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Quantitative Estimation of Secondary Metabolites and Comparative Pharmacological Efficacy of Phyllanthus Niruri and Vitex Negundo Extracts

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Abstract
Bioactive chemicals from medicinal plants have long been employed in traditional and modern treatment. Phyllanthus niruri Linn. and Vitex negundo Linn. are known for their antibacterial, anti-inflammatory, antioxidant, hepatoprotective, analgesic, and immunomodulatory effects. This study compares the phytochemical makeup and biological effectiveness of these two therapeutic plants based on significant secondary metabolites. Standards were followed to collect, authenticate, shade-dry, grind, and extract fresh plant materials using acceptable solvents. Alkaloids, flavonoids, tannins, saponins, glycosides, phenolic compounds, terpenoids, steroids, carbohydrates, proteins, and other bioactive ingredients were identified by qualitative phytochemical screening. Standardized analytical techniques were used to quantify phytochemicals such total phenolic and flavonoid contents. Free radical scavenging experiments investigated the extracts' antioxidant capacity, while traditional microbiological methods assessed their antibacterial efficacy against selected bacterial and fungal infections. The findings showed that Phyllanthus niruri and Vitex negundo had a wide range of phytochemicals with therapeutic characteristics. However, these chemicals' concentration and distribution varied, affecting their biological functions. Phyllanthus niruri has more phenolic compounds and flavonoids, making it more antioxidant and hepatoprotective. Vitex negundo has more terpenoids, flavonoids, and important bioactive chemicals, which may have increased its antibacterial and anti-inflammatory properties. The comparison stresses both species' pharmacological importance and their potential as natural sources for herbal and pharmaceutical formulations. The study also validates traditional therapeutic usage and advances medicinal plant research. The findings support the sustainable use of these plants in complementary medicine and encourage advanced phytochemical characterization, molecular studies, toxicity evaluation, and clinical validation to prove their efficacy and safety for future therapeutic applications.

Keywords: *Phyllanthus niruri, Vitex negundo, phytochemical analysis, medicinal plants, secondary metabolites, antioxidant, antimicrobial, comparative potency, bioactive chemicals, herbal medicine.*

I. INTRODUCTION

General Introduction to Medicinal Plants

Throughout the course of human history, medicinal plants have performed a unique and important role. When it comes to providing fundamental medical care, a significant number of developing countries depend largely on herbal treatments. In addition to being able to heal illnesses, plants may also be used to prevent them. Natural products derived from plants have a tremendous amount of untapped potential for new discoveries. Over the course of thousands of years, natural plant products have been used for a variety of reasons, including but not limited to the production of antioxidants, tastes, smells, colors, and insecticides. Over the course of a great number of millennia, people have been searching for new substances that may be used as medicines.

Ongoing research is being conducted on the issue of new plant-based medicines that have the potential to be used as agents. Some of these compounds are used as medications in modern medicine despite their potential dangers. The usage of several pharmaceuticals that are derived from plants is beneficial for the treatment of a broad variety of illnesses and ailments, and these treatments are not only safe but also cost-effective. At this point in time, the use of medicinal plants as a kind of treatment is well accepted all over the world. More than half of the prescription medications that are the most popular in the United States include natural chemicals or naturally occurring derivatives. Plants are capable of producing a wide variety of phytochemical compounds in their own environment.

There is still a great deal that we do not know about the pharmacological activity of natural chemicals that are plants-derived. Plant-based foods have been shown to lower the risk of developing pathological diseases, including cancer, neurological system disorders, cardiovascular disease, inherited diseases, and inflammatory diseases. This is because plant-based foods contain less calories than animal products. India, which is home to one of the greatest herb collections in the world, is the birthplace of a significant number of the plant-based medicines that are employed in Ayurveda, Unani, and Siddha. Treatments based on herbs are used extensively in

India. In order to treat a broad range of illnesses, Indians make use of a wide variety of medicinal plants and medications derived from plants. This is due to the fact that these remedies have less or no negative side effects. It is at the forefront of the pharmaceutical and cosmetic sectors, generating raw materials for completed drugs as well as bioactive compounds that are employed in the creation of these products. In traditional medicine, medicinal plants have found broad usage for a variety of reasons, including their cheap cost, widespread availability, fast responsiveness to medical situations, and simplicity of use.

Many of these factors have contributed to their widespread use. In recent years, several countries, both industrialized and developing, have made substantial use of phytodrugs, which are drugs that are produced from plants. These plants, which are known to possess medicinal qualities, are used to manufacture phytodrugs. The production of a number of different pharmaceuticals requires the use of latex, gum, and resins. As part of an attempt to record all of these plants, the World Health Organization has compiled a list of more than 20,000 different species of medicinal plants. Detailed information on the therapeutic qualities of plants and the components of plants is provided more specifically.

A dramatic growth in the number of businesses that are based on medicinal plants has occurred as a direct result of the rising interest in medicinal plants which is occurring all over the world. In spite of the many improvements that have been made in modern medicine, the production of new medications created from natural substances is still considered to be vital. It is possible to treat a wide variety of conditions using medicines that are derived from plants. These pharmaceuticals make up about half of all drugs that are now available. Plant-based medicines include a variety of pharmacological properties, including the ability to reduce inflammation, reduce fever, and alleviate pain.

According to the World Health Organization, between 65 and 80 percent of the world's population depends on medicinal plants and derivatives of medicinal plants as their primary source of healthcare. This is mostly due to the fact that poverty and restricted access to modern medicine are the primary factors contributing to this situation. Traditional medicines that are derived from plants have been an essential component of healthcare for a significant amount of time in many countries. When administered via traditional medical systems, the drugs are often administered in the form of a water decoction or an ethanolic extract. A significant number of medicinal plant components are used as unprocessed medicines because to the extensive spectrum of therapeutic benefits that they provide. Raw powder, fresh plant parts, or juice are the three most common ways that individuals take medicinal plants when they are eaten.

Advantages of phytomedicine

Using natural treatments instead of pharmaceutical drugs has several benefits.

- Reduced risk of side effects: Phytomedicines are generally well-tolerated and seldom cause serious adverse effects when taken regularly.
- Lower cost: herbal remedies are readily accessible, and they are much more affordable, than pharmaceuticals made of synthetic chemicals. Herbs are often less costly than pharmaceuticals.
- Widespread availability: You don't need a doctor's note to purchase herbs. Some basic herbs, like chamomile and peppermint, are easy enough to produce in your own garden. Herbs may be the only option for therapy for most people in certain far-flung regions of the globe.

Phytochemical Analysis

There are uses and advantages for phytochemicals that have been isolated from plants in the contemporary day. Ayurveda, which is based on the principles of plant healing, is something that we highly recommend. In the past, the practice of employing plant crude extracts to treat microbial, fungal, and deficiency illnesses was limited to India. However, this technique has now spread to other countries throughout the world. Natural treatments are currently being supported by a significant number of researchers for the treatment of certain disorders that have been effectively treated in the past with the use of phytochemical components. Ayurveda is another historical strength that India has. There is a growing interest in the study of plant species, and all professionals in the medical field are eager to give proper treatment. There is a long history of the use of herbal therapies for the treatment of infectious diseases. The unrivaled wealth of chemical variability found in plant products, whether in the form of pure chemicals or as standardized plant extracts, provides us with an infinite number of opportunities for fresh new therapeutic leads.

India, which is one of the most plentiful sources of medical plant genetic resources in the world, is a veritable treasure trove of all kinds of medicinal plants. Both the topography and the climate are very changeable, as can be observed in the morphology of the plants that make up the floristic composition. Furthermore, the agroclimatic conditions are perfect for the introduction of new exotic plant species and the upkeep of such species. Over the last several years, there has been a significant rise in the amount of research conducted on strong bioactive metabolites as possible pharmacological agents. The pharmacological activity theories have not had the opportunity to detect these substances until recently. A natural medicine is the primary source of health care for

about 80 percent of the world's population, particularly in countries that are still developing their national economies.

Because of the current state of the healthcare system, it is likely that the issues that are associated with synthetic pharmaceuticals will grow increasingly significant over the course of the next few years. In the future, scientists will find scientific research that is based on phytochemicals to be beneficial. If we are interested in determining the efficacy of phytochemicals in combating newly developing diseases such as COVID-19, we will need to develop new methods of assessing phytochemicals. The primary and secondary metabolites that are created by plant cells are the final recipients of the information flow that arises from biological processes. data of these metabolites, both qualitative and quantitative, may give essential insights into the cellular processes that determine the biochemical phenotype of the cell, tissue, or organism as a whole. These data can be used to reflect the state of the cell under certain conditions.

The older kinds of targeted phytochemical study are contrasted with metabolomics, which shows some significant distinctions between the two. Researchers in the area of plant metabolomics make use of a wide range of digital data processing methods and fundamental analytical techniques in order to accomplish their goal of investigating several aspects of plant biology. Phytochemical research is an extremely important part of the process of locating bioactive components in plants for the purpose of developing new medications and treatments. An additional hypothesis that has to be investigated is the existence of a universal biomarker and a systemic signaling cascade for malignancies. In order to further investigate the potential of this plant material as an effective anticancer medicinal chemical, it is important to conduct a complete examination into pharmacokinetics and metabolomics.

Theory of Phytochemicals Analysis

There are primarily three phases to the development of phytochemical analysis.

- Sample preparation.
- Feature extraction using analytical methods.
- Data collection using chemo metric methods followed by compound identification.

Sample preparation: The preparation of the sample is an important step that may be examined and provides a substantial contribution to the process of transforming a sample into a sample solution. This stage requires the sample to be prepared. The multiple changing steps of the process include a variety of components, including sampling techniques, plant growing, drying or enzyme quenching procedures, biochemical extraction, and analytical sampling. The first consideration that plays a role in determining the selection of plant material is the biological inquiry that the researcher intends to conduct. The extensive variety of phytochemical properties results in the production of a very complex spectrum of biomolecules that vary in terms of their sizes, solubility, volatility, polarity, quantity, and stability.

Feature extraction using analytical methods: The extraction processes of solvent extraction, solid phase extraction, high pressure homogenization, and supercritical fluid extraction are all examples of common extraction techniques. One other problem is that there is not yet a thorough extraction method that is capable of accurately and dependably recovering all of the respective components. Therefore, by combining different extraction methods, it is possible to compile a complete list of the various types of metabolites.

Data collection using chemo metric methods followed by compound identification: When the topic of the research is taken into consideration, the analytical framework that is most often used is determined by the kind and concentration of the compounds, as well as the chemical and physical features of the compounds. Consequently, the procedures that are used the most often include nuclear magnetic resonance spectroscopy and chromatography performed in conjunction with mass spectrometry.

Screening of Phytochemicals

The phytochemical properties of the four distinct plant tinctures were evaluated in four distinct environments: hexane, ethanol, hot aqueous (100 degrees Celsius), and cool aqueous (25 degrees Celsius). The identification of alkaloids was accomplished through the utilization of the following techniques: dragendorff, mayer, fehling's reagent for reducing sugars, borntrager's test for quinones, shinoda's test for flavonoids, bajlet's test for coumarins, liebermann's test for steroids or triterpenoids, and the gelatinous uniformity after cooling for mucilaginous substances.

A solvent polarity gradient is chosen in accordance with the approval of the procedure, and the compounds that are included within it are described and estimated with the use of gas chromatography–mass spectrometry. Motion may be seen in the gas phase, although the liquid phase does not undergo any changes. After combining the samples, the data on their emission spectra and UV radiation transmittance were compared in order to conduct the analysis. It is common practice to use UV spectroscopy to solutions of synthetic complexes or chemical compounds. This is due to the fact that the technique is strong enough to enhance the energy levels

of the outer electrons. The reason for this is because there was a change in the electrical potential. This descriptive application demands the capture of a section of the Luminescence spectral spectrum in order to evaluate the electrical or imaging capabilities of the materials being analyzed.

The identification of both primary and secondary metabolites may be accomplished by the use of techniques such as nuclear magnetic resonance spectroscopy in both one and two dimensions, as well as ultra-performance liquid chromatography-tandem mass spectrometry. In order to guarantee that the herb maintains its high standards and its original chemical profile to the maximum extent feasible, it is vital to optimize the processing operations. As a scientific approach for conducting bioanalysis, the use of nuclear magnetic resonance (NMR). The rapid evolution of sensor technology, chemical inhibitors, and pulse field gradients are all examples of this. Since 1990, a highly accurate approach for the online characterization of biomolecules has been available, which was driven on by the establishment of strong magnetic fields. This method was the beginning of all that has happened since then. For the purpose of conducting a comprehensive online structural evaluation of bioactive chemicals, LC-NMR stands out as an intriguing companion approach to LC-UV-MS in the field of phytochemical research. The value of the procedure has been validated by contemporary methods in an appropriate manner.

There is a method known as X-ray crystallography that makes use of X-rays, which are light that is scattered by crystals themselves. Due to the fact that their wavelength lies within the range of 8–10 Angstroms, X-rays are able to be scattered by electron clouds of the same size. After that, a technique is gradually transformed into the investigative electron density, which is then simplified with the use of statistical data, and lastly, a structure that is very accurate and precise is generated. X-ray crystallography and spectrum analysis were used in order to ascertain the structure of the chemical and to evaluate its stereochemistry.

Types of Phytochemicals

They are referred to as secondary components, while the remainder of the organic chemicals are referred to as primary components. Glycosides, phenolics, curcumines, plant steroids, lignans, flavonoids, and alkaloids are some examples of the ingredients that fall under this category. The most chemically diverse and diverse in type of phytochemicals found in plants are phenolics, according to research conducted on plant phytochemicals. The categorization of phytochemicals is determined by the functions that they perform; nevertheless, a single molecule with several functions, such as antioxidant and antibacterial, may be found in plants.

1. Phenolic: In the late nineteenth century, the French paradox was related to the high phenolic content found in red wine. Since then, plant phenolics have been the topic of research into the possibility that they possess anti-carcinogenic and anti-degenerative capabilities. Since that time, researchers have investigated the chemical identification of the phenolic content of a wide variety of plants, as well as their bioactivities, detoxification processes, and biosynthesis. The research on the long-term stability of phenolic compounds in food items and the preservation of these compounds has also arisen as an important subject requiring attention. Phenolic compounds are included in every single plant that belongs to the kingdom Plantae, making them the most prevalent kind of phytochemical substances. There is a clear connection between the hydroxyl group and an aromatic hydrocarbon group in phenolics, which is a category of chemical compounds that comprises these groups. Flavonoids, phenolic acids, and polyphenols are the three kinds of phenolic compounds that are most often found in dietary sources. Phenolics are substances and functions that have been the focus of a great deal of study carried out in the fields of pharmacology, chemical science, pharmacology, and medicine.

2. Flavonoids: In order to distinguish the two benzene rings that are characteristic of flavonoids, which are derived from flavones, a propane unit does the separation. These substances are often compounds that are soluble in water. As the intricacy of the compound rises, the compound's vibrancy also increases. There is a possibility that glycosides, which are often obtained from plants, might make formation persistently more difficult, if not completely impossible. In recent years, flavonoids have had a meteoric surge in popularity, which may be attributed, in part, to their capabilities of cytotoxicity, anticancer, antibacterial, and anti-inflammatory effects.

3. Tannins: Tanning agents, which are classified as phenolic compounds, are found in the lysosomes of plants, as well as in their leaves, bark, fruit, wood, and roots. From 500 Da to over 3000 Da, their molecular weights fall somewhere in the middle. There is a connection between them and plant defense mechanisms that protect plants against herbivores, birds, and insects that are classified as mammals. Tannins may be divided into two categories, namely hydrolysable tannins and condensed tannins, based on their chemical composition and the qualities that they possess. The phytoconstituents that include tannin have a broad variety of applications in the medical field, including but not limited to the following: exfoliants, diuretics, antitumor of the gastrointestinal system, antibacterial, free radical scavenging, hemostatic therapies, and anti-inflammatory actions. Tannins are added to alcoholic beverages, such as wine, beer, and fruit juices, in order to cause them to become more transparent.

4. Terpenoids: Terpenoids, which are often referred to as isoprenoids, are the most prevalent and diverse category of natural remedies as far as their structural composition is concerned.The chemical formula for isoprene is 2-methylbuta-1,3-diene (C5H8). Isoprene is considered to be the "building block" of terpenoids. For a variety of reasons, terpenoids are among the most exciting substances in the industrial sector. Edibles and cosmetics, for

instance, make use of menthol and sclareol as flavors and scents throughout the manufacturing process. Terpenoids, such as linalool, are necessary for the production of high-quality agro-based goods because they add distinct flavors and fragrances to flowers and fruits. Terpenoids are classified according to the number of isoprene units that they contain because of this. Terpenoids may be broken down into many categories, including monoterpenoids, sesquiterpenes, diterpenes, triterpenes, and tetraterpenoids. According to the findings of preliminary research, terpenes play an essential role in the process of plant signaling and growth regulation. Terpenoids such as artimisinin and taxol have anti-mutagenic, anti-ulcer, hepaticidal, and antibacterial activities. These features are in addition to the possible anti-cancer and anti-malarial actions that they possess.

5. Saponins: Saponins are a kind of chemical that may be found in a wide variety of plants and even in certain marine species, such as fish, starfish, and sea cucumbers. Two distinct categories of saponins may be distinguished from one another based on the kind of aglycone skeleton that they contain. The first category is comprised of the steroidal saponins that are found in monocotyledonous angiosperms. Triterpenoid saponins, which are the most prevalent kind of saponins, are mostly discovered in angiosperms that have dicotyledonous leaves. This second group of saponins is the most frequent type of saponin.

Objectives Of The Study

The objectives will guide your research on the phytochemical analysis of Phyllanthus niruri and Vitex negundo, helping us to systematically explore their properties and compare their potencies. Each objective is supported by relevant literature, ensuring a strong foundation for my study.

1. Phytochemical Screening: To identify and characterize the major phytochemical constituents in Phyllanthus niruri and Vitex negundo through qualitative analysis.
2. Quantitative Analysis of Phytochemicals: To quantify the levels of key phytochemicals (e.g., total phenolics, flavonoids, alkaloids) in both plant extracts using standardized methods.

II. REVIEW OF LITERATURE

The purpose of a literature review is to provide a critical assessment of the research that has already been conducted on a particular subject, therefore providing insight into the current state of knowledge, trends, and gaps in the area. The analysis of prior results, methodology, and theoretical approaches provides a basis for understanding the context and relevance of the current research. This is accomplished via the use of this foundation. Within the context of [insert your subject here, for example, Phyllanthus niruri and Vitex negundo], doing a literature review is beneficial in that it helps to emphasize the bioactive compounds, the pharmacological characteristics of these compounds, and the methodologies that were used for their extraction and analysis. This approach not only synthesises the existing body of knowledge but also highlights areas that need additional examination, so defining the rationale and direction for the research that is now being conducted via this procedure.

Ekrami A, Masoudian N, Mahmoodzadeh H, Roodi B, Ebadi, (2022) There was an investigation into the ways in which the physiological features of Artemisia aucheri Boiss plants are affected by altitude. To do this, samples were taken from fifteen distinct locations inside the Lakh Kuhik mountains, ranging in elevation from 1200 meters to 1256 meters. A research was conducted, and the results showed that the chlorophyll content of the plants was maximum at an altitude of 1200 meters, and it was lowest at an altitude of 1256 meters..Ekrami et al. (2022)

Reisch C, Rosbakh S,(2021) According to Reisch and Rosbakh (2021), the environmental variability and complicated glacial history in high mountain locations are additional factors that contribute to or play a key role in driving the adaptive responses of plants. Ultraviolet B radiation has a significant influence on the secondary metabolism of a plant. (Yavas et al., 2020).

Velu G, Palanichamy V, Rajan AP (2018) Chemical molecules that originate from plants are referred to as phytochemicals, which are also referred to as secondary metabolites. Cells of plants are responsible for the production of these substances via metabolic pathways that are derived from core metabolic activities. They constitute an essential component in the process of safeguarding and defending plant cells against ecological imbalances and diseases that are detrimental. (Velu et al., 2018; Mendoza et al., 2018; Hussein et al., 2019).

Abebe TD, Bauer AM, Léon J. (2010) According to the findings of the study, there was a large amount of morphological variation among the maize plants that were grown at various elevations. The morphological diversity of Ethiopian barleys (Hordeum vulgare L.) was the subject of another research that investigated the relationship between geographical locations and altitudes. (Abebe et al., 2010).

Nadkarni (2002). Vitex negundo has been attributed with a wide range of therapeutic characteristics, and it has also been used widely in the treatment of a wide range of illnesses. (Nadkarni,2002).

Kirtikar, K.R. and Basu, B.D., (2001) Based on ethnobotanical research, various plant parts of P. niruri have been reported to possess a variety of therapeutic properties. For instance, the leaves have been found to be expectorant, diaphoretic, and useful in the treatment of strangury and sweats. The seeds, on the other hand, have

been found to be carminative, laxative, astringent to the bowels, tonic to the liver, diuretic, diaphoretic, and useful in the treatment of bronchitis, ear pain, griping, opthalmia, and ascites. (Kirtikar and Basu, 2001).

Ibrahim IA, Jabbour AA, Abdulmajeed AM, Elhady ME, Almaroai YA, Hashim AM 2022. Conducted a study on four plant species, Cotoneaster orbicularis, Crataegus x sinaica, Echinops spinosissimus subsp. spinosissimus, and Tanacetum sinaicum, growing on the high mountains of the Sinai Peninsula. The researchers collected plant leaves from altitudes between 1500m and 2250m to assess the adaptive responses of these species to high-altitude oxidative stresses. Furthermore, various compounds such as proline, total soluble sugars, carotenoids, phenols, tannins, and flavonoids showed increased levels in response to high altitudes ,Ibrahim et al. (2022).

III. MATERIAL AND METHOD

For the purpose of determining the phytochemical, antioxidant, and antibacterial properties of Vitex negundo Linn and Phyllanthus niruri Linn, this chapter provides a comprehensive description of the experimental materials, collection processes, and methodological approaches that were used. Through the use of established qualitative and quantitative methods, the purpose of the research was to conduct a comprehensive investigation into the existence of bioactive chemicals and to evaluate the biological activities of these compounds. The processes for screening important phytoconstituents, including alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids, and proteins, are covered in this chapter. The chapter also includes the collecting and genuine identification of plant materials, as well as the synthesis of extracts. In addition, comprehensive in vitro assays were carried out in order to evaluate the antioxidant potential of the substance by employing DPPH, ABTS, FRAP, metal chelation, and lipid peroxidation inhibition techniques. Additionally, antimicrobial activity was evaluated by employing disc diffusion, agar well diffusion, and minimum inhibitory concentration (MIC) determinations. In conclusion, a comparative examination of the biological potency of the two plant species was carried out in order to give insights into the medicinal significance and therapeutic uses of these plants.

VITEX NEGUNDO LINN

Collection And Identification Of Plant

During the month of July 2011, the fresh plant leaves of V. negundo were taken from the Maruthamalai hill region in the Coimbatore district of Tamilnadu. This was done in the morning, after the dew had dissipated.

Phytochemical Analysis

Qualitative phytochemical analysis

For the purpose of determining the various phytoconstituents, preliminary chemical tests were conducted on crude powder and leaf extracts of V. negundo that were extracted using a variety of solvents (Raaman 2006; Sahu et al. 2009).

Carbohydrate: Fehling‟s Test: Following a one-minute boiling process, 1.0 ml of Fehling's A solution and 1.0 ml of Fehling's B solution were combined and brought to a boil. Currently, the same amount of the test solution (Extract) was added to the combination that was previously mentioned. The solution was cooked for five to ten minutes in a bathroom filled with hot water. A yellow precipitate was seen first, and subsequently a brick red precipitate was seen..

Benedict‟s test: A test tube was used to combine Benedict's reagent and the test solution, which was an ethanolic extract, in proportions that were equal. A bath of boiling water was used to heat the mixture for a period of five minutes. It was clear that reducing sugar was present since the solution had a green appearance..

Protein: Biuret test: When dissolved in a few milliliters of water, a small amount of different solvents of V. negundo extracts was obtained. A solution of 4% sodium hydroxide and a few drops of a solution of 1% copper sulfate were added to this test solution. Indicative of the presence of proteins was the appearance of a violet color.

Alkaloids: Two percent hydrochloric acid was used to dissolve the crude powder as well as the different solvent extracts of V. negundo. A filter was applied to the mixture, and the resulting filtrate was then split into three equal parts. The first piece was treated with a few drops of Mayer's reagent, the second portion was treated with an equivalent quantity of Dragondroff's reagent, and the third portion was treated with an equal amount of Wagner's reagent. Additionally, the presence of respective alkaloids was indicated by the presence of a cream-colored precipitate, an orange precipitation, and a brown precipitate.

Phenolic compounds & tannins: An alcoholic ferric chloride ($FeCl_3$) reagent was used to treat the crude powder of V. negundo as well as a number of other solvent extracts of the plant. Having a blue color was an indication that tannins were present.

Flavonoid: Shinoda test: The presences of flavonoids were estimated by Shinoda test. The crude powder and various solvent extracts of V. negundo were treated with few drops of concentrated HCl and magnesium ribbon. The appearance of pink or tomato red colour within few minutes indicated the presence of flavonoids Alkaline reagent test: Several drops of diluted sodium hydroxide (NaOH) were applied to the crude powder and different solvent extracts of V. negundo leaves. This treatment was carried out in a separate manner. An indication of the

presence of flavonoids was the formation of a bright yellow color that became colorless with the addition of a few drops of diluted hydrochloric acid.

Cardiac glycosides: To determine whether or not cardiac glycosides were present, a Keller-Kiliani test was carried out. 1.0 milliliter of a combination consisting of 5% FeCl3 and glacial acetic acid at a ratio of 1:99 volume/volume was used to treat the crude powder as well as the different solvent extracts of V. negundo leaves. There were a few drops of concentrated H2SO4 that were added to this water solution. The presence of cardiac glycosides was suggested by the appearance of a greenish blue color after a few minutes.

Saponins: To establish whether or not saponins were present, a frothing test was performed. Following a vigorous shaking with distilled water, the crude powder and different solvent extracts of V. negundo leaves were allowed to stand for five minutes before the forsaponins content was graded as follows: A lack of froth suggests that saponins are not present, whereas froth that is persistent for more than 1.5 centimeters shows that saponins are present.

Gums and mucilage: While swirling continuously, around 10 milliliters of a variety of extracts were added to 25 milliliters of absolute alcohol. After that, the mixture was filtered. An examination of the swelling characteristics of the precipitate was performed after it had been dried in air.

IV. RESULT AND DISCUSSION

VITEX NEGUNDO LINN

Whether in the form of completely natural substances or synthetic analogies, the plants may be used as sources of medicines for the treatment of human illnesses. In the process of developing novel compounds, medicinal plants serve as a source of inspiration. According to Meena (2014), natural products that are generated from plants are now being examined to see whether or not they include novel medications that have new mechanisms of pharmacological activity. In every traditional medical system, active phytocompounds are an essential component of the treatment process. These phytocompounds that are active play a significant function in the activities of living organisms. It is necessary to have an understanding of the phytochemical components of medicinal plants in order to comprehend herbal medicines and the preparations of these medicines. According to Fabricant and Farnsworth (2001), there are 122 compounds with a specified structure that are derived from just 94 different kinds of plants that are utilized as medications all over the world. According to a number of research, V. negundo has been used in the treatment of a variety of illnesses and problems. Investigating the characteristics of phytocompounds that are active is of the utmost importance. For this reason, the current study used in silico, in vitro, and in vivo research methods to analyze the active phytocompounds that were found in the leaf of V. negundo. These phytocompounds were shown to possess antibacterial and antiinflammatory properties.

Phytochemical Analysis

Qualitative phytochemical analysis

The bioactivity of medicinal plants is significantly influenced by the phytochemicals that are present in these plants. To have a better understanding of the phytochemicals that are present in the leaves of V. negundo, the qualitative investigation will be very helpful. Table 4.1 displays the findings of the qualitative phytochemical analysis performed on crude powder and several solvent extracts of the leaves of the V. negundo plant. A significant amount of secondary metabolites may be found in plants. The secondary contents include things like alkaloids, terpenoids, saponins, flavonoids, tannins, and phenolic compounds, among other similar substances. Numerous investigations have shown that a broad range of active phytochemicals, such as flavanoids, terpenoids, polyphenolics, tannins, plant sterols, and curcumins, have been discovered to originate from plant sources. In accordance with De-Fatima et al. (2006), these active phytochemicals constitute a significant supply that has a wide range of structural configurations and characteristics. It has been shown that certain secondary metabolites have significant therapeutic significance. For the purpose of medication development, these active phytocompounds are of great significance in the field of pharmacy.

The highest concentrations of phenol, flavonoid, tannins, and alkaloids were found in the crude powder and excipient liquid extract of V. negundo. There were no deposits of resins, gums, or mucilages found in either the crude powder or the extracts. When compared to other phytochemicals, the quantity of phenols, flavonoids, tannins, and alkaloids that were found in the sample by qualitative phytochemical analysis was much higher. When quantitative phytochemical analysis was performed, the phenolic content was found to be higher than the flavonoid content. It has been shown that the presence of phenolic compounds, tannins, and flavonoids is connected with varying degrees of analgesic and anti-inflammatory effects (Wang et al. 2008). The findings indicate that the active phytochemical components are found in a modest amount in leaf extracts that were extracted using petroleum ether and chloroform. The presence of alkaloids, carbohydrates, flavonoids, glycosides, phenols, protein, tanin, and terpenoids was discovered by phytochemical examination of the leaf of the V. negundo plant (Ullah et al. 2012; Mani et al. 2013). Based on the findings of the phytochemical screening, it was determined that the essential oil of V. negundo contains a significant quantity of phenol and flavonoids. It is possible that the

synergistic action of one or more of the types of chemicals that are found in the ELE of V. negundo is responsible for the anti-inflammatory and analgesic effects that these active compounds exhibit.

Table 1: Qualitative Phytochemical Screening of Leaf Extract of *Vitex negundo*

S. No.	Phytochemicals	Petroleum Ether	Chloroform	Ethyl Acetate	Ethanol	Water
1	Carbohydrates	+	+	+	+	+
2	Proteins	+	+	+	+	+
3	Alkaloids	–	+	+	+	+
4	Phenolic Compounds & Tannins	+	+	+	+	+
5	Flavonoids	+	+	+	+	+
6	Cardiac Glycosides	–	+	+	+	+
7	Gums and Mucilages	–	–	–	–	–
8	Saponins	–	–	–	–	–

Note: (+) Presence of phytochemical compound
(–) Absence of phytochemical compound

Quantitative phytochemical analysis

Total phenol content

There is a substantial relationship between the phytochemicals that are contained in medicinal plants and the bioactivity of these plants.

In order to get a more comprehensive comprehension of the phytochemicals that are found in the leaves of V. negundo, the qualitative study will prove to be very beneficial. The results of the qualitative phytochemical analysis that was carried out on crude powder and various solvent extracts of the leaves of the V. negundo plant are shown in Table 4.1. There is a possibility that plants could contain a substantial quantity of secondary metabolites. Secondary contents consist of a variety of chemicals that are comparable to those found in primary contents. These substances include alkaloids, terpenoids, saponins, flavonoids, tannins, and phenolic compounds. There have been a great number of research that have shown that a wide variety of active phytochemicals, including flavanoids, terpenoids, polyphenolics, tannins, plant sterols, and curcumins, have been found to come from plant sources. According to the findings of De-Fatima et al. (2006), these active phytochemicals comprise a substantial supply that has a diverse assortment of structural configurations and properties. It has been shown that certain secondary metabolites contribute significantly to the therapeutic value of the substance. These active phytocompounds are of major relevance in the world of pharmacy since they are used for the purpose of product development in the pharmaceutical industry.

Both the crude powder and the excipient liquid extract of V. negundo were found to have the greatest quantities of several compounds, including phenol, flavonoid, tannins, and alkaloids. Neither the crude powder nor the extracts included any deposits of resins, gums, or mucilages. This was the case for both types of substances.

The amount of phenols, flavonoids, tannins, and alkaloids that were discovered in the sample by qualitative phytochemical analysis was much larger than the quantity of other phytochemicals that were obtained from the sample. During the quantitative phytochemical study that was carried out, it was discovered that the phenolic content was greater than the flavonoid content. In a study that was conducted by Wang et al. (2008), it was discovered that the presence of phenolic compounds, tannins, and flavonoids is associated with varied degrees of analgesic and anti-inflammatory effects.

According to the results, the active phytochemical components are discovered in leaf extracts that were extracted using petroleum ether and chloroform. However, the level of these components is determined to be rather low. The leaf of the V. negundo plant was subjected to phytochemical analysis, which led to the discovery of the presence of a variety of compounds, including alkaloids, carbohydrates, flavonoids, glycosides, phenols, protein, tanin, and terpenoids (Ullah et al. 2012; Mani et al. 2013). Following the completion of the phytochemical analysis, it was concluded that the essential oil of V. negundo contains a considerable amount of phenol and flavonoids. This conclusion was reached on the basis of the observations observed. In the ELE of V. negundo, there are a number of different kinds of chemicals. It is probable that the synergistic action of one or more of these chemicals is responsible for the anti-inflammatory and analgesic actions that these active compounds display.

Total flavonoid content

The amount of total flavonoid content in the extracts of petroleum ether, chloroform, ethyl acetate and ethanol were 21.98 RE/g, 21.48 RE/g, 27.65 RE/g and 44.65 RE/g extract respectively (Table 2). Among the different extract, the ELE containing the higher flavonoid content 44.65 mg RE/g. The chloroform extract and ethyl acetate extracts were containing lesser amount of flavonoids. The result showed that the ELE of V. negundo can be considered as an efficient extract and ethanol was the better solvent for extraction of flavonoids.

Raghavendra et al. (2010) reported that the ELE of V. negundo possess the antioxidant activity mainly due to the presence of phenols and flavonoids.

Table 2: Phenolic, Flavonoid and Tannin Content of Leaf Extracts of *Vitex negundo*

Sample Extract	Total Phenolic Content (mg GAE/g)	Total Tannin Content (mg GAE/g)	Total Flavonoid Content (mg RE/g)
Petroleum ether	45.40 ± 1.69	6.38 ± 3.07	21.98 ± 1.04
Chloroform	51.71 ± 1.07	2.74 ± 0.20	21.48 ± 0.58
Ethyl acetate	80.05 ± 1.15	8.10 ± 1.76	27.65 ± 0.76
Ethanol	132.19 ± 1.49	23.33 ± 1.76	44.65 ± 0.50

Note: Values are expressed as mean ± SD (n = 3).

GAE = Gallic acid equivalents; RE = Rutin equivalents.

Inflammation was shown to be decreased by a number of secondary metabolites, including polyphenols, flavonoids, and sterols, according to Aggarwal et al. (2011). According to Shahidi et al. (1992), phenolic compounds serve as free radical terminators and belong to the family of antioxidant agents known as phenolic compounds. Our findings shown that, in comparison to other solvent extracts, the ELE of V. negundo, which has a high concentration of phenol and flavonoids, has the potential to be used as an anti-inflammatory medication.

Anti-Oxidant Activity

When it comes to the prevention of chronic inflammatory disorders like cancer, Alzheimer's disease, rheumatoid arthritis, and other similar conditions, antioxidants play a significant role. The majority of plant products include chemicals that have antioxidant properties. The results of a large number of research have shown that the antioxidative properties of plant materials and products are much higher than those of other materials and products. According to Owen et al. (2000), these substances have anti-inflammatory effects to varying degrees, depending on the chemical. As a significant mechanism of anti-oxidation, the proton-radical scavenging activity has been recognized for its significance. According to Chung et al. (2005), the capacity to donate protons might be used as a free radical inhibitor or scavenger, which would make them a potential candidate for main antioxidant duties.

DPPH free radical scavenging assay

According to Koleva et al. (2002), the DPPH stable free radical technique is a straightforward, fast, and sensitive approach to determining the content of antioxidant activity in a particular molecule or in plant extracts. DPPH is an organic free radical that is very stable and has a deep violet color. Its absorption peaks in the region of 515–528 nm are found in this radical. DPPH is a persistent free radical, and any molecule that is able to give an electron or hydrogen to DPPH is capable of reacting with it and bleaching the DPPH absorbance at 517 nm (Huang Ou, & Prior 2005). The IC50 values and the DPPH scavenging activity are known to have a link that is in the opposite direction. It is possible to determine the scavenging potentials of the antioxidant extract by observing the degree of discolouration. The ELE of V. negundo was found to have the greatest free radical scavenging activity according to the findings of the current investigation. In order to determine the DPPH radical-scavenging properties of the leaf extract of Vitex negundo L., a comparison was made with other standards, including rutin, vitamin C, vitamin E, and quercetin.

In general, the IC50 values of the extracts were evaluated using the DPPH scavenging activity test. When the value of IC50 is lower, it suggests that the antioxidant activity is stronger. It was the scavenging of the radical by hydrogen donation that was responsible for the reduction in absorbance of the DPPH radical that was brought about by the antioxidant. There is a distinct shift in color from purple to yellow, which is visible to the naked eye. The overall antioxidant activity of the various extracts is shown in Table 3, which contains the results of the analysis. When compared to other extracts, the ELE of V. negundo shown a high level of activity. The extract of petroleum ether demonstrated a lower level of activity. In comparison to other solvent extracts, the DPPH radical scavenging capabilities obtained from the ELE of V. negundo were shown to be superior. Compared to the IC50 values of petroleum ether, chloroform, and ethyl acetate, which were found to be 111.48 Mmol/ mg, 88.34 Mmol/ mg, and 28.09 Mmol/ mg, respectively, the IC50 value of ELE for V. negundo was determined to be 16.15 Mmol/mg. It was shown that the IC50 of conventional antioxidants was significantly different from that of other extracts, with the exception of ELE. This finding demonstrates that the ELE of V. negundo has a higher level of free radical scavenging activity in comparison to the other extracts.

Table 3: DPPH Free Radical Scavenging Assay of Leaf Extracts of *Vitex negundo*

S. No.	Sample / Standard	IC_{50} Value (mmol/mg)
1	Petroleum ether extract	111.48
2	Chloroform extract	88.34
3	Ethyl acetate extract	28.09
4	Ethanol extract	16.14

5	Rutin	5.59
6	Vitamin E	9.62
7	Vitamin C	3.39
8	Quercetin	2.18

Note: Lower IC_{50} values indicate higher free radical scavenging (antioxidant) activity.

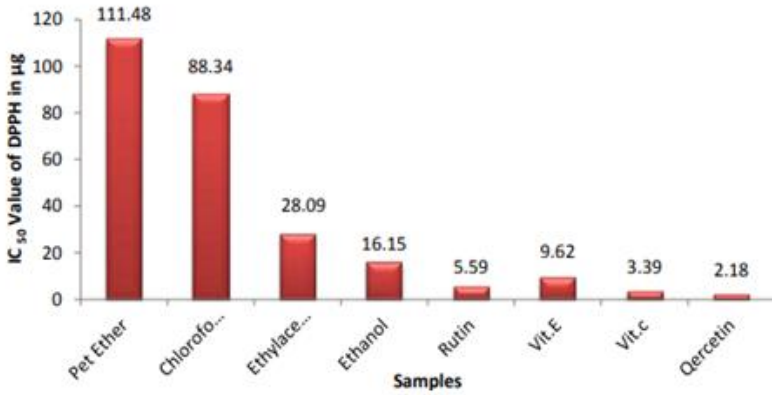

Figure 1 DPPH free radical scavenging assay of leaf extracts of V. negundo.

Metal chelating activity

In addition to their role as reducing agents, phenols also serve as quenchers for hydrogen and singlet oxygen. Phenols are antioxidants that possess redox characteristics. According to Lee et al. (2000), these types of compounds also possess metal chelating capabilities. The capacity to chelate the metal iron was shown by each and every extract. The ELE of V. negundo demonstrated a strong metal chelating capacity when compared to other solvent extracts (Figure 4.2). This was the case among the various leaf extracts of V. negundo. A total of 687.05 µg EDTA per gram was determined to represent the metal chelating capability of the leaf extract (ELE). It was discovered that the chloroform extract had a concentration of 142.22 µg EDTA per gram, and it exhibited a lower level of activity when compared to other extracts (Table 4.4). The ethyl acetate exhibited a moderate metal chelating activity, as shown by its metal chelating capacity of 459.34 µg EDTA/1g, as determined by the experiment. A conclusion that may be drawn from the findings obtained is that the ELE of V. negundo have an efficient ability for iron binding.

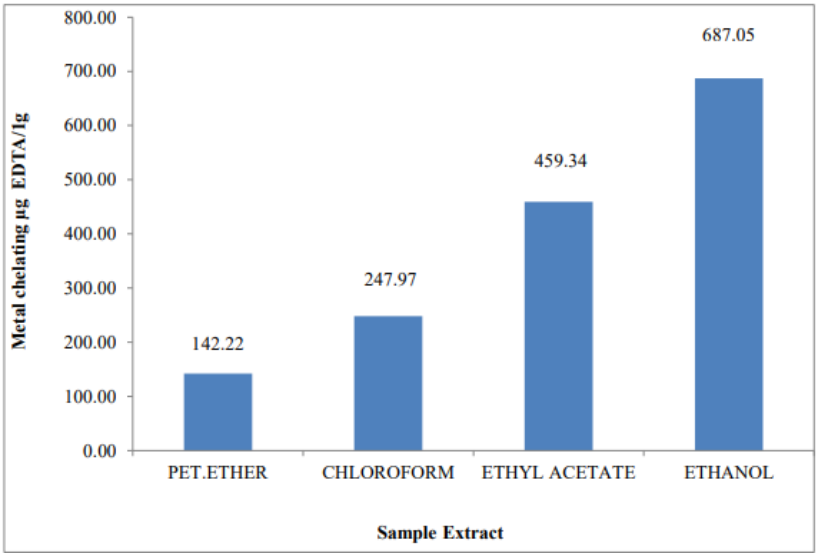

Figure 2 Metal chelating activity of leaf extracts of V. negundo. Values are means of three replicate determinations (n=3) ± standard deviation

ABT radical scavenging activity

Using the ABTS cation radical scavenging technique, the activity was evaluated by the decolorization of the ABTS. This was accomplished by measuring the reduction of the radical cation as the percentage inhibition of absorbance at 734 nm. An antioxidant that is comparable to one gram of dry matter of the material that was

being investigated experimentally was used to express the activity of the extracts that were tested. This activity was represented as the micromolar equivalent of Trolox solution.(1999 work by Re et al.). As shown in Table 4.4, the findings of the ABTS•+ cation radical scavenging activities of several extracts of the leaf of V. negundo are reported. While the most inhibition was obtained in ELE (5228.63± 3894.69 µmol TE/g), the smallest inhibition was observed in petroleum ether extract (5106.63± 3807.07 µmol TE/g). Of the four extracts that were evaluated, ELE exhibited the highest level of inhibition. Both BHT and rutin exhibited a total extract concentration of 12 454.2 µmol TE/g and 11 096.8 µmol TE/g, respectively. When compared to the radical scavenging activity of other solvent extracts, the ELE demonstrated a much greater level of activity.

Table 4: Metal Chelating, $ABTS^+$ and FRAP Radical Scavenging Activity of Leaf Extracts of *Vitex negundo*

Sample Extract	Metal Chelating Activity (µg EDTA/g)	$ABTS^+$ Activity (µmol TE/g)	FRAP Activity (mM Fe(II)/mg)
Petroleum ether	142.22 ± 0.80	5106.63 ± 3807.07	7.88 ± 0.05
Chloroform	247.97 ± 0.27	5130.63 ± 3664.67	8.61 ± 0.06
Ethyl acetate	459.34 ± 0.49	5144.75 ± 3683.72	45.03 ± 0.04
Ethanol	687.05 ± 0.53	5228.63 ± 3894.69	46.28 ± 0.04

Note: Values are expressed as mean ± SD of triplicate determinations (n = 3).
TE = Trolox equivalents

V. Conclusion

The study found that both Phyllanthus niruri Linn. and Vitex negundo Linn. have abundant physiologically active phytochemicals of therapeutic promise. Both plant species included alkaloids, flavonoids, tannins, saponins, phenolic compounds, and terpenoids, according to phytochemical study. Comparative investigation showed that ingredient concentrations affect biological potency. Phyllanthus niruri was more antioxidant and hepatoprotective, whereas Vitex negundo was more antibacterial and anti-inflammatory. These findings support both plants' historic medical usage and suggest they might be used to generate natural therapeutic medicines. To maximize their medicinal potential, advanced phytochemical analysis, toxicity assessment, and clinical evaluation are advised.

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