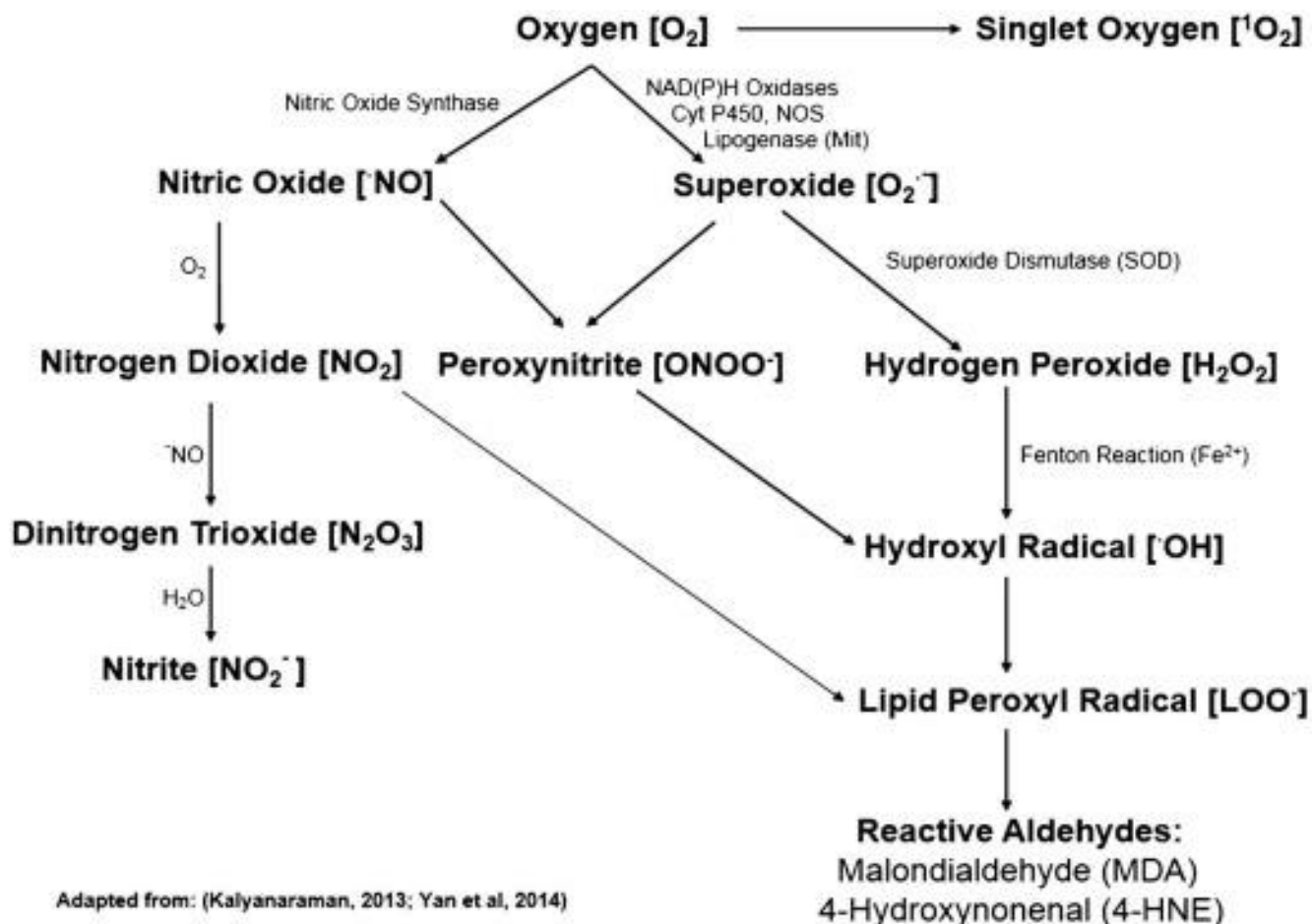
Assays that are based on chemical reactions can be divided into two categories: those that are based on the hydrogen atom transfer (HAT) reaction and those that are based on the same electron transfer (ET) reaction. However, there are several that combine the two systems (for example, DPPH and ABTS). It is important to note that a number of techniques, such as ABTS, DPPH, and FRAP, have limited biological relevance. This is due to the fact that these techniques evaluate the inhibition of radicals that do not exist in biological systems (for example, DPPH• and ABTS•+) or the reducing capacity based on a single particular ion (Fe<sup>3+</sup>). This is not the case with the oxygen radical absorbance capacity test (ORAC), which measures the inhibition of the biologically relevant peroxy radical (ROO•), and chemiluminescence, which may detect the quenching capacity of various oxygen and reactive nitrogen species [24]. Both of these assays come with their own unique set of advantages and disadvantages. Generally speaking, the selection of a particular antioxidant method is mostly determined by the ease of use, the cost, and the kind of material being examined. In spite of this, the linking of numerous chemical tests to high-throughput or automated systems (for example, high-performance liquid chromatography) [25] makes the analysis easier

and saves time, which in turn makes it possible to employ simultaneous applications of more than one approach. Given the lack of a biological system and the components that make up that system, it is advised that the antioxidant activity of natural products be tested using assays of biological relevance or complementary assays (for example, chemical and biochemical). This will prevent the possibility of over- or under-estimating the antioxidant activity of natural products.

On the other hand, the utilisation of methodologies that are biologically irrelevant has the potential to offer insights into the antioxidant characteristics and to direct the research towards quantifying methods that are more suitable, such as in vivo assessments. The development of electrochemical sensors and biosensors is the primary focus of research that will be conducted in the future. These sensors have the potential to be utilised for the rapid quantification of complicated samples in tiny amounts, and they also have the potential to provide new information regarding the kinetics and mechanisms involved in the antioxidant activity [26].

### **Antioxidant, Oxygen Radical Absorbance Capacity (ORAC), Description and Advantages:**

It is the so-called reactive oxygen species (ROS) that are responsible for oxidative stress. Free radicals are included in this category. According to Halliwell and Gutteridge (1984), a free radical is defined as any atom (such as oxygen or nitrogen) or group of atoms or chemical species that is capable of independent existence and contains at least one or more unpaired electrons in the configuration of the outermost shell. Molecular oxygen yields reactive oxygen species (ROS), which are metabolites of molecular oxygen that have a higher reactivity than molecular oxygen and are continuously produced as by-products of normal aerobic metabolism.



When a cell is subjected to a greater quantity of reactive oxygen species (ROS) than it is able to destroy in an instant, oxidative stress emerges. Stress and disease circumstances both lead to an increase in the production of reactive oxygen species (ROS). It is also possible for ROS to be absorbed from the surrounding environment. Damage to nucleic acid bases, lipids, and proteins is a significant consequence of oxidative stress. This damage can severely compromise the functioning and viability of cells, or it can induce a variety of cellular responses through the generation of secondary reactive species, which ultimately results in the death of cells through necrosis or apoptosis. There is a well-established fact that oxygen free radicals and their metabolites have the ability to cause direct cell damage, which can then set off a chain reaction of radical reactions that can lead to the development of illness. There are a number of degenerative disorders that are associated with ageing that can cause oxidative stress, and oxidative stress can also be a factor that contributes to the

development of other diseases. A number of diseases, including but not limited to arthritis, atherosclerosis, cancer, diabetes, infection, ischemia, and Parkinsonism, are all associated with the presence of reactive oxygen species or oxidative stress as a component of the clinical condition [27]. A component of oxidative stress can also be found in other conditions, such as shock, frostbite, and damage caused by radiation. There are endogenous mechanisms or antioxidants that are present in the human body. These mechanisms serve the purpose of protecting the body from the formation of oxidative stress and of maintaining redox equilibrium within the cells of the body.

### The Fourier Transform Infrared (FTIR) spectroscopy:

In addition to being utilised for the examination of synthetic mixes, molecular absorption spectroscopy has been utilised extensively for the quantitative determination of chemicals in a variety of formulations. Due to the fact that the majority of active compounds absorb in the

ultraviolet region and exhibit heavily overlapped spectra, the utilisation of these techniques has the intrinsic problem of preventing the simultaneous determination of these compounds. Through the use of Fourier Transform Infrared (FTIR) spectroscopy, it is possible to analyse a significant quantity of information regarding the composition and structure characteristics of plants. Although the analysis can be carried out on both pure substances and complex mixtures, it does not

require the separation of the components into their constituent parts. When compared to colorimetric approaches, infrared spectrometry proves to be more sensitive and selective. The Fourier transform infrared spectroscopy (FT-IR) is a well-established approach that saves time in the process of characterising and analysing microorganisms, as well as monitoring biotechnological activities. Here are some examples: **Figure 1, 2, 3.**

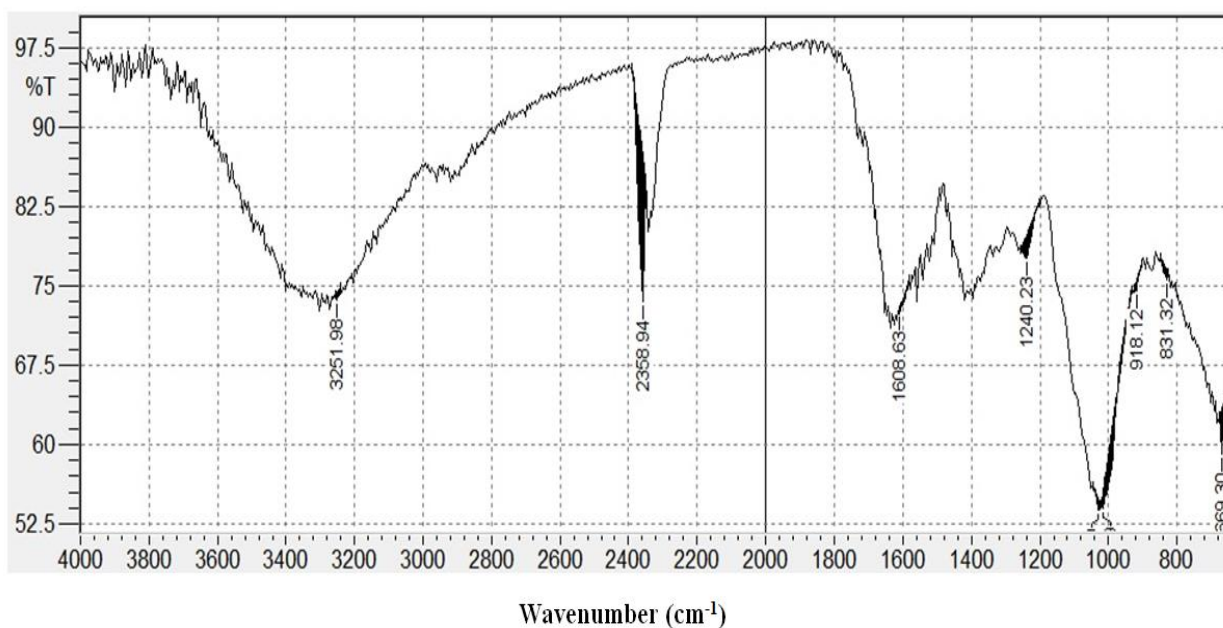


Figure 1. Fourier-transform infrared spectroscopic profile solid analysis of *Harpagophytum procumbens*.

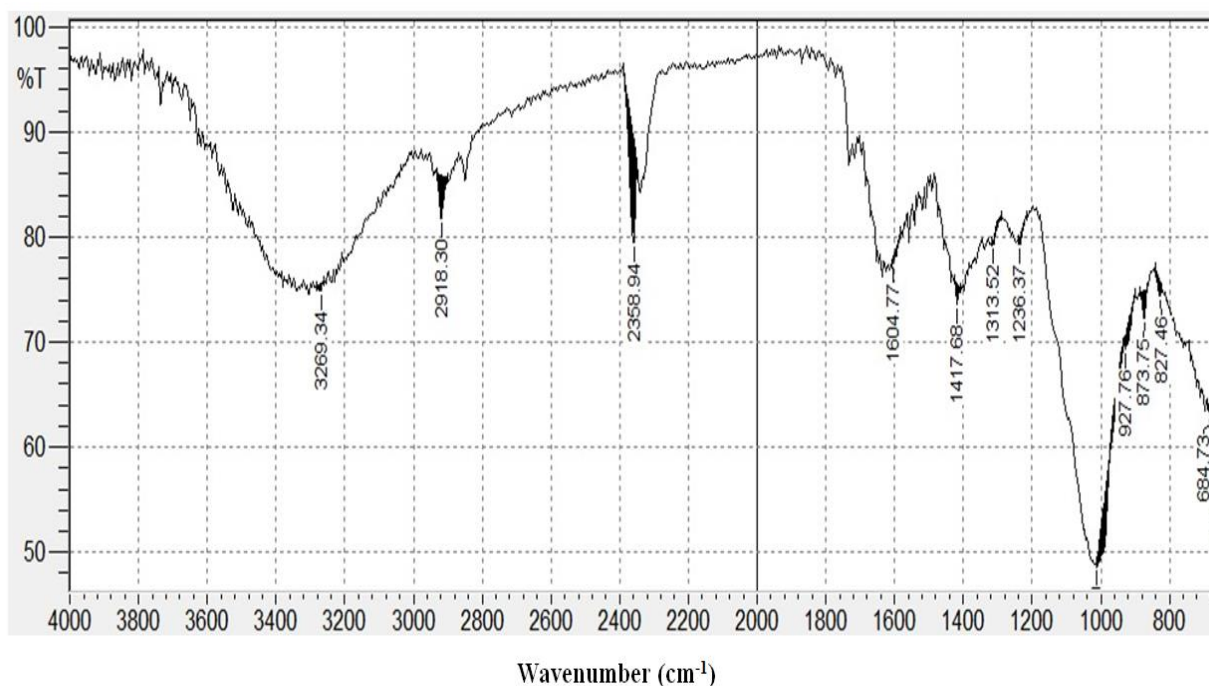


Figure 2. Fourier-transform infrared spectroscopic profile solid analysis of *Cordia myxa*.

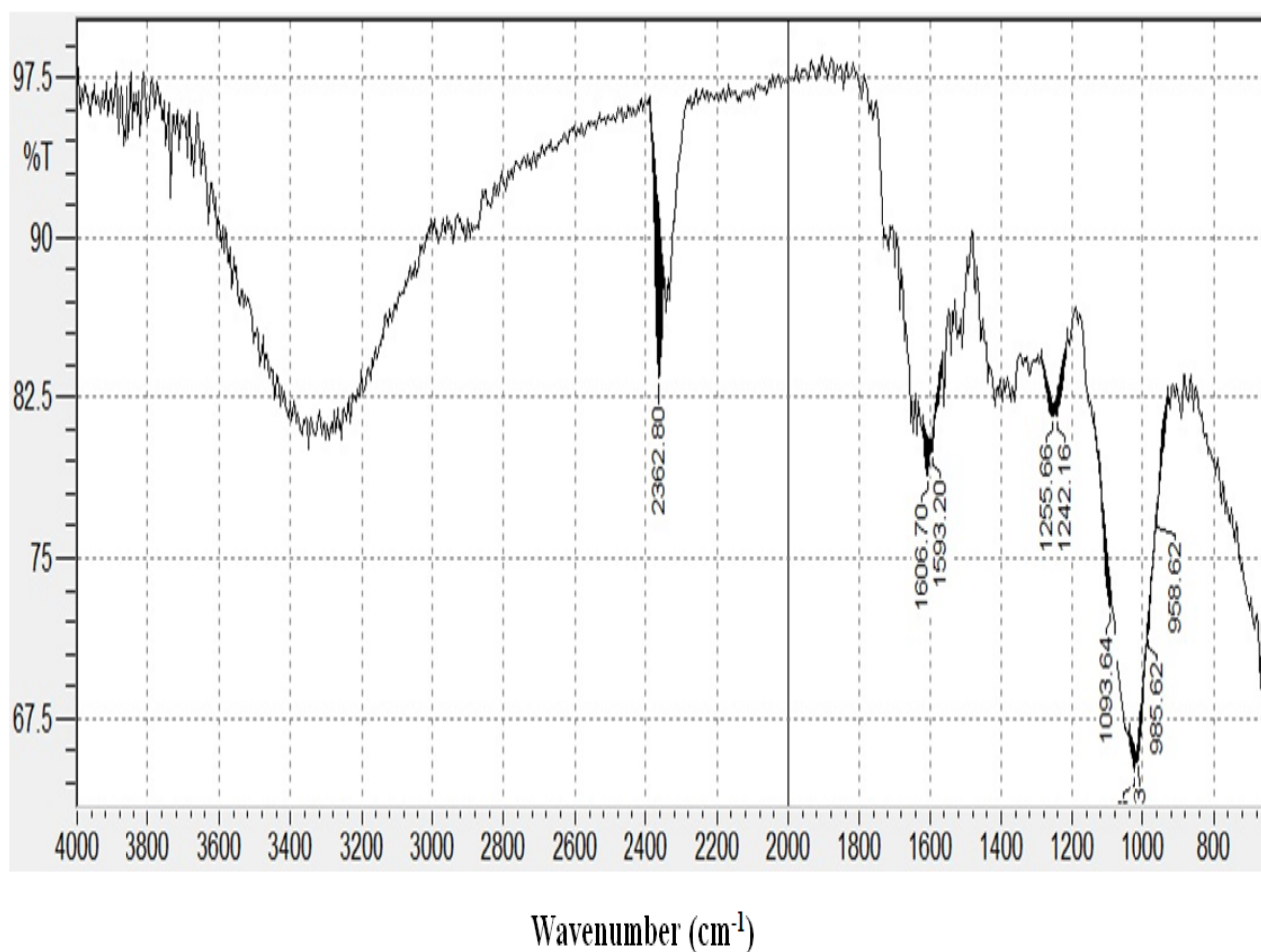


Figure 3. Fourier-transform infrared spectroscopic profile solid analysis of *Mentha pulegium*.

### Ferric reducing antioxidant power (FRAP), Description and Advantages:

The Ferric Reducing Antioxidant Power assay, also known as FRAP, is based on the reduction of a colourless Fe<sup>3+</sup>-TPTZ complex into a vivid blue Fe<sup>2+</sup>-TPTZ after the complex interacts with a possible antioxidant. This technique proved to be useful for screening antioxidant capabilities and comparing the efficacy of various chemicals, and it did so at a reasonable cost. In light of this, the FRAP method was utilised in this study for the purpose of determining the antioxidant activity of a number of different phenolic acids. It is interesting to note that the precise mechanism of the antioxidant activity of these compounds, as well as the influence of the structure of the compounds on their activity, has not yet been fully explored and is a contentious topic in the scientific

literature [28, 29]. During the course of the experimental research, computational chemistry was found to be an effective help. In order to expound on the most likely mode of action of antioxidants, not only the hydrogen atom transfer (HAT) mechanism, but also the sequential proton loss-electron transfer (SPLET) and the single electron transfer-proton transfer (SET-PT) mechanisms have been extensively researched using density functional theory (DFT) techniques [30-33]. The obtained results made it abundantly evident that the SPLET mechanism of action is the most advantageous one in a polar solvent, whereas the HAT mechanism is the most dominant one in benzene. By providing an explanation of the rationale that was employed in the experiment, the presented computational results are a complement to the experimental studies. The fact that this is the case demonstrates the significance of

computational chemistry approaches as a supplementary instrument in any modern-day research endeavour.

### **Antimicrobial Activity:**

Since ancient times, there has been widespread optimism over the potential of plant extracts as a potential antibacterial medicine. On the other hand, over the course of the past few years, approximately 250,000 higher plant species have been described. Of these, only 17% have been evaluated according to biological aspects, and only 10% have been evaluated for antimicrobial activity and several classes of secondary metabolites (such as alkaloids, flavonoids, saponins, and so on) [34]. The phenomenon known as antimicrobial resistance (AMR) poses a substantial risk to human health all over the world.

There were approximately 4.95 million deaths that were attributed to antimicrobial resistance in the year 2019, with 1.95 million of those deaths being connected with diseases caused by fungi, primarily *Candida*, *Aspergillus*, and *Cryptococcus* respectively [35]. *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* are the major bacteria that are responsible for the majority of fatalities that are attributed to antibiotic resistance. Antibiotics when combined with plant extracts have a synergistic impact against bacteria that are resistant to antibiotics, which may result in the development of novel treatment options for infectious diseases in the event that the antibiotic is no longer effective during therapy [36].

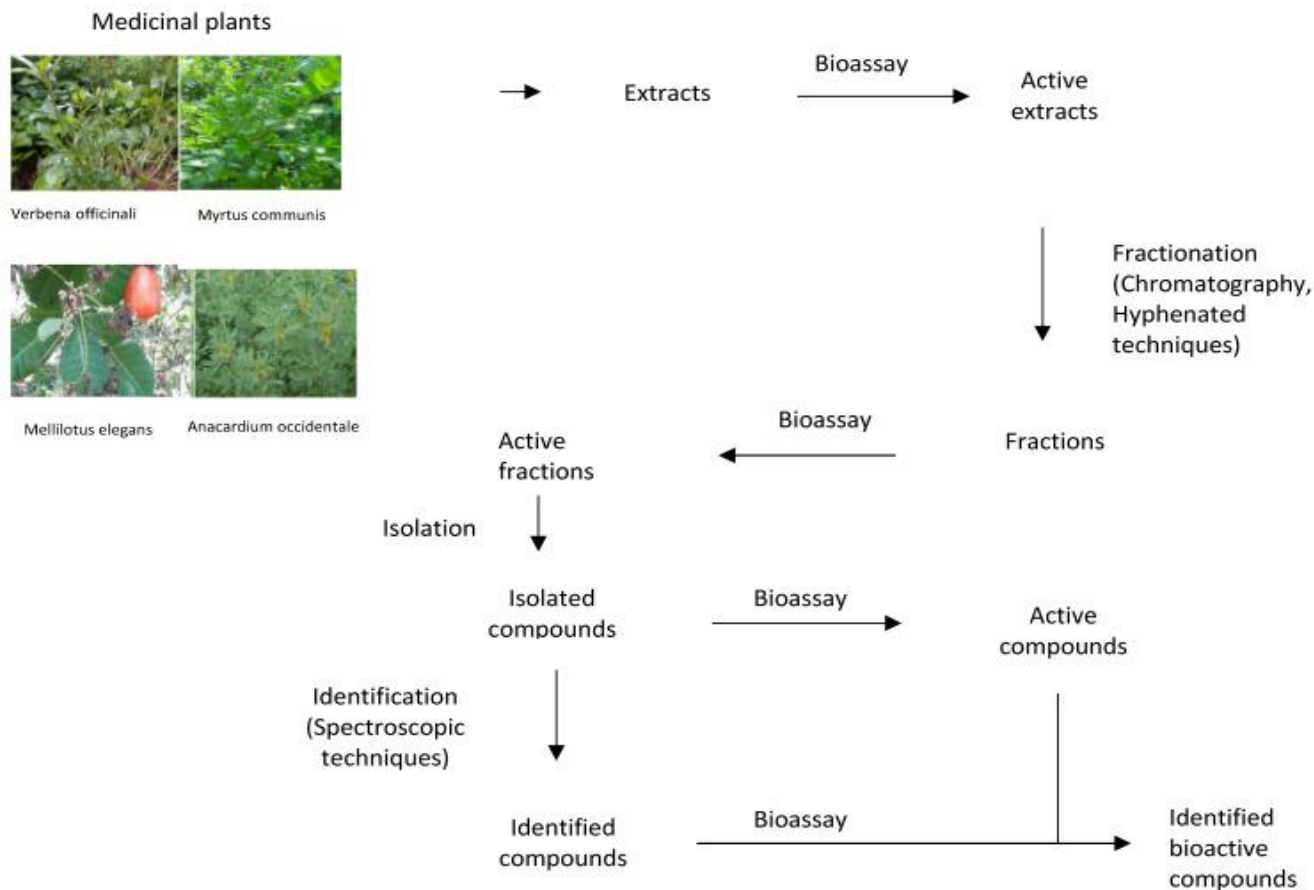
The Clinical Guidelines of the European Committee on Antimicrobial Susceptibility Testing (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) are designed to standardise testing for clinical purposes. However, when it comes to natural extracts, these guidelines are also utilised because there are no standards that are specific to natural

extracts [37]. Therefore, it is of the utmost importance to take into consideration the constraints that this entails.

Natural extracts, for instance, might not be hydrophilic, and there is no established database of lowest inhibitory doses for combinations of molecules, thus it is difficult to compare the outcomes of these experiments. Because there is a lack of standardisation regarding the methods that are utilised to establish antimicrobial activity, it is not always possible to compare the results of different investigations that are focused on natural compounds [38]. In spite of the fact that new technologies have been developed to evaluate antimicrobial activity, the old technologies are still the ones that are utilised the most for analysing bacteria and fungus. One example is the development of in vitro antimicrobial assessment methods in the 1960s. These methods were developed in response to the need for quick and straightforward tests that analyse a wide range of plant extracts or pure chemicals at a low cost [39]. Disc diffusion and broth or agar dilution assays are the reference methods; however, in the last several decades, new assessment methods have evolved to solve some of the shortcomings of old methods, [40] such as the reaction time, limited sensitivity, and repeatability. These methods have been developed to alleviate these disadvantages.

### **Utilisation of Antimicrobials obtained from Extracts of Medicinal Plants:**

The antimicrobials that are extracted from medicinal plants are natural, safer than synthetic alternatives, readily available in local communities, less expensive to purchase, and simple to administer. Furthermore, they have the potential to provide significant therapeutic effects and are more cost-effective than other treatment options. On top of that, extracts of medicinal plants have the potential to be an effective alternative treatment in the event that there are significant adverse effects and drug resistance [41].



**Figure: The process of discovering bioactive compounds.**

The current percentage of antibacterial compounds derived from medicinal plants that have been approved does not adequately reflect the potential of these compounds for future usage as antimicrobial medicines.

The utilisation of natural extracts from plants as antimicrobial medications presents a number of intrinsic problems, including the following:

According to the findings of recent research, medicinal plant components have to be utilised with caution in the lack of precise proof demonstrating their efficacy [42]. Rare are the instances in which well-controlled, double-blind toxicological and clinical investigations are conducted to demonstrate their efficacy and safety [43]. The utilisation of medicinal plants has been linked to the adulteration of valuable compounds, inadequate cultivation and collecting methods, a lack of standardisation during the preparation process, and inadequate storage conditions, which

eventually has an impact on the process of developing novel antimicrobials [44]. The quantities and actions of various chemicals in extracts can be affected by a variety of factors, including the season of harvest, the region of agriculture, the plant parts that are used, and the type of processing that is performed. It is possible that the comparison of the various literature data for the antibacterial activity of plant extracts could be troublesome due to the fact that the composition of plant extracts varies depending on the local climate and environmental factors. It is important to note that rainfall and humidity levels vary from one geographical region to another, which might have an effect on the composition and production of chemicals in medicinal plants of the same species that are grown in different geographical places. Furthermore, the global warming of the environment is another difficulty that is affecting the climatic conditions and, as a result, putting the composition of compounds and

their production in jeopardy, even in the same geographical regions.

It is challenging for scientists to create a map that takes into account all of the intricate interactions that could take place between the many different compounds that are present in a medicinal plant. Another intrinsic challenge is the ability to have a comprehensive understanding of the composition of plant extracts. This is due to the fact that plant extracts contain a large number of components, making them difficult to interpret [45, 46]. The process of isolating individual compounds that possess the requisite antibacterial activity can be time-consuming and may need a significant quantity of plant material. In addition, there are challenges associated with the rediscovery of the same molecules from multiple sources. It is therefore possible to achieve standardisation, stability [47, 48], and quality control; however, this is not a simple task. On the other hand, the possibility of doing research on a large number of chemicals that have not yet been discovered could be a factor in the renewed focus on medicinal plants. Synergism among compounds in a complex mixture provides a unique set of challenges due to the fact that the technology necessary to investigate several compounds acting on potentially multiple biological targets has not yet reached its full potential. When dealing with medicinal plant species, it might be challenging to make the necessary arrangements, particularly when dealing with a situation that involves overseas travel. Regulations pertaining to the collection of plants and the export and import of plants vary from one location to another, depending on where the study is being carried out.

As biotechnology continues to advance, it is inevitable that we will be able to conduct additional research into the chemical composition of medicinal plants and develop more advanced methods for the extraction, fractionation, and identification of bioactive compounds. These compounds are distinguished by a wide variety of chemical structures and mechanisms of action. The search could be more methodical if extraction and in vitro testing methods were standardised.

This would also make it easier to evaluate the results of the search. Standardisation would be beneficial. Furthermore, reference models have not yet been utilised in the research being conducted on plant extract mixes; nevertheless, subsequent research will disclose whether or not they are applicable to this course of action.

### **Conclusions:**

The natural molecules that are produced by plants, microorganisms, and insects have a high structural and physicochemical diversity, which makes them a virtually inexhaustible source. This diversity has the potential to be used for the discovery and development of novel bioactive compounds that have applications in a variety of industries, including the pharmaceutical industry, the food industry, and the biotechnological industry, to name just a few. In addition, the selectivity, specificity, and potency of many natural compounds are characteristics that are shared by all of them. This is because natural compounds have undergone continuous evolutionary specialisation over the course of millions of years. Considering the vast number of different biological molecules, as well as the numerous promising actions and evaluation methods, it is of the utmost importance to choose which tests are the most pertinent for the initial screening stage. It is usual practice to evaluate the compound in a variety of in vitro biochemical and pharmacological assays as the initial step in the process of biologically evaluating natural compounds.

There is a fresh hope for combating the serious threats that are posed by the growing evidence of antibiotic resistance, and that hope is the antibacterial activity of medicinal plants. As a result, there is an immediate requirement to recognise and isolate new bioactive chemicals from medicinal plants, which have not yet been thoroughly investigated. Antimicrobials and antimicrobial resistance modifiers have been shown to have therapeutic potentials, and the great diversity of these compounds has demonstrated that they have such potentials. The possible application of newly discovered bioactive

chemicals is still a difficult task. It cannot be overstated how important it is to emphasise the need of conducting thorough in vitro and in vivo experiments in order to guarantee the selection of antimicrobial plant-derived chemicals that are both effective and non-toxic. Exploiting the possible synergistic or antagonistic effects of chemicals within and amongst medicinal plant extracts is another significant hurdle that must be overcome.

Following the first identification of the biological potential derived from an extract or a natural source, it is also essential to identify the particular molecule that is accountable for the activity in question. By utilising techniques such as fractionation, nuclear magnetic resonance (NMR), high-performance liquid chromatography-mass spectrometry-mass spectrometry (HPLC-MS/MS), high-resolution Fourier transform mass spectrometry (FTMS), and photo-diode arrays, in order to identify specific metabolites or active chemicals that are present in the sample, this initial screening can be further supplemented.

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