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Isolation and characterization of bioactive compounds from medicinal plants

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Abstract

Pure compounds or standardized extracts of medicinal plants include an unprecedented range of chemicals, giving up endless possibilities for new therapeutic leads. Edible plant research has recently seen a surge in interest throughout the globe, driven by rising demands for chemical variety in screening programs and the search for medicinal medicines derived from natural sources. There are many different kinds of bioactive chemicals found in medical botanicals and herbal remedies. Analytical techniques, such as those used to extract, isolate, and characterize active components in botanical and herbal medicines, are the main subject of this article. We will go over the most typical issues and major obstacles that arise when trying to isolate and characterize the active components in botanicals and herbal remedies. We will discuss the benefits and drawbacks of various extraction procedures since they are crucial to the research of botanicals and herbal medicines. Examining the bioactive compounds present in plant extracts include standard phytochemical screening tests, chromatographic techniques, and non-chromatographic techniques.

Keywords: Bioactive compounds, extraction methods, medicinal plants, natural products

Introduction

The phytochemicals found in medicinal plants have recently attracted a lot of attention since they may serve as a springboard for new chemical entities by serving as a lead ingredient in the selection process. It is well-established that the majority of phytochemicals found in herbal plants, including phenolics, coumarins, and flavonoids, have biological and pharmacological effects on illnesses. Researched phytochemicals included with antioxidant-rich diets generated from fruits and vegetables are components of modern nutraceuticals, dietary supplements, and the DASH diet plan to control hypertension. Research into medicinal plants and the industrial production of phytomedicine are both enhanced by the current emphasis on natural product usage in the identification, creation, and formulation of novel compounds to supplement traditional pharmaceuticals and synthetic products. Biological activities including anti-inflammatory, antioxidant, antibacterial, and anticancer properties have been linked to natural products with high concentrations of phenolic compounds and flavonoids, according to studies. Some age-related diseases, especially those brought on by oxidative stress, may be treated or prevented with the help of these active metabolites. The pharmaceutical and nutraceutical sectors are heavily invested in research and development, and medicinal plants contain bioactive compounds that have great therapeutic value. As a result, medicinal plants are also a key source of conventional pharmaceuticals.

A long history of human contact with the environment is reflected in the usage of herbal remedies throughout Asia. The vast variety of compounds found in medicinal plants makes them useful in the treatment of both acute and chronic illnesses (Duraipandiyan *et al.*, 2006) [7]. When chemically manufactured medications began to cause side effects and germ resistance, people began to look to ethnopharmacognosy as an alternative. As an alternative with fewer side effects, they discovered hundreds of phytochemicals derived from plants. Anticancer, antibacterial, antioxidant, antidiarrheal, analgesic, and wound healing activities were among the several favorable biological effects that were documented. Many individuals claim that certain herbal or natural items have beneficial

effects. To back up this long-standing assertion, however, clinical studies are required to show that a bioactive substance is beneficial. To understand the pharmacokinetics, bioavailability, efficacy, safety, and drug interactions of newly manufactured bioactive compounds and their extracts, it is essential to conduct comprehensive assessments prior to starting clinical trials. Thorough clinical studies are necessary for a pharmaceutical to be approved for widespread use in patient treatment. These trials not only guarantee the safety of the participants, but also address particular research concerns about the drug's short- and long-term effects.

Nearly 20,000 medicinal plants are known to occur in 91 countries, with 12 of those nations being classified as having mega biodiversity, according to the World Health Organization (WHO). In order to use biologically active substances derived from plants, the following steps must be taken: extraction, screening for pharmacological effects, separation and characterization of bioactive components, toxicological evaluation, and clinical evaluation. This work details methods for isolating and characterizing bioactive compounds in plant extracts using chromatographic methods including high-performance liquid chromatography (HPLC), Fourier transform mass spectrometry (FTMS), and standard phytochemical screening assays.

Literature Review

Bhuyar, Prakash *et al*. (2020) [4]. As a source of valuable therapeutic remedies, medicinal plants help alleviate human illnesses. This study used the disc diffusion technique and the broth dilution method to assess C. asiatica's anti-fungal efficacy against C. *albicans*, Aspergillus niger, and *Penicillium* sp. Crude methanol from C. asiatica was shown to be the most effective extract in inhibiting fungal activity. Broth dilution, rather than disc diffusion, was the preferred method for quantitatively assessing the anti-Candida activity of plant extracts since it enabled the calculation of the MIC values of the crude extracts According to the findings of the disc diffusion test, the plant extracts impede the process. However, proof of C. asiatica's anti-fungal effect is further supported by the findings of the broth dilution method, which reveal that the crude methanol extract of the plant has lower MIC values. Bioactive data showed that n-Hexadecanoic acid (99%), cis-Vaccenic acid (91%), 5-Hydroxymethylfurfural (88%), and tetra decanoic acid (86%) were the primary chemical components of Centella asiatica. These bioactive compounds were found in the plant's leaves, stems, roots, and overall extract. To determine which phytochemical is responsible for its therapeutic benefit, further research is needed.

Rasul, Md. (2018) [2]. The unparalleled accessibility of chemical components in natural products derived from medicinal plants opens almost endless possibilities for novel pharmaceuticals, whether in the form of pure compounds or standardized extracts. Natural remedies have a long history of use as an alternative to hormone replacement therapy, anti-inflammatory, analgesic, and treatment for chronic illnesses such as cancer, diabetes, asthma, and others. There aren't enough bioactive natural ingredients in natural remedies, even if they're widely available. Finding better, more selective ways to extract and isolate novel natural compounds is of the utmost importance right now. This review study aims to provide a thorough overview of the analytical approaches used in the field. These methodologies include topics such as common phytochemical screening assays, natural product extraction, isolation, and characterisation from medicinal plants.

Kumari, Sumona & Singh, Rajvir & Gurav, N.P. & Mehta, Naresh. (2017) [1]. Isolation of 21α-hydroxyfriedel-4-(23)-en-3-one, β-sitosterol, 1-triacontanol, friedel-1-ene-3-one, pelargonic acid, and lignoceric acid was the outcome of a phytochemical study of the Nyctanthes arbor-tristis (Harsingar) stem. The findings showed that the chloroform fraction had an 83.96% growth inhibition rate against the Mycogone perniciosa fungus, whereas the ethyl acetate fraction had an 82.29% growth inhibition rate. The bacteria Xanthomonas axonopodis pv. citri and Pseudovorax sp. were most effectively inhibited by the methanol extract at a dose of 2000 µg/mL, with inhibition zones of 11 and 3.50 mm, respectively.

Rajkumar, P. & Velmurugan, Devadasan & Kumaresan, S. & s.selvaraj, & Suganya, R. & Chinnadurai, Renuga. (2017) [3] For thousands of years, medicinal plants have been used for their medicinal properties, particularly in underdeveloped nations. The World Health Organization in these countries has emphasized the importance of medicinal floras, despite the lack of scientific explanations for their therapeutic uses. This has led to a surge in the recording of therapeutic plants, with researchers increasingly looking to them as potential sources of drugs to treat various illnesses, including viral infections. This review focuses on Siddha medicinal herbs with strong antiviral activity, examining traditional Siddha literature and evidence-based research data. Dengue infection is currently untreated due to the lack of an authorized vaccine or targeted pharmacological therapy. The complicated lifecycle of dengue viruses and the genome's low coding capacity have contributed to the difficulty of developing a vaccine against the disease. The lethal dengue hemorrhagic sickness is thought to be produced by a combination of infections, with antibodies and immunity acquired from the first infection helping with the second subtype's infection. No vaccination has been developed that successfully stimulates protective antibodies against all four types simultaneously. This highlights the critical need for the rapid discovery of a safe and effective dengue virus treatment. This mini-review primarily focuses on the recently identified peptidyl and non-peptidyl inhibitors of dengue and their workings.

Extraction Methods

Extraction is the process of separating the parts of plants that have therapeutic properties using established and selected methods. The most crucial first step in studying medicinal plants is extracting their components from plant materials. Only then can they be further isolated and characterized. Many methods exist for obtaining valuable compounds from plants. One way to classify these methods is by how long they've been around; some are more modern, while others are more traditional. Conventional procedures usually employ water or organic solvents and are carried out at atmospheric pressure, while modern methods use pressure and/or very high temperatures. Solvent extraction, distillation, pressing, and sublimation are some of the extraction processes that rest on the extraction principle.

Solvent extraction is the method that is used most often. Natural product extraction consists of the following steps: first, introducing a solvent into the solid matrix; second, dissolving the solute in the solvents; third, diffusing the solute out of the solid matrix; and lastly, collecting the extracted solutes. Fluids such as water, ethanol, chloroform, dichloromethane, hexane, ethyl acetate, methanol, and others are often used as solvents in the extraction process. Traditional extraction techniques often include organic solvents, necessitating a substantial amount of solvent and a considerable amount of time for the extraction process.

Maceration

Here, the solid plant components are mixed with the solvent in a sealed container and left to steep for three to seven days, stirring occasionally, until the soluble components dissolve. The next step is to pass the mixture through sieves or nets to remove any debris. After pressing the marc, the mixed liquids are purified by filtering or decantation. A tiny amount of alcohol may be added when the solvent is water and the maceration duration is extended in order to inhibit the formation of microbes.

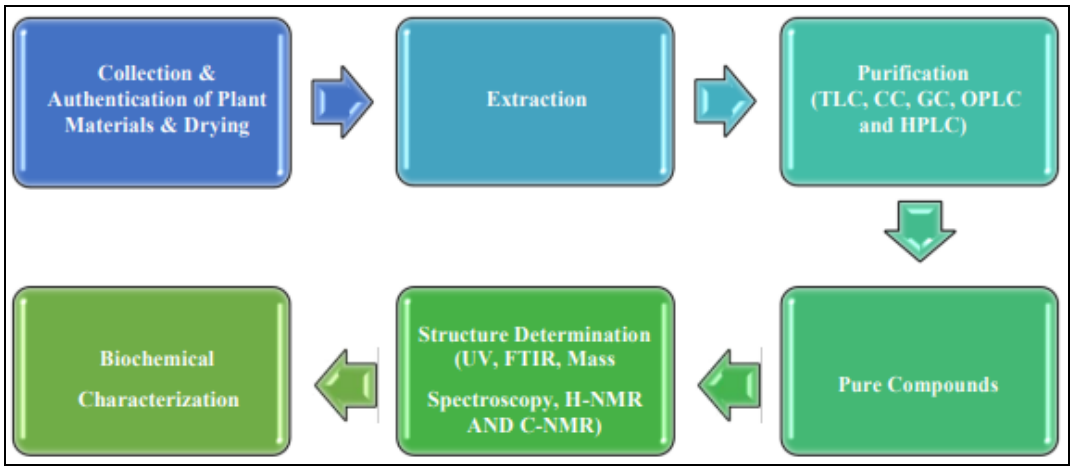

Fig 1: Medicinal Plant Natural Product Extraction, Isolation, and Characterization

Percolation

When making tinctures or fluid extracts, this is the method that is utilized most often to get the active components out. To collect the plant matter, a percolation tube is used, which may be either cotton-plugged or equipped with a filter and a stopcock.

Digestion

The extraction procedure in this maceration method involves the application of mild heat, between 40 and 60 degrees Celsius. When somewhat higher temperatures are tolerable, it is used. Using a magnetic stirrer, a mechanical stirrer, or even just stirring the mixture every so often may change the process.

Infusion

This extraction process begins with a short maceration of the plant material in water, either at room temperature or at boiling point. The crude medications' more water-soluble components are diluted in this solution.

Pressurized Liquid Extraction (PLE)

Improved solvent extraction system (ESE) and accelerated solvent extraction system (ASE) are alternative names for the same procedure. This technique makes use of high pressure and temperature to speed up the extraction process by making the solvent more diffusive; at the same time, the pressure keeps the organic solvent from boiling and forces it to permeate the matrix pores.

Isolation and Purification

The aforementioned extraction techniques yield complicated mixtures of components, including many distinct kinds of naturally occurring substances with varying degrees of polarity. Additional separation and purification steps are required to acquire a bioactive molecule in its pure form. The identification and characterization of pure bioactive natural products continues to be hindered by the difficulty of separating them. New methods for isolating and purifying natural compounds have emerged in the last few years. A number of bioactive natural chemicals have been separated and purified using various separation techniques, such as thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC), gas chromatography, paper chromatography, column chromatography, high-performance liquid chromatography (HPLC), and organic phase liquid chromatography (OPLC). Column chromatography and thin-layer chromatography (TLC) in various stationary phases remain the most used procedures because to their accessibility, cost, and convenience of use.

Thin Layer Chromatography (TLC)

When studying natural products, TLC is the chromatographic technique that is employed most often. Analysis, isolation, and parameter setup for column chromatography may all benefit from this simple and inexpensive method. In most cases, organic solvents (less polar) are used as the mobile phase, with more polar silica or alumina serving as the stationary phase. Normal phase chromatography describes this scenario.

Column Chromatography (CC)

The most efficient method for separating raw plant extracts into their individual components is column chromatography. In this preparative chromatographic technique, the extracts are loaded onto the stationary phase, The stationary phase,

consisting of silica gel, and the mobile phase, eluent, are carried through the column.

High performance thin layer chromatography (HPTLC)
It is a kind of planar chromatography that uses detection and data capture to separate natural substances on high performance layers. Layers of high-performance sorbent with a particle size of 5-7 microns and a thickness of 150-200 microns are created by coating these pre-coated plates. Reducing particle size and layer thickness improves the plate's efficacy and the separation's nature.

High Performance Liquid Chromatography (HPLC)
Isolation of natural products with this method is commonplace since it is durable, adaptable, and efficient. There are a number of analytical methods, but this one is quickly becoming the gold standard for fingerprinting studies used to ensure the safety of medicinal herbs. Picking the right detector is the first step in using high-performance liquid chromatography (HPLC) to detect any chemical. The separation degree is primarily dictated by the stationary phase and mobile phase choices.

Bioactive compounds from medicinal plants of Ethiopia
Both traditional health practitioners (THPs) and women who practice self-administration in the home rely on a rich but unstandardized pharmacopoeia of plants used throughout Ethiopia's long history of traditional healthcare. Scientific research has established the effectiveness of a handful of these plants in treating tapeworms and controlling schistosomiasis, but the safety and efficacy of numerous others in treating a wide range of diseases is still in its early stages.
Environmental deterioration, agricultural development, loss of forest and woodland, and urbanization are among the many current threats to these priceless plant species. People have depended on these resources for decades to treat human and domestic animal illnesses; therefore, this is a worrying indicator. Many Ethiopians lack access to quality health care because traditional medical facilities are too concentrated in major cities and have not kept up with the country's rapidly expanding population. Up to 80% of Ethiopians utilize traditional medicines as their main source of health treatment because they are culturally entrenched, accessible, and economical. Table 1 shows that herbs, shrubs, and trees make up the bulk of therapeutic plant species. Since the majority of medicinal plant species occur in natural habitats (Table 2), they face comparable challenges and tendencies to forest plant species. According to Table 3 of the List of Medicinal Plants, Preserving these plant species must be prioritized immediately via the use of biotechnology, tissue culture, and the extraction of vital medicinal compounds.

Table 1: Medicinal plants by their growth forms

Growth Habit	No. of Species	Percent
Herbs	271	30.5
Shrubs	168	19
Trees	110	12.4
Climbers	74	8.3
Reed	2	0.2
Unidentified	262	29.6

Table 2: Medicinal plants by their state of existence.

State of Existence	No. of Species	Percent
Wild	357	40.2
Cultivated	89	10
Weed	52	5.9
Undetermined	389	43.9

Table 3: Important Medicinal plants and disease treated

Medicinal Plant	Disease
Allium sativum	Malaria
Aloe barbadensis	Ascariasis
Achyranthes aspera	Tonsillitis
Carica papaya L	Hepatitis
Citrus aurantifolia	Diarrhea
Citrus aurantifolia	Hepatitis
Cordia africana	Acute febrile illness (AFI)
Cyphostemma auriculata	Snake bites
Citrus aurantifolia	Hyper tension
Datura stramonium	Tenea versicolor, wound
Datura innoxia	Leishmaniasis
Dodonaea viscosa	Vitiligo
Eucalyptus globulus	Acute febrile illness (AFI)
Entada abyssinica	Herpefoster
Euclea natalensis	Rabies
Euphorbia tirucalli	Tuberculosis (TB)
Linum usitatissimum	Swelling around neck (Megegha)
Ocimum lamiifolium	Acute febrile illness
Phytolacca dodecandra	Abortion
Rumex abyssinicus	Hypertension
Sida schimperiana	Tuberculosis (TB)
Schinus molle	Asthma
Solanum insanum	Anthrax
Solanum dulcamara	Goiter
Trigonella dulcamara	Spider poison
Whithania somnifera	Common cold
Zingiber officinale	Abdominal cramp

Conclusion
It is very difficult to identify and characterize natural compounds derived from plant extracts due to the fact that these products often comprise combinations of components with varying polarity. The separation and characterization of many natural compounds rely heavily on extraction. In order to extract the natural products, almost all of them need purification using a mix of different purification processes, some of which use chromatography and others of which do not. Some of these plants have been scientifically proven to be effective in treating tapeworms and schistosomiasis (Phytolacca dodecandra as a molluscicide), but research on the safety and effectiveness of many others is still in its early stages. Environmental deterioration, agricultural development, loss of forest and woodland, and urbanization are among the many current threats to these priceless plant species. The public takes this as a warning, as they have depended on these resources for many years to treat human and animal diseases.

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