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A Review Of Modern And Conventional Extraction Techniques And Their Applications For Extracting Phytochemicals From Plants Abstract

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ABSTRACT

The first important stage of plant formulation preparation are extraction processes. Modern methods of extraction, highly effective and advanced, have played a crucial role in the progression of traditional herbal remedies. Significant strides in the development of modern techniques for extracting and analyzing medicinal plants will ensure the availability of top-tier herbal products to consumers globally. In the development of analytical techniques for identifying constituents in botanicals and herbal preparations, various extraction methods are used for sample preparation. In this thesis, the principles of operation, influencing factors, research advancements, and strengths and weaknesses of different extraction approaches are discussed. Natural medicines have long relied on methods that save solvents and energy, especially for thermolabile phytocompounds. For centuries, natural products have been the sole means of preventing and curing human diseases. They serve as an essential source of drugs. The quantities of bioactive natural products in natural medicines are typically quite low. Today, methods for extracting and isolating bioactive natural products are crucial and highly preferred. The paper provides an overview of techniques used in the past for extracting and isolating natural products. In this paper, the techniques used in research on natural products, both conventional and modern, are compared with their advantages, disadvantages, and practical examples presented.

Key Words: *Efficacy, Extraction Efficiency, Microwave Assisted Extraction (MAE), Supercritical Fluid Extraction (SFE), Ultra sonication Assisted Extraction (UAE), Natural products, Extraction, Isolation, Natural medicine, Chromatography, Phytochemical investigation*

Introduction :

Medicinal plants have been an integral component of traditional healthcare systems in Africa since the beginning of time. They are perhaps the earliest and most potent curative entities in this region. [1]

Approximately USD 100 billion is the estimated global market value for all medicinal plant commodities each year. In pharmaceutical chemistry, there is a significant advancement in the creation of synthetic drugs. However, nearly 75 to 80 percent of the global population still rely on herbal drugs as their medicines, In third world countries, primary health care is mostly provided using medicines that have better tolerability with the human body and minor side effects, and are easier to obtain. [2]

Nearly 44% of all new drugs are synthesized using these natural products, primarily as lead compounds, to formulate and prepare partially synthetic medicines. [3]

Plants have become more fundamentally involved in the discovery of new lead molecules and drugs. New drugs are discovered in medicinal plants by screening their extracts for novel compounds and determining their biological activities. The molecular structure and pharmacological or toxicological properties of the suspected new molecules or bioactive compounds are determined after they are separated and purified. [4]

The phytochemicals are extracted from plant parts such as roots, stems, barks, leaves, or fruits using a suitable solvent. This process is then followed by identification of the plant species by a botanist at a herbarium, using well-conserved

leaves, flowers, or fruits obtained from natural settings. The solvent is removed from the extract through various processes, leaving a concentrated version behind. This concentrated extract is then subjected to chromatographic techniques for the isolation and purification of bioactive compounds. The isolated compounds undergo spectroscopic analyses, such as UV/Vis, IR, carbon and proton NMR, and mass spectrometry, to determine their structures. Chemical methods may be used for pharmacological and toxicological testing after that. The bioactive compounds, once elucidated, may be synthesized or semi-synthesized. Extraction is an integral feature in natural product research. Goings-on relate to the unyielding advancement and discovery of more efficient and cost-effective extractive techniques. This review delves into various conventional and contemporary extraction methods, their optimization conditions, and their comparative advantages and disadvantages. These techniques have been critically reviewed for a wide range of recent applications. This literature review will contribute to the current knowledge and discover new extraction methods. Bioactive compounds are produced in large quantities by a plant's life, falling into two categories of enormous phytochemicals. [5]

Primary metabolites are produced by a plant for its normal growth and development. They include nucleic acids, carbohydrates, fatty acids, proteins, and ubiquitous molecules such as growth regulators and cell wall components. The surroundings and threats in the niche require the plant to enhance its ability to survive by producing secondary metabolites within the second category. Secondary metabolites function as compounds in plants, enabling adaptation to local environments. They serve various roles in plant physiology and biochemistry. Biomolecules with insecticidal, fungicidal, antibacterial, or antiviral properties are produced by certain plant species in unfavorable niches, such as warm and moist humid tropical forests, in order to save themselves. [6]

Some plant roots can produce antifungal phytochemicals, even when there are no visible signs of invasion among its leaves. These defensive bioactive molecules can protect the plant against insects and microorganisms in the soil. Additionally, these secondary metabolites may exhibit antifungal reactions against human pathogenic fungi. [7]

Phytochemicals, with their diverse functions in plant cells, are of interest to pharmacologists and biochemists. Some specific secondary metabolites act as bioactive compounds, causing pharmacological or toxicological effects on animals and humans. Terpenes and terpenoids, alkaloids, and phenolic compounds can be broadly classified as bioactive phytochemicals. [5-7]

Medicinal plants have been used extensively in healthcare systems around the world for centuries. In Africa, they remain a primary component of traditional healthcare and represent the earliest and most resilient curative entities.[8]

In rural Africa, plant remedies prescribed by traditional practitioners are the most accessible and affordable medicinal drugs for communities, and at times, the only treatments available. Plant remedies have been studied worldwide to establish their efficacy. Some promising potential results have led to the synthesis of plant-based medicines. [9]

Approximately USD 100 billion is the estimated global market value for all medicinal plant commodities each year. In the current scenario, about 75 to 80% of the global population rely on herbal drugs as their medicines, despite the significant advancements in the creation of synthetic drugs through pharmaceutical chemistry, In third world countries, primary health care is mostly provided using medicines that have better tolerability with the human body and minor side effects, and are easier to obtain. [10]

Novel drugs are developed primarily as lead compounds using natural products, with an estimated 44% being derived from them. These natural products are used to prepare and create partially synthetic medicines.11]

Novel lead molecules and drugs have become the focus of research, shifting from traditional methods to using plants. Plants are screened for new compounds through extract analysis, followed by biological activity tests. New molecules or bioactive compounds are then isolated and purified for elucidation of their molecular structures and further pharmacological or toxicological tests.[12]

Extraction :

Desired natural products are separated from raw materials through the process of extraction. The extraction method depends on the principle involved, which can be solvent extraction, distillation, pressing and sublimation. The steps involved in the extraction of natural products are commonly carried out through solvent extraction. [13]

The solid matrix can absorb the solvent. [14] The solvents cause the solute to dissolve. [15]

The solid matrix releases the solute. [16] The solutes are collected as they are extracted. The properties of the extraction solvent, the particle size of the raw materials, the solvent-to-solid ratio, the extraction temperature, and the extraction duration will affect the efficiency of the extraction process.[17-21]

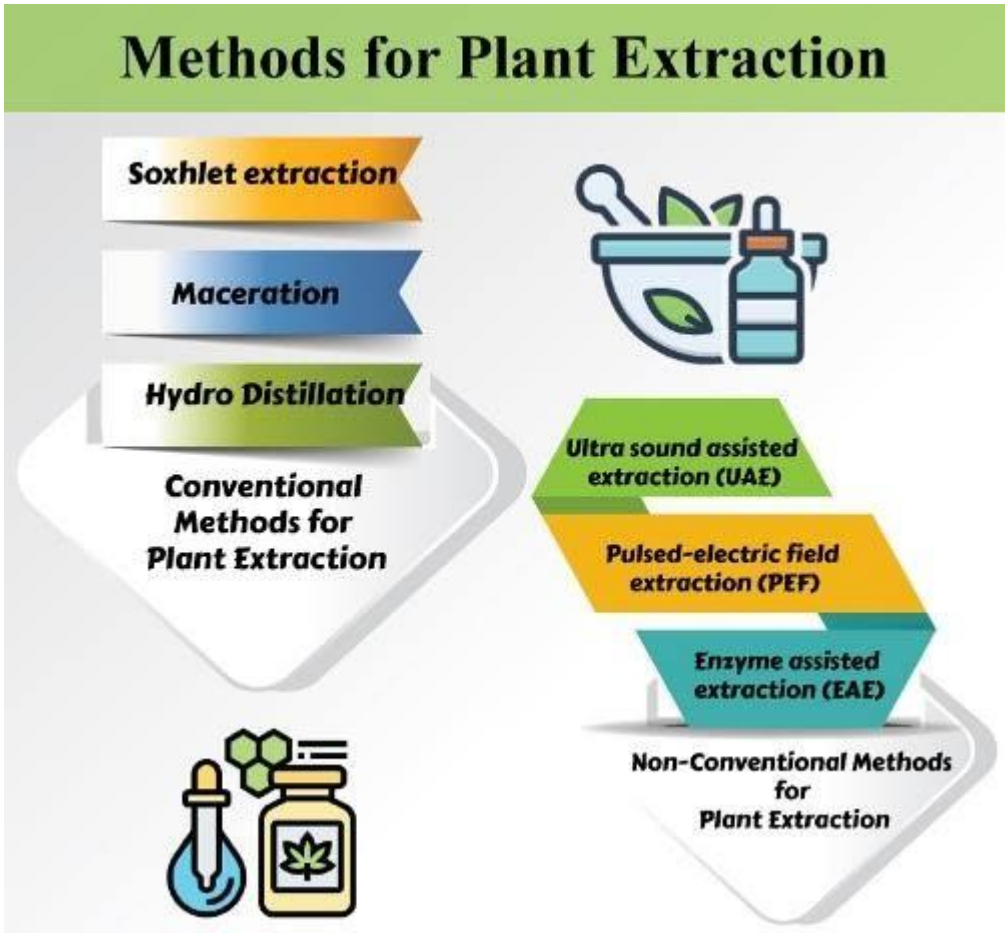

Figure – 1 (Types of Extraction)

Conventional extraction techniques :

Meceration

The solvent soaks the plant material in the maceration extraction method for at least three days with intermittent agitation at room conditions. [9] The mixture is strained after the extraction process is complete, either through sieves or a net with tiny holes. Later, it is pressed and filtered or decanted, standing aside. Marc is processed in this manner. Maceration is preferably conducted in a stoppered container to minimize solvent loss through evaporation. The solvent should not evaporate during extraction, resulting in already concentrated extract being obtained. Vacuum evaporation is used frequently to concentrate the product. An appropriate solvent is necessary for maceration as it determines the categories of phytochemicals obtained from the samples. Thermolabile phytochemicals can be recovered with the help of the solvent. The process, however, has drawbacks, including low efficiency and lengthy extraction time. [22]

This procedure can have substantial efficiency under optimized conditions, resulting in high yields of phenolic compounds and anthocyanins from chokeberries.[23]

The extractive methods for a study on C. cajan leaves to obtain flavonoids, including microwave-assisted, reflux, ultrasound-assisted, and maceration, were employed. The yield was observed to be the least using maceration techniques. Triton X-100, a nonionic surfactant, was used at 35 °C and pH neutral to extract flavonoids from rhizomes of turmeric, fruits of Arbutus unedo L. raised at a temperature of 79.6 °C in 3.7% diluted ethanol, leaves of Ficus carica, and Euphorbia neriifolia using 75% concentrated ethananol at room temperature.[25-27]

These studies used solvents based on the polarity of the phytochemicals for extraction. Triton X, with its hydrophilic side chain despite being nonionic, was employed for extracting less polar flavonoids. In contrast, more polar flavonoids were extracted using ethanol or a mixture of ethanol and water further, 50% ethanol has been used as a solvent to extract polyphenols, including anthocyanins, from dried chokeberries. [28].

Digestion

The extraction process in digestion involves slight warming, which is similar to maceration, for extracting the desired compounds.[9] The extraction solvent's efficiency improves as it is warmed, but care must be taken not to change the temperature and alter the bioactive phytochemicals of the given plant material. The temperatures are mostly set between -18 °C and 50 °C, but can be raised to 50 °C for harder plant materials and those containing poorly soluble phytochemicals. The solvent is pre-heated to the indicated temperatures and the desired plant parts are introduced into it in a container. The

extraction process lasts between half an hour and 24 hours, during which the optimum temperature is maintained. This is achieved by shaking the container at regular intervals. [22]

Infusion and percolation

The plant material is steeped in hot solvent, primarily water, for about 15 minutes in a capped container. The extract (tea) is then poured off and separated from the marc using a filter. Infusion is a method used to extract the readily soluble constituents of the plant material in a weak solution. [22]

The dried crushed leaves of tea brands such as alokozay, lipton, tapal, and tetley can extract caffeine within the temperature range of 30 to 90 °C during brewing times that range from 2 to 30 minutes. (The infusion process extracts caffeine from the tea leaves.) [29]

Phenolic compounds are obtained from Tilia cordata fruits at an optimal temperature of 95 °C. Some infusions are used as remedies for health conditions like diarrhea, bronchitis, and asthma. For example, antioxidants, phenols, and flavonoids are extracted from different ginger rhizomes through boiling water for 10 minutes.[30]

The most common process for making liquid extracts, known as tinctures, is percolation. This method involves the solid material passing through the liquid drop by drop. The plant material is slowly saturated with ethyl alcohol as it is gradually added, and the solvent pushes the previously added solvent down through the material. [22]

The plant material must be shredded before being put into the percolator to avoid shredding it too finely. The fine particles could then easily separate from the extraction solvent. The extraction solvent causes the plant matrix to pre-moisten and stretch out, allowing phytochemicals to easily diffuse into it. However, this process results in the extract becoming cloudy with residue settling at the bottom of the percolator.[22-30]

The plant material is introduced into the percolator, and then the extraction solvent is added from the top. The solvent passes through the plant material at a speed determined by the nature of the plant material. It must not flow too rapidly to allow for solvent penetration into the plant cells and the extraction of phytochemicals. However, the solvent's percolation rate should not be too sluggish. For incomplete extraction, a greater quantity of solvent is necessary. Generally, 5 mL/min is the recommended solvent flow rate for extracting 1 kg of plant material. The selection of an extraction solvent relies on the chemical characteristics of the secondary metabolites being isolated. The plant material is widely extracted using a water-alcohol solvent mixture. The water hydrates the plant walls, and the alcohol extracts active components from the plant material, which are chemically close to it. 70% ethanol was used for the extraction of phenolics, specifically elicitation. Petroleum ether was used for the extraction of phenols and flavonoids type antioxidants. [31-32].

Inorganic aqueous solutions of hydrochloric acid were used for extracting alkaloids from various wild fruits through percolation, besides alcohol.[33]

The plant material acts as a preservative through the absorption of alcohol, which in turn preserves the extract. The resulting liquid is referred to as leachate. After extraction, the plant material is pressed to separate the residually absorbed solvent, and solution is added to the leachate. The extraction process is considered complete once a colorless liquid, free of phytochemicals, has been eluted from the percolator. [22]

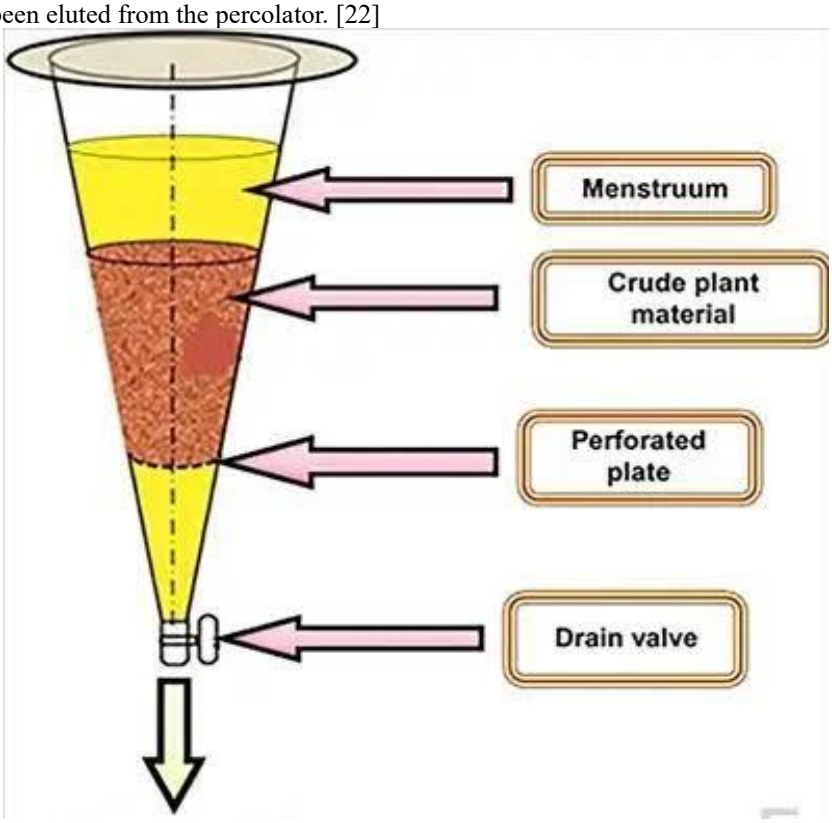

Figure – 2 (Method of percolation)

Decoction

Decoction involves boiling plant materials in water for 15 to 60 minutes, resulting in the extraction of phytochemicals that are not degraded or altered by the increase in temperature.[22].

The tender parts of the plant, such as leaves, roots, flowers, and young shoots, are typically boiled for 15 minutes to extract phytochemicals. Decoction and infusion are used to extract phenols and flavonoids from fruits, rhizomes, and leaves at specific temperatures. [32-34]

The liquid extract is obtained by filtering the solution after decoding astringent plant parts with branches and tree barks for an hour. The decoction is cooled before filtering to achieve the desired volume. Undesirable products are likely to be present in the extract produced through decoction. Decoction is not the optimal technique for thermolabile compounds. It has been found that the bark extract of S. Cumini, when extracted using decoction, possesses substantial antiglycation and antioxidant potential.[35]

Soxhlet extraction

The thimble, filled with ground plant material, is used for the continuous extraction of phytochemicals using a hot solvent. [22]

Phytochemicals are extracted from the ground plant material in the thimble, using ethanol or methanol as the solvent in the bottom flask. Heated in the apparatus, the solvent vaporizes and condenses in the condenser, then drips back into the thimble, completing the extraction process. This technique enables the achievement of improved yields compared to maceration extraction methods. At 70 °C for eight hours, fatty acids are recovered from hemp seeds. Phenolic compounds are extracted from leaves using 60% ethanol for a two-hour extraction time. [36-37]

Numerous studies have demonstrated the effectiveness of the Soxhlet extraction technique. It extracted 38.21 mg g−1 of ursolic acid from traditional Chinese medicine cynomorium. However, this method poses a risk of degradation for thermolabile compounds due to the higher temperature. The amounts of polyphenol and alkaloid extracts decreased in the Soxhlet method, as found in an article comparing it to maceration. [38]

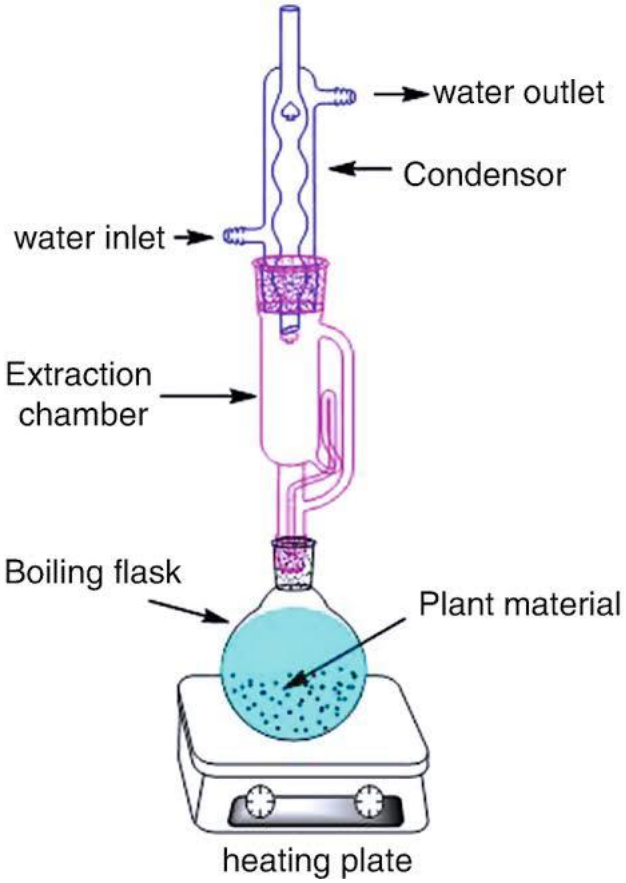

Figure – 3 (Soxhlet Extraction)

Modern extraction techniques

Accelerated solvent extraction (ASE)

Under such conditions, the solvent is maintained in its liquid phase by using elevated temperatures ((50 to 200°C) and pressures (10 to 15 MPa). This results in an enhanced diffusivity of the solvent, which in turn leads to a faster extraction process. [39]

Significance has been achieved by this method due to its advantages, including low solvent consumption, high yield, and relatively shorter time span. Favorable conditions for Accelerated Solvent Extraction operations are indicated by higher

solvent temperature and pressure. This technique of solvent extraction is more robust than maceration or Soxhlet extraction. Significant ASE performance is demonstrated through various examples. For instance, ASE has been shown to recover more lipophilic and hydrophilic phytochemicals from raspberry pomace than Supercritical Fluid Extraction. ASE had less impact on temperature and extraction time than SFE, yet it recovered 25% of both lipophilic and hydrophilic compounds, which was less than the yield of 15% in SFE.[40]

This technique involves charging an extraction cell made of stainless steel with a sample, followed by charging it with a solvent and placing it between inert silica layers, which are separated by cellulosic filter papers. The system is then heated at high temperature and pressure for a specified duration to promote extraction. The heating process increases the diffusion coefficient and decreases the viscosity, thereby facilitating the extraction process. The solvent and nitrogen are used to clean the extract in vials, with fresh solvent and nitrogen pumped in. Inert packing material keeps the plant matrix from forming aggregates that might obstruct the system. [41]

The extraction conditions of ASE - temperature, pressure, and extraction time – can be adjusted to achieve higher recovery yields of phytochemicals. For instance, the effectiveness of ASE was assessed in relation to cocaine and benzoylecgonine extracted from coca leaves. At an optimum temperature of 80 °C, 20 MPa pressure, and after 10 minutes, the extraction process exhibited its strongest effect. [42]

Phenolic acids and flavonoids are extracted differently by temperature. High temperatures yield maximum content of phenolic acids, while lower temperatures optimize extraction efficiency for flavonoids.[43-44].

The critical conditions for extraction can decide the efficiency of extraction, singly or in combination. In some situations, one parameter alone determines the effect on extraction. The recovery of carbohydrates and phenolic compounds from barley hull did not depend on the pressure and time of extraction. Instead, only the temperature was found to significantly influence the yield of phytochemicals during extraction. [45]

The nature of phytochemicals influences the extraction yield in different ways, as some researchers claim that various extraction conditions can affect the yield of two distinct types. The discovery was made during the ASE process of extracting Beta-glucans and phenolic compounds from waxy barley. At high temperatures, the β-glucans exhibited lower extraction yields, but the phenolic compounds showed higher recovery yields. The reason for the lower β-glucan yields at the extraction's lower end might be due to their fragmentation at high temperatures. [46]

Microwave-assisted extraction

Microwaves and solvent collaborate in the new extraction method for natural products, known as microwave-assisted extraction or microwave extraction. Microwaves span the frequency spectrum from 300 MHz to 300 GHz. During extraction, microwaves heat the solvent and plant tissue, thereby enhancing the extraction's rate. The process of dipolar rotation in microwaves results in the conversion of energy to heat. Heating is directly proportional to the dielectric constant of the solvents. The viscosity of the solvent influences the extraction process, as it affects the dispersion of ions and therefore solvation. The solvents diffuse into the sample, extracting the solute from the functional site, and the released solutes then join the solvents. This method effectively maintains the biological activities of the extracts. The antioxidant activity, total phenolic content, and targeted color quality of green tea extracts were improved through the optimization of MAE , resulting in confirmed enhancements of the phytocompounds' antioxidant properties. [48,49]

Saponins, sterols, flavonoids, and polyphenolic antioxidants have been isolated using the MAE technique from seeds, leaves, dried mushrooms, and leaves, respectively. [50-52]

The microwaves directly strike the polar compounds, such as flavonoids, polyphenols, and saponins, which are extracted by MAE as phytochemicals. Available are various progressive and powerful MAE instruments and methods, such as solvent-free microwave-assisted extraction (SFMAE) and pressurized microwave-assisted extraction (PMAE).[51]

The plant materials' drying process heats the infinitesimally small traces of moisture in the plant cells, resulting in evaporation once the microwave begins to heat the water within the cells. Phytochemicals are released from dried plant cells when cell walls are pushed outward due to pressure caused by evaporation. The cell walls stretch and burst, increasing the yield of bioactive substances.[51].

The solvent pre-treats the plant material by soaking it, resulting in increased amounts. It facilitates the hydrolysis of glycosidic (ether) bonds of cellulose, producing fractions soluble in the solvent. The solvent also aids in deeper penetration into cell walls as temperature rises, enhancing phytochemical yield. The extraction techniques, specifically heat–reflux (HRE), cause a series of cellular penetration and phytochemical solubilization, resulting in the release of bioactive compounds from the plant cells. In MAE (Micro-Assisted Extraction), on the other hand, the plant cell walls are completely ruptured, as evident in the SEM (Scanning Electron Micrographs) images. The plant material in MAE undergoes cell wall expansion, causing phytochemicals to flow out and come into direct contact with the solvent. In contrast, during MAE, the infinitesimally small vapor traces in plant glands and vascular tissues are heated, resulting in desorption of active components and the exposure of phytochemicals to the solvent. The dielectric susceptibility of the solvent and plant material, as well as microwave power and extraction time, influence the efficiency of extraction in MAE, with temperature being another factor. Carotenoids were effectively extracted from carrot waste through the use of specific microwave powers and extraction times. [53-54]

In such cases, solvents with a lower dielectric constant may be preferred for extracting thermo-labile samples to avoid increasing the heating of the solvent-sample mixture. N-hexane or other microwave transparent solvents suspend the plant material to preserve thermolabile phytochemicals. The following conditions were used to extract Curcuma oil via MAE

(Microwave-Assisted Extraction): extraction time of 29.99 minutes, Wattage of 160, and a Curcuma powder and ethanol ratio of 1:20 (w/v), resulting in an optimum yield of 10.32%. [55-56]
Advantages of microwave-assisted extraction (MAE) over conventional solvent techniques are: it is faster, uses less solvent, is economical, and has a higher extraction rate. Suitable for smaller phenolic molecules such as quercetines, isoflavines, MAE exhibits these features due to their stability at microwave temperature ranges.[57]
The study demonstrated that the yield of triterpenes from Centella Asiatica was doubled compared to the soxhlet extraction. Optimal time and wattage were found to be very crucial, as shown in a study associated with Dioscorea hispida.[58-59]

Ultrasound-assisted extraction, UAE (sonication extraction)

Ultrasounds travel through a medium with expansions and contractions, following the wave nature, as electromagnetic waves with frequencies above the audible range of sound waves (20 kHz to 2000 kHz). The ultrasound-induced cavitation increases the contact surface area between plant samples and solvents, as well as enhances the permeability of cell walls. This phenomenon is known as cavitation. It involves the formation, growth, and collapse of bubbles. Some studies have observed that the frequency used can influence the extraction of compounds from the sample in a favorable manner. [60]
Interestingly, a study observed that the highest yields of phenolics at lower frequency were 40 kHz than 120 kHz. Such an observation prompts studies to evaluate ultrasonic parameters simultaneously to enhance and optimize extraction efficiency. A study using UAE reported the extraction of phenols from rhizomes with 75.3% ethyl alcohol over an extraction time of 40 min as having higher yields than one solvent in context. [61-62]
At lower frequencies, a study observed that phenolics were extracted most efficiently with ultrasound at 40 kHz, rather than 120 kHz. This finding prompts researchers to conduct studies on the simultaneous optimization of ultrasonic parameters to enhance and improve extraction efficiency. Ultrasound modifies and disrupts the physical and chemical characteristics of plant materials, expedites the release of phytochemicals, and reinforces the solvent system's mass movement in plant cells. [63-65]
The extraction process was sped up, energy consumption was lowered, and more phytochemicals were recovered from annatto seeds using the UAE method. In this study on annato, UAE efficiently retained the isolated phytochemicals, thereby supporting their functional activities. Phenolic compounds are extracted significantly from strawberries and oranges in the UAE. [66-68]
This method has been reported to efficiently extract phenolic derivatives and anthocyanins from grape peels within 30 minutes using Ultrafiltration Assisted Extraction (UAE), as opposed to the maceration method which took NUM minutes and yielded lower extracts. [69-70]
The UAE's process enhances the extraction rate, involving a relatively lesser volume of solvent. [71-72]
UAE (ultrasonic-assisted extraction) is preferred for extracting triterpenoids from food grade seeds, resulting in the maximum amplitude of sonication as 60%. It also extracts phenolic compounds from pumpkin slices at lower ultrasonic power, yielding 44.60%. [73-74]
Using an ultrasonic output power of 100 W, anthocyanins are extracted from red cabbage leaves. A notable characteristic of UAE is its ability to preserve the natural state of compounds that degrade at high temperatures. These compounds – carotenoids, phenolics, and vitamin C – are isolated from spices like ginger, garlic, and turmeric without being altered chemically. [75-76].

Supercritical fluid extraction (SFE)

Valuable compounds of various origins can be extracted commercially using SFE technology. This technology has great potential for obtaining valued compounds from food products. SFE is characterized by the changes in temperature and pressure necessary for the transformation of a gas into a liquid, resulting in two indistinguishable phases. At the critical point of a supercritical fluid, the temperature and pressure are defined, and the substance exhibits properties of both gas and liquid phases indistinguishably. The critical region becomes accessible above the critical temperature (Tc) and critical pressure (Pc).The extract solvent-free process occurs when the solvent, having passed through the packed bed and come into contact with extractable chemicals, causes their solubilization. Subsequently, the solvent separates from the extract as temperature rises and pressure drops. A supercritical fluid, such as carbon dioxide, exhibits the liquid-like property of solvating when its temperature exceeds 31.1 °C and pressure surpasses 7380 kPa. Several recent studies can be referred to wherein supercritical carbon dioxide has been used. At optimal pressure of 57 °C and temperature of 80 MPa, oil lipids (consisting mainly of glycerol-lipids, sterol esters, and phospholipids) are extracted from seeds.[77-78].
At a pressure of 27 MPa, seeds release alkaloids. At a pressure of 30 MPa, leaves of sweet cherry yield polyphenols, vitamins, anthocyanins, dietary phenolic compounds, and carotenoids. Essential oils are extracted from a herb at a pressure of NUM MPa.[79-81]
At a pressure of 350 bars, supercritical CO2 extracts phenolics from potato peels uniquely, enabling the technique to capture thermolabile phytochemicals. Supercritical CO2 functions by strongly solvating nonpolar phytochemicals, while polarized phytochemicals typically exhibit low solubility in it. Such additives as ethyl alcohol or methanol, water, acetone, ethyl acetate, and acetonitrile can increase the solubility of polar phytochemicals in supercritical-CO2, thereby enhancing the yield of the phytochemical extract. 30% was increased by the addition of ethanol as a co-solvent to scCO2 in the extraction of main cannabinoids from industrial hemp residues. The ethanol co-solvent also facilitated the recovery of better yields of phenolic compounds and flavonoids, which exhibited superior antioxidant capacity. [82-84].
The extraction yield of antioxidant activity from Phyllanthus niruri was directly proportional when ethanol-modified scCO2 was used. Ethanol-water co-solvent was also employed to boost scCO2 extraction. [85-86]

CO2 is easily available and cheap, and it has several advantages, including low toxicity. The supercritical CO2 extraction method is commercially used for extracting natural resources. However, the temperature and pressure parameters must be critically adjusted to improve yield and maintain biological activities. Supercritical CO2's solubility for solutes increases with higher temperatures. However, caution is advised when dealing with thermolabile molecules, as their temperature should be carefully considered to avoid compromise.[87-89].

Phytochemicals are kept at constant low temperature values and experience increased pressure, which helps preserve them from degradation. For instance, by applying elevated pressure to tomato skin, an enhanced yield of thermolabile phytochemicals is extracted. Carbon dioxide flow rates, carbon dioxide flow, and extraction time remain consistent throughout the process.This extractive process requires proper sample preparation to maximize yield and maintain product quality, as the absence of moisture is essential to avoid negatively impacting the yields.[90-91]

The use of ethanol-modified supercritical carbon dioxide (ScCO2) and ethanol-water co-solvent has been reported to increase the extract recovery yield from Phyllanthus niruri. This yield positively correlates with the antioxidant activity. [85-86]

CO2 is readily available, cheap, and has the advantage of low toxicity. The supercritical CO2 extraction method is used in commercial extractions from natural resources. However, the yield and biological activities are enhanced by carefully adjusting the temperature and pressure parameters. Supercritical CO2 takes in more solutes as the temperature rises, but care should be taken with thermolabile molecules, as their temperature should not be compromised.[87-89]

At constant temperature, carbon dioxide flow rate, and extraction time, increasing the pressure helps preserve thermolabile phytochemicals in tomato skin from degradation, resulting in a significant yield. In this extractive process, proper sample preparation without moisture is significant for achieving maximum yield and maintaining product quality. Moisture can adversely affect yields. [90-91]

Enzyme-assisted extraction (EAE)

Phytochemicals are dispersed and stay inaccessible in the cell cytoplasm of some plants due to the stabilization of polysaccharide lignin networks through hydrogen bonding, hydrophobic interactions including van der Waals forces. The separation of these compounds is a significant challenge. The specific enzymes pre-treat the plant material, breaking down cell walls and hydrolyzing carbohydrates like cellulose and lipid bodies, thereby releasing bound phytochemicals efficiently and increasing their yield during extraction. The plant seeds are hydrolyzed using enzymes in the EAAE (Enzyme Assisted Acidic Ethanol Extraction) process, while in the EACP (Enzyme Assisted Cold Pressing) method, oils are extracted from seeds. Cellulase, pectinase, and amylase are the specific enzymes employed in these processes.

Phytochemicals that were originally inaccessible to the solvent become extractable when enzymes from the EAE are used in combination with other extraction techniques. The conventional solvent extraction using water resulted in a low phytochemical recovery yield, while the use of enzymes during microwave processing increased the extractability of phenolic compounds from olive pomace. At higher extraction temperatures and faster heating strategies, phenolic compounds were more effectively extracted. [93]

The result of sequential treatment of sisal waste with enzymes and ultrasonication is higher yields of pectin, compared to lower yields obtained through other extraction techniques without the use of enzymes. [94]

The nature of enzymes determines their concentration and optimal pH for maximum activity. For carbohydrases, for example, the highest extract yield is achieved at 50 °C and an acidic pH optimum, using a 0.1 M acetate buffer, in the presence of a multi-enzyme complex, Viscozyme L, which contains arabinase, cellulose, β-glucanase, hemicellulase, and xylanase. The yield of the extract is influenced by the enzyme concentration. For instance, a higher concentration of cellulase leads to an increased licorice extraction yield.[95-96]

Phytochemicals are detached from plant material, releasing them from the enzymes so that the extraction method can be performed. The pectinase treated Pomelo peels released flavonoids after incubation at various times. These flavonoids were then extracted using the ultrasound-assisted method at an optimal temperature of 30°C. The 1-hour pre-treatment of rosemary leaves with pectinolytic enzymes and the 24-hour solid-liquid conventional extraction with 50% hydroethanolic solvent were found to be the optimum conditions for the extraction of rosemary leaves to occur. The extract showed greater radical scavenging activity of antioxidants when subjected to enzyme pretreatment than without it. A study demonstrated that enzymatic protein hydrolysis is an effective method for increasing the extraction of phenolic compounds from wine lees and obtaining extracts with enhanced functionalities. [97-99]

Conclusion

Natural product research is receiving significant global attention. The extraction of bioactive compounds is a crucial step in this field, but it has historically been a bottleneck, slowing down the screening process. Inert plant components are separated from medicinally active molecular components during extraction. This process is accomplished using traditional solvent extraction techniques or modern, green extraction procedures. The choice of extraction technique significantly impacts the dependability and quality of subsequent analytical activities. Modern techniques offer manifold advantages over conventional methods in achieving economic viability, environmental friendliness, shorter extraction times, and better yields of bioactive compounds without compromising their biological activities. Modern techniques offer advantages over conventional extraction methods, such as shorter extraction times, lower solvent demand, preservation of biological activities, higher yields, and less energy consumption. The plant matrix, economic viability, environmental impacts, and targeted phytochemicals determine the choice of extraction technique.

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