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Methods of Extracting Useful Components of Plants and their Application in Biological Research

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Abstract
Plants and herbs have been used as food and medicine for thousands of years. It is hard to get the bioactive parts of plants out for studies in phytochemistry and biology. Herbalists and scientists have come up with a number of ways to get bioactive constituents out of crude medicines to make sure they work. New plant leads like morphine, quinine, taxol, artemisinin, and alkaloids need careful extraction of active phytochemicals to reduce synthesis and separation costs. Thus, bioactive ingredient-rich extracts and fractions must be extracted from plants using proper procedures. Thus, extraction methods affect yield, phytochemical content, etc. This chapter describes and compares herb and medicinal plant bioactive component extraction methods.

Keywords: Herbs, Medicinal plants, Plants extracts, Extraction, Bioactive ingredients, Phytoconstituents, Secondary metabolites, Phytochemicals

Introduction

Manufacturers of medicinal plant extracts and essential oils use the best extraction methods to meet the demand for herbal medicines, nutraceuticals, and natural products used for primary healthcare around the world. There are many ways to make extracts and essential oils of consistent quality. Since ancient times, herbs and medicinal plants have been a source of many chemicals that have effects on the body. Indigenous people have been using crude plant parts or pure plant compounds to treat a wide range of illnesses for hundreds of years. A lot of research has been done on them, but solvent extraction for phytochemical and biological studies has its own problems. The variety of chemicals in natural products makes them great for finding new drugs. Natural products are important sources of phytochemicals because they work well, are safe, and have few side effects. Plants have alkaloids, terpenoids,

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coumarins, flavonoids, compounds with nitrogen, and compounds with sulphur, phenolics, and other chemicals [1]. These compounds reduce inflammation, boost the immune system, fight cancer, and fight free radicals, kill germs, and more. This chapter talks about plant extraction methods and compares them based on how they work, how the solvent affects the extraction process, their strength, their limits, their economic viability, and their pros and cons. This chapter will also talk about common problems and how to fix them. Only a few natural products that come from plants are shown.

Herbal remedies

A plant is "medicinal" if it has one or more substances that change the way sick mammals' bodies work and are used to treat illness. Farnsworth and Soejarto said that therapeutic plants are "all higher plants with health-related medicinal properties, or those that have been proven to be medicines by Western standards, or those that have parts that are called "hits." The term "medicinal plant" (MP) refers to any plant that, in one or more of its parts, has chemicals that can be used to treat health problems or that are used to make medicines. Plants or parts of plants may be good for you [2]. MPs are important because they give us therapeutic molecules that could be used to make new medicines. MPs are plants that are used in both conventional and alternative medicine. MPs include species used for food, fragrance, and beauty.

Metabolites

Small molecules are the steps in the process of metabolism. Primary metabolites are needed for plants to grow, develop, and have babies. Primary metabolites are things that cells need to grow and develop, like carbohydrates, polysaccharides, amino acids, sugars, proteins, lipids, and fermentation products like ethanol, acetic acid, citric acid, and lactic acid. Secondary metabolites have a job to do, but they are not necessary for the organism to live (e.g. phenolic, steroids, lignans, etc.). They show how unique a species is and are only found in some animals. These substances aren't always made, and it's usually not known what they do or how they help the body. Some are made for obvious reasons, like to keep predators away or to attract the same or a different species, but it's safe to assume that all of them are

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important to the producer [3]. Secondary metabolites help plants stay alive and deal with problems in their surroundings. Bioactive substances include terpenoids, alkaloids, organosulfur, chemicals with nitrogen, and phenolic chemicals.

Bioactive compounds

For a long time, no one knew what bioactive substances were. Rarely is bioactive defined. bio-active. The prefix "bio-" comes from the Greek word "bios," which means "life," and the suffix "-active" comes from the Latin word "activus," which means "active" or "dynamic." When something is bioactive, it means that it is physiologically active. Bioactive compounds are things that can affect living things. A plant extract is a substance or active ingredient that is taken from plant tissues, usually with the help of a solvent, and used for a certain purpose. Bioactive substances are chemicals that are important to the body's function but are not needed as nutrients. Vitamins, polyphenols, alkaloids, and other bioactive compounds are found in nature and are part of the food chain [4]. They are made by plants, animals, fungi, and sea creatures (e.g., lichens). Bioactive natural compounds are rare in nature. There are active chemicals in plant matrices. All plant organs and parts, including leaves, roots, barks, tubers, woods, gums or oleoresin exudations, fruits, figs, flowers, rhizomes, berries, twigs, and the whole plant, make active chemicals in small amounts and varying concentrations. After chemicals have been extracted, more steps may be needed to clean or separate them.

Fresh or dried plants

Fresh and dried samples are used to make medicines. For phytochemical analysis, plant parts should be put into boiling alcohol right after they are picked. Dry the plants out first. Most of the time, dried samples are better than fresh ones because they last longer. Fresh things fall apart faster than dry ones. Dried samples have less phytoconstituents like Essential Oils (EOs). Before extracting fresh plant material with organic solvents like methanol or ethanol, plant enzymes must be turned off. Organic solvents that don't mix with water can separate the extractive, which may contain a lot of water [5].

Drying

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When plant matter is dried, enzymes like glucoside hydrolysis can't break it down. It should be dried quickly in an open space at room temperature with air flow to keep it from getting too hot or wet. They were put in shallow trays that had good air circulation and were put in the sun or shade, depending on what they were. But direct sunlight is avoided to reduce the chance of chemical reactions that could lead to the artefact after it has been exposed to UV radiation. Plants can be dried in the oven or at 40–50°C. Plant material is dried below 30°C so that thermolabile compounds don't break down. Lyophilize plants that are sensitive to heat or cold (freeze-dried). For freeze-drying, you put something that is frozen in a chamber that is empty and between 60 and 80°C. The frozen substance's water vapour quickly moves to the cold surface to dry it [6].

Grinding plant materials

Smaller particles make it easier for the sample and extraction solvent to work together and increase the rate of yield. Grinding made the samples coarser and smaller, while powdering made the particles more uniform and smaller, so they had better contact with the solvents used for extraction. Some of the things that are done to plants before they are extracted are drying and grinding. Because it takes longer to get rid of bigger particles, they must be as uniform as possible. Solvent molecules touch larger analytes, and for extraction, particles with a size of less than 0.05 mm are best [7]. Smaller pieces of dried plant samples can be made with a mortar and pestle, an electric blender, or a mill.

Getting active compounds from plants and herbs

With the traditional method of extraction, certain solvents are used to separate the medicinally active mixture of several naturally active chemicals from plant tissues. It could also be said that the plant material is treated with a solvent to dissolve the medicinally active parts and leave most of the inert parts. So, extraction separates the soluble plant compounds from the cellular marc, which is not soluble. The combination of liquid, semisolid, or dry powder metabolite is meant to be taken by mouth or put on the skin. Extraction is based on how well the solute, matrix components, and stabilizing solvent mix. Liquid/solid,

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liquid/liquid, and acid/base extractions are popular. To get at these active chemicals, you have to follow steps that take into account the plant parts used as starting material, the solvent, the extraction time, the size of the particles, and stirring. The extraction principle is the same for solvent extraction, distillation, pressing, and sublimation. Most people utilize solvent extraction [8].

Conventional ways

Most extraction techniques involve liquid–solid extraction. They are easier to use if you heat them or use solvents with different polarities.

Maceration

Plant extracts are made by soaking coarse or crushed plant parts in a solvent for 2–3 days in a closed container at room temperature while stirring them often. A sealed extractor keeps solvent from evaporating into the air. Soluble phytoconstituents are released when the cell walls of plants are softened and broken. After a certain amount of time, the mixture is filtered or poured out. The easiest and most common way is to use maceration. This method is used to extract by letting molecules move around, which takes time. Maceration breaks up the buildup of concentrated solution around particles and adds new solvent to their surfaces so they can be extracted later [9].

Digestion

This maceration is done with low heat. Menstruum works better because the active parts of plants don't change with temperature (solvent or mixture of solvent used for extraction). When a somewhat higher temperature is okay, it makes the menstruum solvent work better. Most days are between 35 and 40°C, and the highest temperature is 50°C. The part of the plant to be extracted is put in a container with liquid that has already been heated to the right temperature, and the container is shaken for 30 minutes to 24 hours [10]. This process is done on herbs or plant parts with chemicals or polyphenolic compounds that are hard to dissolve.

Percolation

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It is done by slowly passing the boiling solvent through the plant material (5–7 drops per minute) until the extraction is done and then letting the solvent evaporate. At the bottom of the vessel, concentrated plant extracts are collected. Repeated percolations with new solvent and pooling extracts may give a lot of extract. Most of the time, this is how the active ingredients for tinctures and fluid extracts are taken out. It takes a long time, needs a lot of solvents, and may need workers with special skills.Technology for extracting is getting better all the time. They are the most recent new ways of doing things [11].

Extraction helped by microwaves (MAE)

Microwaves have frequencies from 300 MHz to 300 GHz and wavelengths from 1 cm1 to 1 m1. The waves are made up of two fields that move in opposite directions. This extraction heats up faster because of microwaves. Each molecule that is exposed to the microwave field reduces thermal gradients [12]. Heat creates volume, which makes equipment smaller because process rates go up. This makes the same equipment space more productive by making better use of it. MAE uses high heat and pressure and water or alcohol to get the solvents out (Figure 1).

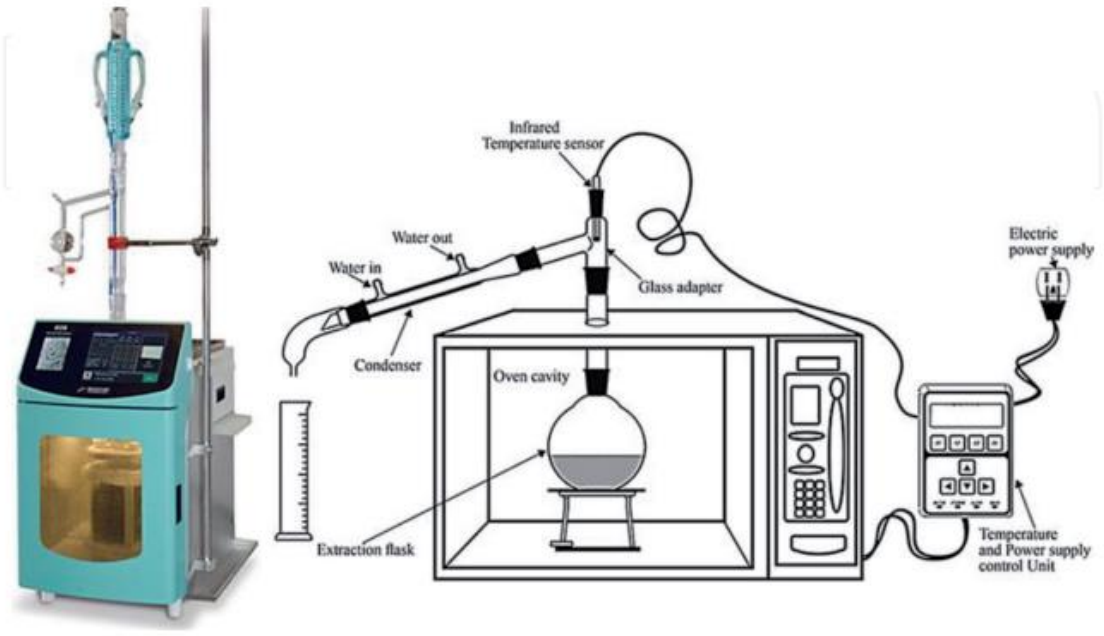

Fig.1 Diagram of how microwave-assisted extraction works

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Sonication or extraction with the help of ultrasound (UAE)

In this extraction process, ultrasound at 20–2000 KHz makes the cell walls more permeable and helps the cavitation process. The procedure is only useful in certain situations because it is expensive. By making free radicals and changing drug molecules, ultrasound waves may damage the active parts of medicinal plants [13]. The diagrams of the equipment are below (Figure 2).

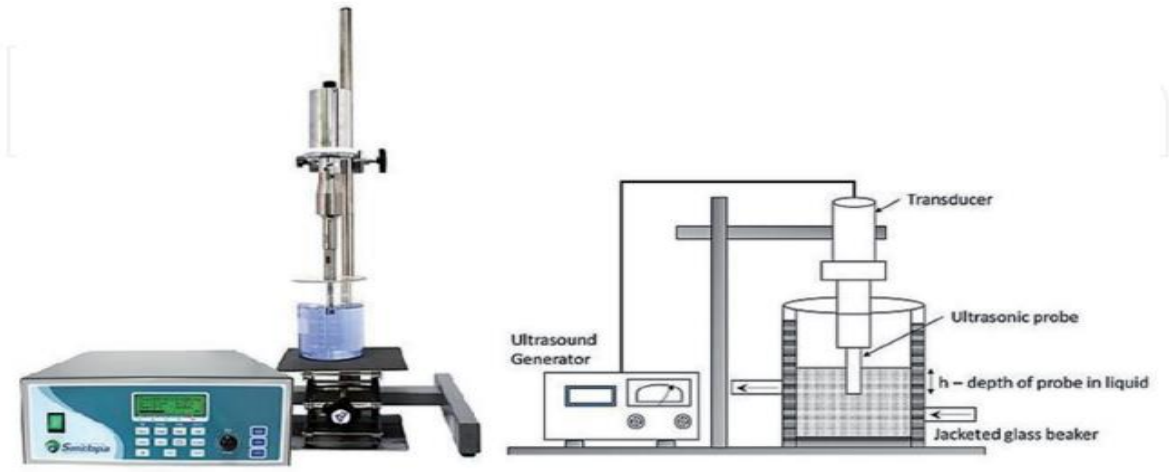

Fig. 2: A diagram of a piece of equipment that uses ultrasound to help pull out teeth

Chromatographic techniques

These methods use the size, shape, and charge of the chemicals to separate them from mixtures. A mobile phase (extraction solvent) and a stationary phase are used in chromatography (silca gel or sephadex with calcium sulphate as a binder). Silica gel separates amino acids, carbohydrates, lipids, and alkaloids. Proteins and amino acids are taken apart by Sephadex. Vitamins, carotene, phenols, steroids, and alkaloids are all separated by aluminium. Alkaloids, food colours, and amino acids can be separated with cellulose powder. Organic cations and steroids are kept apart by celite. Chromatography used adsorption, partition, affinity, ion exchange, or size exclusion to separate chemicals. PC, TLC, CC, LC, GC, and HPLC are all types of chromatography [14].

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Thin Layer Chromatography (TLC)

Adsorption is used to separate compounds from mixtures with this method. Mixtures and stationary phases work together to make things separate. It separates chemicals with low molecular weight. The stationary phase is a sludge made of 100g of silica gel and distilled water. Sometimes, Sephadex is used. On a 20cm x 20cm glass plate, a 1.5mm silica gel solution is poured. It gets hard at 105°C for 1 hour. After that, 10mL of extract is injected onto the bottom plate and spread out. The plate is carefully put into the separation chamber with the mobile phase and left for 30 minutes. Based on how well they mix, chemicals that are mixed will rise on a plate. Each separated part is named by figuring out its retardation factor, which is the ratio of the distance between the compound and the solvent, and comparing it to a known compound [15,16]. The chemicals are scraped off with a spatula and taken out again with different solvents. It takes less time, gets rid of spots, and is resistant to acid.

High Performance Liquid Chromatography (HPLC)

In this method, adsorption is split up. It divides things into organic and inorganic parts. The stationary phase is made up of tightly packed solid particles, and the moving phase is made up of a solvent. Mixture parts interact with solid particles in the stationary phase to start the separation process [17]. People use a solvent reservoir, a sample injector, a pressure pump, an HPLC tube, and a diode detector. The mixture that needs to be separated is put into the bottom of the HPLC. The right solvent is put into the solvent reservoir. A pressure pump forces the solvent down the tap so that it can mix with the sample that was injected. The diode detector sorted the chemicals, got rid of the trash, and sent the last substance to the equipment that would process it [18].

Identification techniques

Medicinal plant extract parts were found in many different ways. It found the functional group, many bonds and rings, the arrangement of hydrogen and carbon, and the structure. People use MS, UV, NMR, and IR (IR).

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Mass Spectrometry (MS)

This method helps find chemicals based on how they are made and how heavy they are. Order and name the unknown ingredient in a mixture. Peptides and oligonucleotides are found. When electrons hit an organic molecule, very strongly charged ions are made. At first, the signal was found by using a 70eV electron ionisation energy and a sample spectrum as a percentage peak [19]. Compounds can be identified by their relative molecular mass and weight. This is done by plotting the masses of fragmented ions against their charges. MS gives a lot of information about organic molecules. So, MS/HPLC is used to make medicines from plants [20,21].

Ultra Violet Spectroscopy (UV)

This method can be used to look at both the quality and quantity of the parts of a plant extract. Certain amounts of phenols, anthocyanins, tannins, and polymer dyes were found. With this method, the total amount of phenolics and secondary metabolites can be found [22]. Specific frequencies were used to find flavonoids (320nm), phenolic compounds (280nm), anthocyanins (520nm), and phenolic acids (360nm) [23].

Conclusion

Extraction, isolation, identification, and use of natural products are becoming more popular. Analytical methods that are modern, economically viable, and good for the environment depend on new research and safe extraction. In plant extraction, it is important to get rid of components that may be extracted along with the target molecules, avoid contamination, and keep needed metabolites or artefacts from breaking down because of the extraction conditions or impurities in the solvent. Filter the solution you've made to get rid of any particles. Researchers have looked at medicinal plants to see if they have biological effects or if they work like they do in traditional medicine. Many medicinal herbs have been taken out, split up, and separated. In biological or pharmacological tests, most substances showed signs of being useful. But success and validity depend on the choice of solvent, the extraction process, screening for phytochemicals, fractionation, and identification methods. Lastly, it is important to know and use these strategies [24, 25]. These methods should be updated often

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to make research easier and better. Plant extracts should only be kept at room temperature or in the sun for a short time to reduce the risk of artefacts being made and extract components breaking down or changing into something else. The plant matrix and type of compost should be taken into account when choosing the best extraction method.

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