WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

A Review: An Overview of Common Extraction, Isolation, UV, and Characterization Technique in Herbal Drug Discovery

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Abstract

Techniques for the extraction, isolation, UV, and characterization of compounds from plant tissue are the foundations performed using these techniques, and publications about them are available. However, it is important to put them together and discuss a basic description with comparative strengths and weaknesses. The goal of this work is to provide a selection of drug discovery and related research areas for the best technologies that can be adopted. Through a basic method of reviewing the literature on technologies systematically used in natural source drug discovery, students, researchers, and physical and analytical chemistry, spectroscopy is frequently used to identify chemicals by looking at the spectrum that they emit or absorb. Additionally, astronomy and remote sensing make extensive use of spectroscopy. The majority of large telescopes are equipped with spectrometers, which can be used to determine an object's velocities based on the Doppler shift of its spectral lines or its chemical composition and physical characteristics. Absorption spectroscopy includes UV-Visible spectroscopy

Keywords: Extraction, Isolation, characterization, Elucidation, drug discovery.

Introduction

Drug discovery from natural resources requires pursuing a multifaceted approach to scientific investigation. Understanding drugs or drug-like activities requires a good understanding of areas such as ethnic use, chemistry, pharmacology, biology, and toxicology [1]. Plant-derived drugs have been successfully embedded in complex scaffolds in plant tissues. Drug discovery from these natural plant sources requires knowledge of the actual active ingredients responsible for one or more pharmacological activities. its nature and characteristics. The first step is to acquire knowledge of its ethnographic uses and instruct scientists on the appropriate extraction methods to apply. Chemistry and other areas of scientific investigation play a central role in drug discovery, where active ingredients must be isolated, characterized, and tested from complex scaffolds of plant material [2]. Various techniques are currently used to release, isolate and identify active ingredients in plants. Depending on the type of compound group of interest, compounds are extracted at hot, cold, or ambient temperatures if known. Drug discovery standards include therapeutic and qualitative evaluation of drugs and pharmaceuticals. Their purity, homogeneity, potency, physical and chemical stability, bioavailability, and identity characteristics [3] that require isolation of the extracted phytopharmaceutical materials are identified and further developed. Evaluation of the use of plant ethanol for a disease is done through community surveys, previous literature reviews, results of community interventions, media reports, and community observations [4,5]. Extraction is performed by different techniques in different solvent systems [6]. Separation is carried out using chromatographic techniques, mainly using spectroscopic techniques to characterize the separated compounds [7].

2. Extraction method

Plant matter extraction is an important process in isolating and purifying natural plant compounds. Plant matrices are complex in nature and contain various compounds with different physical and chemical properties [8]. Therefore, it is imperative to carefully separate the matrix from the rest of the plant to generate the desired pure compound for plant characterization. There are several ways to classify extraction methods [9]. In this chapter, they are classified according to the operating temperature

2.1 Low or room Temperature methods

2.1.1 Cold extraction method

This method is described in Refs [10, 11]. Briefly, he puts samples of dried plant parts in different solvents for 7 days, shaking them every 24 hours. The sample is then filtered through Whatman filter paper, vacuum dried on a pre-weighed watch glass at room temperature, and the yield is determined by difference. A common example of cold extraction is maceration. In this process, coarsely ground plant parts or whole plant material is stored in contact

with a solvent for some time with periodic shaking. In the process, soluble substances dissolved in the solvent are released [12]. The overall advantage of cold extraction methods is that they are simple, inexpensive, and environmentally friendly [6]**.**

2.1.2. Enzyme-Assisted Extraction (EAE)

This method uses a solvent containing different enzymes, chosen according to the environment in which the solvent works best and how the scientist needs to catalyze the compound. Commonly used enzymes for extraction include proteases, lipases, and phospholipases, effectively reducing solvent usage [13]. Especially for essential oils, pectinase and α-amylase are the most commonly used enzymes. This method does not drop the connection, but it is expensive to set up. They are also too demanding in terms of required nutrients, the presence of oxygen, and temperature optimization.

2.1.3 Plant tissue homogenization

Fresh, wet, or dry plant parts are ground and soaked in a solvent. Shake the mixture vigorously for 5-10 minutes or leave it for 24 hours with periodic shaking, then filter the extract. The extract filtrate can be concentrated by centrifugation and dried. In some cases, the filtrate can be dried directly under a vacuum and then redissolved using the desired solvent [14]

2.1.4 Ionic Liquid Extraction

In this method, organic salts in liquid form selectively interact with polar and nonpolar compounds using π-stacking interactions, hydrogen bonding, ion exchange, and hydrophobic interactions [15]. This is an excellent method for recovering organic and inorganic ligands in high yields. Ionic interactions make the extraction quality and efficiency very high. High-temperature extraction should be performed for compounds known to be thermally stable [16, 17]. Many people worry that high-temperature extraction denatures essential compounds, but not all compounds are destroyed. Research on the extraction of Asiatica Cantele.

2.2 High-temperature extraction methods

in phenolic compounds and antioxidant activity has been reported [18].

2.2.1 Decoction

This procedure involves boiling, cooling, straining, and pouring the right amount of cold water into the medicine to produce the required amount. This method can be used to extract heat-stable and water-soluble compounds of interest from herbal plants [17]. Due to high temperature, this method yields more oil-soluble compounds than leachate or maceration [18].

2.2.2 Soxhlet extraction

This is ideal when the compound of interest is known to have limited solubility in the solvent, or when an impurity is insoluble in that solvent. Thermally labile compounds may decompose and are not subject to this procedure. The advantage of this method is that multiple passes through the hot solvent are not required, and one pass is sufficient for it as it is reused [16].

2.2.3 Microwave extraction

This is essentially a conventional solvent extraction, but the solvent-sample mixture is heated using microwave energy. Microwave energy penetrates plant material and selectively evaporates even traces of water within plant cells. The pressure created by the heating inside the cell causes the cell wall to rupture. This pleasure secretes active ingredients from cells and in this way increases the yield of plant components [10].

2.2.4 Automated solvent digester extraction method

Recent use of general-purpose extraction systems (such as the BUCHI system) uses thimbles containing test samples in small vials containing various extraction solvents, with temperatures set just below boiling points. The extract is filtered, concentrated in a vacuum concentrator, and ready for phytochemical measurements [10, 11]

2.2.5 Hydraulic Oil Extraction (PLE)

Devices using heat up to 200°C and pressures between 35 and 200 bar are used [13]. The sample is placed in a holder and water is typically used as a solvent. As temperature increases, heat causes the solvent to become less viscous and penetrate the plant matrix more easily. High pressure keeps the solvent in the liquid phase. structure of the device protects oxygen and photosensitive compounds from decomposition. Although this process is environmentally friendly, it is expensive. Selectively suitable for thermally stable compounds.

2.3 Optional temperature extraction

The following steps are optional, depending on your knowledge of the nature of the desired components in your sample.

2.3.1 Sequential Exhaustive Sampling

This is an extraction with solvents ranging from the least polar solvent (usually n-hexane)the to most polar solveusualally methanol), ensuring that compounds with a wider range of polarities are extracted. It is one of the most common extraction methods that can be performed at elevated temperatures, especially for heat-labile compounds [17].

2.3.2 Injection and digestion

This method is only used when the drug ingredients are readily available in the whole form, such as tea in tea bags. Infusions are freshly prepared by softening the finished drug component with hot or cold water. A closely related method is digestion, during the preparation of the extract, uniform, mild heat is applied to the maceration process to facilitate the release of the active ingredients [17].

2.3.3 Supercritical fluid extraction process

This method uses a liquefied gas (usually CO2) that is pumped through a cylindrical channel containing the test sample material at approximately 32 °C or higher and 74 bar [13].

The mixture is then sent to a separation chamber where the gas is recovered for reuse and the extract is completely separated from the solvent. This makes the method superior as it leaves no traces of solvent in the extract [20]. At low temperatures, thermally labile compounds such as terpenes and terpenoids (boiling point ~150 °C) are obtained in high yields [13]. It is non-toxic, easily extracted primarily from essential oils, and non-flammable.

2.3.4 Sonication

This sonochemistry-based method uses ultrasound (20-2000 kHz) to penetrate the sample material. It can be used at room temperature, but in most cases can be used on a variety of high-temperature hotplates [21] to increase cell wall permeability. Solvent selection is important based on viscosity, polarity, surface tension, and vapor pressure, which affect the cavitation phenomenon [13]. Methanol, ethanol, and hexane are the most common solvents, sometimes water is added. Although this method is effective in liberating components, its drawbacks include high installation and running costs, modification of some active ingredients, and formation of free radicals in the sample that can confound the results. [22]

3. Analytical technology for separating and refining plant components

Due to the variability of phenolic compounds (polarity, chemical structure, glycosidic linkages, and spectral properties), no single method is universally suitable for the separation of all extracts and must be chosen carefully [23]. The identification and isolation of bioactive compounds from herbal extracts is the starting point for drug development for potentially new mechanisms against human disease [24]. Purification exposes the sample to different solvents of different polarities and separates them using chromatographic techniques [25, 26]. Broadly speaking, he has two types of chromatography. liquid and gas chromatography. Liquid chromatography techniques are techniques in which the mobile phase is a liquid.

3.1 Liquid chromatography technology

This chromatographic technique separates molecules within a test sample based on shape, size, and charge [27]. The mobile phase, the solvent containing the analytes, is passed through a molecular sieve called the stationary phase to separate the components of the extract. Chromatographic techniques can be classified based on phase states and mechanisms, phase polarity, separation region geometry, experimental parameters and duration gradients, and column dimensions. This chapter describes techniques based on phase states and mechanisms.

3.1.1 Adsorption chromatography

Also called liquid/solid or displacement chromatography, it is based on interactions between solutes and active sites on a solid stationary phase. In particular, the stationary phase interacts with specific functional groups of the mobile phase through non-polar interactions, non-covalent bonds, hydrophobic interactions, and van der Waal forces [28].

3.1.2 Partition chromatography

Also known as liquid/liquid chromatography, this process is based on the interaction of the molecules to be separated with two solubility-immiscible liquid phases, the stationary liquid phase being adsorbed onto a solid. soluble components in it are strongly retained by it and are the first to elute when they are in the mobile phase, whereas they are delayed in the system when they are more strongly retained by the stationary phase [29].

3.1.3 Affinity and ion chromatography

The extract is introduced onto the column and interacts with stationary phase ligands. If they have a high affinity for their ligand, they will be attracted toward the stationary phase. If there is little or no affinity, buffers with different pH or higher ionic strength will be easily washed out of the system and will elute faster. Typically, the component of interest binds to a ligand [10]. On the other hand, closely related ion chromatography separates ionic components and polar molecules in extracts based on their electrical properties [30].

3.1.4 Linked stages

In this method, the stationary phase is an organic species attached to a solid surface. When the mobile phase is liquid, the process is called led the liquid bond, ed phase,e, and equilibrium are based on the separation between the liquid and bonding surface [33]. When the mobile phase is gas, the process is called the gas-bonded phase and the equilibrium is based on the distribution between the gas and the bonding surface [7].

3.2 Examples of Liquid chromatography

3.2.1 Planar chromatographic techniques

This is a technology that allows the solute in the solvent to pass through the surface of the adsorbent arranged in a plane.

3.2.1.1 Paper chromatography

Special paper is used to provide an inert platform for separation. Carefully place the test sample near the bottom of the paper and place the paper into a chromatography chamber containing a solvent that moves upward by capillary action carrying the appropriate soluble molecules.

The paper should have high porosity for high capillary action and be thick to hold more samples

[34]. The advantage of paper chromatography is that it is relatively inexpensive and has good reproducibility of retention factor (RF) values directly on paper [35].

3.2.1.2 Thin layer chromatography

This is adsorption chromatography [36] and the separation is based on the interaction of the sample with a thin layer of adsorbent attached to a plate. This is ideal for small molecule compounds. Adsorbents are carefully selected according to the components they are good at separating. The most commonly used adsorbents to separate components are silica gel (especially alkaloids, amino acids, lipids, sugars, and fatty acids), aluminum (phenols, alkaloids, carotenes, steroids, vitamins), celite (inorganic cations and steroids), Starch. (amino acids) and Sephadex (proteins and amino acids) [10].

3.2.1.3 Columns and pressurization techniques.

These are chromatographic techniques that use packed solid-phase columns as separation housings

It is based on the interaction of solutes in the mobile phase (usually polar combinations) with tightly packed solid stationary phase particles (usually non-polar particles) High pressures of 250-400 bar are required to elute the analytes from the column to the detector. A diode array detector (DAD) is one of the most commonly used detectors for measuring analyte spectra. This technique is suitable for thermally labile and non-vaporizable samples and is a good complement to gas chromatography techniques in sample analysis [38].

3.2.1.3 High-performance thin layer chromatography

This technique is an advanced technique of TLC with features that enable better efficiency and resolution, autosampler and automatic visualization of spots, and quantitative analysis [39]. This technique uses a chamber containing a diaphragm rather than a column. HPTLC manufacturing has a plate sorbent size of 5-7 microns and a coating layer of 150-200 microns thicker than TLC. The mobile phase is pumped over the plate under constant pressure [40]. HPTLC is also used in combination with spectroscopic techniques to maximize the analytical potential of these techniques [40]

3.2.1.4 Lamina chromatography with optimal performance

This technique combines TLC and HPLC principles and is a preparative and analytical tool suitable for both research and quality control laboratories. The liquid mobile phase is pumped through a column supporting a solid stationary phase of silica gel or bonded phase media (amino, diol, cyano, ion exchange, C8, and C18). Pressures up to 50 bar are sufficient to force the mobile phase through the planar column at a constant velocity. [10].

3.3 Gas chromatographic techniques

The species are dispersed between gaseous (mobile) and liquid (stationary) phases. The sample is vaporized and injected into a chromatographic column transported by an inert gas. The stationary phase is embedded in an inert solid material. The distribution of species in the test sample gives a measure of separation, with some mixing well in the stationary phase, being retarded in the gas phase or not eluting at all, and those well distributed in the gas phase eluting as gases. [17, 43].

3.4 Non-chromatographic techniques

3.4.1 Immunoassay

This method uses monoclonal antibodies directed against low molecular weight bioactive natural products and drugs [44]. They offer extremely high sensitivity for receptor binding assays, qualitative and quantitative analyses, and enzymatic assays. Compared to chromatography, enzyme-linked immunosorbent assay (ELISA) is more sensitive in most cases [8]. Although this method is efficient, it is very laborious to obtain reagents, ethically acceptable for in vivo assays, and expensive for in vitro assays in cell lines.

3.5 Structural elucidation by spectroscopy

Structural elucidation essentially involves using spectroscopic techniques to characterize the chemical constituents in a sample [25] This is done using a combination of spectroscopic techniques, each providing key information that must be linked to the other's data. Public repositories of compounds such as ChemSpider [45] and PubChem [46], and drug and metabolism databases such as HMDB [47], DrugBank [48], KEGG [49], MZedDB [50], ChEBI [51], provide a website. - Search based on the formula and mass of known molecular compounds and available biological test results [52]. This section presents techniques for identifying the structures and names of compounds isolated from plant extracts.

3.5.1 Fourier-transform infrared spectroscopy (FTIR)

This technique helps identify functional groups present in isolates from plant extracts [53, 54]. He a drop of his liquid sample he places it between two sodium chloride plates, forming a small film. For solid samples, they are ground to the finest possible powder and mixed homogeneously with potassium bromide (KBr). The mixture is then compressed into a pellet, placed in a sample holder, and ready for analysis. The analysis produces peaks at specific wavenumbers that are characteristic of the functional groups detected in the sample [17]. 3.5.2 Nuclear magnetic resonance spectroscopy (NMR)

This technique helps determine their orientation relative to the structural framework and the central isotope carbon. This technique uses magnetic fields to determine structural geometry. C NMR helps identify the types of isotopes present in a region and how they are positionally related to each other. 1 H NMR largely captures the relative positions of protons in the structure. Another type of NMR is time-domain NMR, which provides information about molecular dynamics in solution. If the sample is solid, solid-state NMR can be used to directly determine the structure of the solid sample. In industrial applications, NMR techniques are replacing X-ray crystallography techniques because of their versatility [10].

3.5.3 Mass Spectrometry (MS)

This technique is useful for elemental determination of plant extracts and other products. It is also important to partially understand the partial structural organization using mass spectral fragmentation [55].

Triple quadruplex MS is very sensitive and is typically used for target samples. For very high mass accuracy and resolution, Fourier transform instruments are used for structure determination, which provides information about the mass of the entire compound and the masses of various fragments of the compound [10].

3.5.4 UV Spectroscopy

The investigation of how electromagnetic radiation interacts with matter is known as spectroscopy. It is employed for a variety of sample analyses The term "spectrum" refers to a depiction of the reaction as a function of wavelength or, more typically, frequency This process involves measuring these reactions, and a spectrometer or spectrograph.

Conclusion

The cost of some handling equipment and equipment can be a limiting factor for extraction and isolation. A combination of simpler and cheaper methods can overcome this limitation. Plants have a wide variety of compounds with the potential for drug development, so extracting and isolating them with a single method may not be ideal. Combining two or more methods during the extraction and separation stages may improve efficiency. Structural elucidation has already taken place with a combination of different techniques for meaningful interpretation of spectral data. However, we need a single, robust device that can perform all techniques at once to determine compound structures. Using UV equipment, you can calculate lambda max, which is

unique to each compound A limitation of this work was that it did not include a commercially available rapid test method. The method still uses the basics described in this overview.

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