

Article

Advancement in extraction and phytochemical analysis of medicinal plants: A review

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Abstract

Medicinal plants are a real gift of nature to human beings and have been used in traditional medicine for centuries. Medicinal plants possess a vast array of biologically active compounds, rendering them invaluable resources for maintaining health for the majority of the world's population. Moreover, they serve as crucial resources in the research of alternative new drug active ingredients. The development and utilization of medicinal plants has become a hot research topic in recent years. Extracting compounds from these plants forms the cornerstone of natural product research. There is a relentless effort to improve and discover better extraction technologies that are more efficient and cost-effective. Phytochemical analysis is essential to determine the biological activity of new therapeutic drugs through the analysis and identification of the extracted chemical components, as well as the qualitative and quantitative determination of the main active ingredients in medicinal plants. This review aims to discuss the current extraction technology and analysis methods of phytochemical components, providing a reference for the research and development of medicinal plants.

Keywords: Medicinal plants, extraction techniques, phytochemical analysis, quantitative, qualitative, bioactive compounds.

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Introduction

Plants are autotrophs, and in addition to the primary metabolites present in all living organisms, they have secondary metabolites that allow them to produce and accumulate compounds of very diverse chemical properties (Legrand et al., 2016). Natural compound ingredients have the characteristics of various skeletons, broad activity, high efficiency, and low toxicity (Lu et al., 2019). These chemicals are sometimes used directly, and sometimes they are raw materials in forming other critical medicinal compounds. So finding and developing new drugs from plants has become the leading strategy of modern new drug research and development.

Phytochemicals can be separated from the plant material by various extraction techniques. The most commonly used conventional methods include maceration, percolation, infusion, decoction, hot continuous extraction (Soxhlet extraction), etc. Modern green extraction methods, such as supercritical fluids, ultrasound, accelerated solvents, microwave, and enzyme-assisted extraction methods, are becoming increasingly important.

Extraction is only the first step in phytochemical analysis in medicinal plants, and the search for new drugs in medicinal plants involves screening the plant extracts for new compounds and then conducting bioactivity tests. Suspected new molecules or bioactive compounds are then isolated and purified for molecular structure elucidation and further pharmacological or toxicological testing (Bitwell et al., 2023).

At present, the methods of phytochemical analysis include chemical experiments, spectroscopy, chromatography and combined methods. A rapid and effective selection method based on new technical means is the premise of identifying and selecting active ingredients in the "natural combination library" and developing new drugs. In recent years, with the cross-application and integration of multiple disciplines, the effect of biological affinity is rapidly improved. Rapid selection models are emerging, and the combined application of multiple technologies makes the study of active ingredients of medicinal plants more efficient, sensitive, and specific (Verma, 2021). It thus provides help for the development of new drugs to a certain extent.

In view of this, the extraction technology and analysis method of phytochemical components were introduced in this paper in order to provide a reference for further study of phytochemistry.

Methods of Extraction

Conventional Extraction Techniques

Decoction

The decoction was made by boiling the mixture in distilled water at 100°C for some time. The boiling extraction method is also called the "Water boiling method" or "water extraction method" because the extraction solvent is usually water. The advantage of the water decoction method is that it can fully extract significant polar chemical components. However, the disadvantage is that the extraction of small polar chemical components is incomplete, and the extract is usually mixed with many water-soluble impurities. Due to it requiring high-temperature heating, it will cause the change of heat unstable compounds and the loss of volatile substances. The study about decocting-induced chemical transformations of Du-Shen-Tang found a total of 45 major ginsenosides were identified in Du-Shen-Tang, 21 of which were determined to be newly generated during the decoction of ginseng (Li et al, 2010). This extraction technique is helpful for phytochemicals that do not decompose or modify with increasing temperature. The extraction time of the decocting method is generally 15–60 minutes (Azwanida, 2015). Boiling time depends on the phytochemicals to be extracted and the nature of plant tissues. Leaves, flowers, roots, and tender stems are usually boiled for 15 minutes. For example, fruits, roots, and leaves are decoctions at 100°C to extract phenols and flavonoids (Chanda and Kaneria, 2012; Mahmudati et al, 2020).

Maceration

Maceration is developed based on the decoction method. The active components were extracted with the principle of similar phase dissolution. The powder of natural products is soaked with the corresponding solvent (Azwanida, 2015). Maceration is generally carried out in a sealed container to avoid losses caused by solvent evaporation. After extraction, the mixture is filtered to obtain the solvent, and the plant residue is pressed and filtered to collect the impregnating solvent. Some thermolabile phytochemicals can also be extracted by Maceration. Concentrating the extract cannot be used to volatilize the solvent under average temperature and pressure but to recover the extract by vacuum evaporation.

Low extraction efficiency and long extraction times are the underlying disadvantages of the method (Wilson et al, 2022). A comparison of the extraction process of polyphenols from *Thymus serpyllum* L. herb using maceration, heat-and ultrasound-assisted techniques found that the maceration method was lower than the other two methods (Jovanović et al, 2017).

Selecting a suitable solvent during the maceration process is crucial, as the solvent will distinguish between the classes of phytochemicals recovered from the sample. During the carob macerate produced by maceration, phenolic and aroma compounds are extracted as ethanol concentration changes, and the samples' color, acidity, and phenolic content are affected (Hanousek et al, 2020).

However, optimized conditions may attribute significant efficiency to this technique. In this study, by comparing the physicochemical and aromatic characterization of carob macerates produced by different maceration conditions, optimal maceration process parameters are determined: a maceration of carob pods at room temperature for eight weeks in a 50% v/v hydroalcoholic base (1:5 solids to liquids) (Hanousek et al, 2020).

Percolation

Percolation is the development of the maceration method, based on the maceration method, a dynamic leaching method is adopted. First, carefully shred plant material before introducing it to the percolator, but not too fine because it is challenging to separate fine particles from the extraction solvent. Then slowly add the penetrating solvent to the top of the plant material. A percolating solvent depends on the plant's nature, commonly ethyl alcohol, as it passes through the plant material, it gradually absorbs phytochemicals and is slowly pushed downwards by a fresh solvent added from the top. It is essential that the solvent flows slowly to allow time for the solvent to penetrate plant cells and extract the constituent phytochemicals. However, if the solvent percolation rate is too slow, it will require more solvent for complete extraction. It is generally recommended that 5 mL of solvent should be used per kg of plant material (Bitwell et al., 2023).

The fresh solvents continuously displace the saturated leaching solution, allowing for fuller extraction of the chemical components in the natural raw material so that the extraction efficiency of percolation is higher than that of maceration. Using 95% ethanol as an extracting medium, The yield of total decarboxylated ("total CBD") from percolation is significantly higher than that from maceration (Wilson et al., 2022).

The end of percolation is marked by the elution of the colorless liquid from the filter. At this point, the eluent is free of phytochemicals. Once the procedure is complete, the botanical matter is compressed to absorb any remaining solvent, and the remaining solution is subsequently incorporated into the leachate (extract). The extract obtained by percolation is relatively clean, but the extraction is

time-consuming.

Reflux Extraction

The reflux extraction method is a method in which volatile organic solvents such as ethanol are used to extract raw materials, the leaching liquid is heated and distilled, the volatile solvent is distillate and then cooled, and the raw materials repeatedly flow back into the leaching container. This cycle is repeated until the effective components are completely extracted by reflux. Compared with impregnation and percolation, reflux extraction can shorten the extraction time and save the solvent. It is the most commonly used extraction method in the chemical research of natural products.

Study on the optimal extraction of phenolic compounds from *Pleioblastus amarus* (Keng) shell by optimizing the heating reflux extraction method, while the optimal test process conditions were determined as a liquid-to-solid ratio of 20:1 mL/g, an ethanol concentration of 75%, and an extraction time of 2.1 h. On the other hand, Studies on the extraction of essential oils from rosemary, sage and bay laurel after different pretreatments showed that heating reflux extraction (soaking the plant material at 40°C for one hour) pretreatment could improve the yield of each plant essential oil (Miljanović et al., 2020).

However, because this method requires heating, it is unsuitable for extracting heat-unstable substances. Optimization of extraction conditions from multiple flavonoids and phenolics in *Acanthopanax* Leaf extracts, compare the efficiencies of ultrasonication and reflux extraction (with 70% methanol for 60 min). The results have shown that ultrasonication extracted more than reflux extraction (Zhang et al, 2016).

Soxhlet Extraction

Soxhlet extraction is a method of extracting natural substances by cyclic heating using solvents. The natural material is placed in a thimble (porous bag) made from firm filter paper or cellulose, which is placed in the compartment of the Soxhlet paraphernalia. A suitable extraction solvent (usually methanol or ethanol) was selected and placed in a round-bottom flask. Then the solvent is heated and vaporized, meets the condenser and condenses into a liquid, and drops into the soxhlet extractor. When the liquid level of the solvent under the reflux in the extraction cylinder exceeds the siphon of the Soxhlet extractor, the solvent in the extraction cylinder flows back into the round-bottom flask, that is, siphoning occurs.

Based on thermal reflux extraction, a siphon device is added so that the natural products can be continuously extracted by new solvents. Therefore,

the soxhlet extraction method has the advantages of thermal reflux and percolation, which can shorten the extraction time and reduce solvent consumption. However, it is also not suitable for the extraction of heat-unstable substances. The values of density, oleic acid, peroxide, and unsaponification of the oil extracted by the soxhlet method were lower than those extracted by the cold pressing method (Özcan et al., 2020).

In a Comparison between traditional and soxhlet extraction of *Salvia officinalis* extracts, soxhlet provided extracts with higher antioxidant activities than traditional extraction (Vieira et al., 2020). A study was to evaluate various extracts prepared from *S. terebinthifolius* by soxhlet extraction and by maceration, samples obtained by soxhlet had higher phenolic content, antioxidant activities, and antibacterial activities (D'Sousa'Costa et al., 2015).

Steam Distillation

Steam distillation is often used to extract insoluble components that can be distilled with steam and will not be destroyed. The plants containing volatile components were crushed, soaked, heated, and distilled, the volatile components in the plants were distilled with steam, and the distillate was collected after condensation.

Steam distillation is often used to extract essential oil from plants. Based on this, the experimental conditions are optimized to screen the best extraction technology and improve extraction efficiency. A Box-Wilson experimental plan in this study to determine the optimal operating conditions for obtaining high *Myrtus Communis L.* essential oil volumes: boiler occupancy rate 100%, distillation duration 75 min, and particle size 20 mm (Kaya et al., 2020). Persian lime juice (*Citrus latifolia* Tanaka) was extracted by continuous distillation to obtain a higher extraction rate (Padilla-de la et al., 2021). Compared with hydrodistillation and cellulase-assisted hydrodistillation, steam distillation is still the most commercially valuable method for extracting lavender essential oil (Wainer et al., 2022).

Sublimation

Sublimation is an extraction method in which solid substances in natural products are directly gasified without a liquid state and then re-solidified into a solid state after cold treatment (Zhong-Ping et al., 2005). Due to the high sublimation temperature, it is easy to cause the carbonization of natural products, and the carbonization of natural products will produce volatile tar-like substances attached to the sublimation, which is inconvenient to refine and remove (Xiao-Geng et al., 2010).

Squeezing Method

The squeezing method is an extraction method that separates the oil or juice in fresh, natural products from the mixture of liquid and solid by physical methods.

Modern Extraction Techniques

Supercritical Fluid Extraction (SCFE)

Supercritical fluid extraction is an extraction method using supercritical fluid as solvent. When approaching the critical point, small changes in pressure and temperature can cause significant changes in the density of the supercritical fluid. The viscosity is smaller than that of liquid solvents, so the dissolution range of natural products is wide. The diffusion degree of natural products in the solvent can be increased, and the extraction efficiency can be improved. Due to its low critical temperature (31°C) and pressure (7.3MPa), as well as its chemical inertness, low cost, and non-toxicity, supercritical carbon dioxide is the most commonly used supercritical fluid, which has a good extraction effect on non-polar compounds such as lipids and volatile oil components. Optimization of supercritical fluid extraction for lutein extraction from *Scenedesmus almeriensis* demonstrated that the maximum lutein recovery (~98%) with purity of ~34% was achieved operating at 65 °C and 55 MPa with a CO₂ flow rate of 14.48 g/min (Mehariya et al., 2019).

The flavonoids in *Pueraria lobata* were extracted using supercritical carbon dioxide extraction. The optimal conditions were determined by response surface methodology: the highest flavonoid yield of *P. lobata* was a pressure of 20.04MPa, a temperature of 50.24°C, and a co-solvent amount of 181.24 ml (Wang et al., 2008).

However, for the extraction of polar substances, the extraction effect can be improved by adding modifiers to the supercritical carbon dioxide.

In the study on the extraction of triterpenoids from the deciduous bark of *Eucalyptus*, supercritical fluid extraction of carbon dioxide and modified carbon dioxide was studied, and it was found that pressure and auxiliary solvent affected the extraction results to varying degrees, the addition of 8% (wt) of ethanol increases triterpenoids yield more than threefold at 160 bar and 40°C (Domingues et al., 2012).

This study compared the yields of khellin and visnagin obtained with different conventional solvents and with SCFE with carbon dioxide (containing 5% methanol as a co-solvent). Among the test extracts, 30% ethanol yielded the highest extraction (15.44%), while SCFE yielded the lowest

(4.50%). Furanochromones were highest in SCFE (30.1%) and lowest in boiling water extract (5.95%). In conclusion, supercritical fluid extraction provides a more selective and cytotoxic extract than traditional extraction methods are relatively safe and relatively inexpensive (Khalil *et al.*, 2021).

Pressurized Hot Water Extraction (PHWE)

Pressurized hot water extraction (PHWE) is an extraction method according to the property that the boiling point of the solvent increases with the increase of pressure, in the pressurized solvent extraction method, the temperature of the solvent can be raised to above its boiling point at room temperature by increasing the pressure of the system.

This method has better permeability and can ensure a high solubility and diffusion rate of solutes in the solvent, effectively reducing the extraction time and thus improving the extraction efficiency. The PHWE technique used to extract cannabinoid compounds from *Cannabis sativa* seeds found that the optimal conditions were set at 150°C (extraction temperature), 160°C (collection vessel temperature), and 45 min (extraction time). This method was more efficient and environmentally friendly due to extract more cannabidiol and reduce delta-9-tetrahydrocannabinol and cannabinol, thereby decreasing the psychoactivity of cannabis products (Nuapia *et al.*, 2020).

Due to temperature at 121 °C and pressure at 15 p.s.i, Solvent properties of water are significantly increased, the study showed PHWE extracts of hydrolyzable tannins from *Phyllanthus tenellus* Roxb produced almost twice as many hydrolyzable tannins as pure methanol did (Mohd Jusoh *et al.*, 2019). PHWE enabled the recovery of extracts with phytochemicals from avocado seeds yields remarkably higher than those extracted using methanol-based extraction (Ong *et al.*, 2022).

Ultrasonic Assisted Extraction(UAE)

Ultrasonic-assisted extraction method, also known as the ultrasonic extraction method, is a method that uses ultrasonic technology to assist solvent extraction. Due to the cavitation effect of ultrasound in the solvent, the diffusion and dissolution of solutes in the solvent can be promoted, it can also effectively transfer heat, thereby shortening the extraction time and improving the extraction efficiency. In addition, UAE is easy to operate, can save solvent, and reduces energy consumption.

The antioxidant capacity and total polyphenol content of spruce bark were evaluated to compare UAE and solvent extraction(SE) methods, results

showed that the amount of phenolic compounds extracted by the UAE was more than that by the SE, indicating that the extraction efficiency of the UAE was higher than that of the SE (Chmelová *et al.*, 2020).

In comparison between conventional extraction (CE) and ultrasonic-assisted extraction (UAE) of carotenoid extraction from cashew apple, total cashew carotenoids obtained from UAE are more efficient than those derived from CE due to higher yields, shorter processing times, and easier extraction procedures (Coelho *et al.*, 2022).

Using ionic liquids instead of conventional organic solvents, developed an aqueous ionic liquid-based ultrasonic-assisted extraction (ILUAE) method to extract the eight ginsenosides from ginseng root. The proposed approach exhibited 3.16 times higher efficiency and 33% shorter extraction time, which indicated use of ionic liquids as solvents greatly increases extraction potential (Lin *et al.*, 2013).

Microwave-Assisted Extraction (MAE)

Microwave-assisted extraction (MAE), also known as microwave extraction, is a recent way that incorporates microwaves and solvents during the extraction process. Microwaves interact with polar molecules and produce heat, heat transfer and mass transfer are consistent, and the two functions accelerate the extraction process to improve extraction efficiency. MAE method can shorten the extraction time, reduce the use of organic solvents, avoid the heat degradation of the extraction component and the selective heating of medicinal materials, and is considered an environmental extraction method.

MAE was used for the extraction of isoalantolactone and alantolactone from *Inula helenium*. Optimization of the optimal extraction process was 1 g plant sample (sifted through 140 mesh) mixed with 15 ml of 80% ethanol solution, microwave radiation 120 s at 50°. Compared with ultrasound-assisted extraction, and heat reflux extraction, MAE was more efficient and time-saving for the extraction of alantolactone and isoalantolactone from *Inula helenium* (Zhao *et al.*, 2015).

Optimization of catechin and proanthocyanidin recovery from grape seeds using MAE: 94% ethanol, 170 °C temperature, and a duration of 55 min. It was found that MAE yielded 3.9-fold more monomeric catechins and 5.5-fold more proanthocyanidins than conventional extract (94% ethanol, shaking at 25°C for 55 min). It shows that MAE is a promising extraction method (Chen *et al.*, 2020).

This study aimed to optimize the MAE of the volatile oil from *Pterodon emarginatus* fruits. The oil

yield of optimized MAE is several times higher than that of conventional extraction (CE). Moreover, the solvent used is small, and the extraction time is short. The MAE method uses less energy and is more bio-sustainable than conventional cleverger apparatus extraction (CE) (Vila Verde et al., 2018).

MAE was compared with conventional, ultrasound-assisted, and accelerated solvent extractions by the TPC (total phenolic content), TAA (total antioxidant activity), and IPA (individual phenolic acids) of peel extracts from *Citrus sinensis*. There was a greater TPC and TAA concentration in MAE extracts than in the other three (Nayak et al., 2015).

Pulsed Electric Field Extraction (PEFE)

Pulsed electric field-assisted extraction uses the raw material as an electrolyte, destroys a plant cell membrane or cell wall by the perforation effect pulsed of electric and magnetic fields, and promotes the natural ingredients to be dissolved efficiently. The method is a non-heating extraction method, so it is suitable for extracting thermally unstable substances. The method is mainly affected by the electric field intensity, input-specific energy, and pulse number.

Some researchers have studied the application of pulsed electric fields in the fermented wine industry, there are several advantages: reducing the maceration time of brewing materials, increasing the color of fermented wine, enhancing the extraction of key functional components, promoting the production of aroma compounds, inactivating spoilage microorganisms (Feng et al., 2022).

Researches show that in combination with mechanical pressing, PEF treatment will improve apple, grape, and carrot juice yields and quality (Grimi et al., 2021; Jaeger et al., 2012; Luengo et al., 2014). PEF also helps enhance the extraction rates of colorants (carotenoids, anthocyanins, betaines, etc.) and bioactive compounds (polyphenols) from foods and food by-products. Furthermore, PEF can reduce-solvent consumption, lower extraction temperatures, and shorten extraction times (Boussetta et al., 2012; Puértolas et al., 2013).

Enzyme-Assisted Extraction (EAE)

Enzyme-assisted extraction is a method that degrades and destroys the cell wall structure of natural products by enzymes to promote the dissolution of intracellular active ingredients. Cellulase, α -amylase, and pectinase are commonly used in enzyme-assisted extraction. With complex enzyme-assisted extraction (CEAE), the soluble phenolics, flavonoids, DPPH, ABTS, and FRAP contents were improved by 103.2%, 81.6%, 104.4%, 126.5%, and 90.3%,

respectively. Specifically, quercetin and kaempferol with higher bioactivity were increased by 3.5- and 2.2-fold, respectively. As a result of CEAE, total soluble phenolic extracts of guava leaves showed the highest antioxidant activity and protection against supercoiled DNA damage. Enzyme-assisted extraction technology can be used to extract biochemical components from plant matrices and is useful in the pharmaceutical and food industries (Wang et al., 2017). For example, EAE has been employed for the extraction of anthocyanins and phenolic compounds from multiple matrices such as blackcurrant (González et al., 2022), bilberry (Syrpas et al., 2021), pistachio green hull, or habanero chili pepper (*Capsicum chinense*) seeds (Cortes-Ferre et al., 2022). And EAE has been employed for the extraction of polysaccharides from *Cornus officinalis* (You et al., 2013), brown macroalgae (Nguyen et al., 2020), *Dictyophora indusiata* (Wu et al., 2013).

Accelerated Solvent Extraction (ASE)

Accelerated solvent extraction (ASE) is an extraction technology for extracting solid or semi-solid samples with solvent at a temperature of 50~200°C and a pressure of 1 000~3 000 psi. Its advantages include low solvent usage, high output, and a short processing time.

ASE was applied to extract saikosaponin a, saikosaponin c, and saikosaponin d from the roots of *Bupleurum falcatum*. The optimized procedure set 120°C of extraction temperature, employed 70% methanol as extraction solvent, 10min of static extraction time, 60% of flush volume, and the extraction recoveries of the three compounds were near 100% with one extraction cycle. As compared to heat-reflux extraction and ultrasonic-assisted extraction, the proposed ASE method was more efficient and faster (Li et al., 2010).

Using ASE method to study camptothecin from *Nothapodytes nimmoniana* and piperine from *Piper nigrum*, the temperature-based extraction not only reduces the time but is also simple, fast, and accurate. In herbal products, the ASE is a suitable tool for quality control or standardization (Upadhyya et al., 2014).

A study about antioxidant compounds from *Gardeniae Fructus* and its acetylcholinesterase inhibitory and PC12 cell protective activities, the results indicated that ASE is a proper extraction method compared to three other extract methods (heated refluxing extraction (HRE), ultrasound-assisted extraction (UAE), and microwave-assisted extraction (MAE) (Fan et al., 2021).

Aqueous Two-Phase System Extraction (ATPS)

Aqueous two-phase system (ATPS) extraction comprises two kinds of insoluble polymer or polymer and inorganic salt solution. It uses the difference in the partition coefficient of the solute in the two phases to extract.

The Vietnamese herb *Peristrophe bivalvis* (L.) Merr. (Acanthaceae) contains natural pigment anthocyanin, the ATPS was used to extract and purify anthocyanins from leaf extracts. The study results revealed that it is possible to recover 80% of the anthocyanin in leaves while removing 80% of the contaminated nitrogen compounds. In addition, the shelf-life of pigment in the purified extract was significantly longer than that in the crude extract (Le et al., 2023).

Using an ATPS based on ethanol and NaH_2PO_4 , quercitrin, hyperoside, rutin, and afzelin from *Zanthoxylum bungeanum* were extracted and purified. ATPS conditions optimized were: 29% NaH_2PO_4 , 25% ethanol concentration, 1% leaf extracts, no pH adjustment, and repeated 1 h extractions at 25°C. In comparison with 60% ethanol extracts, quercitrin (44.8 mg/g), hyperoside (65.6 mg/g), rutin (56.4 mg/g), and afzelin (6.84 mg/g) in the extracts were increased by 49.9%, 38.8%, 45.6%, and 36.8%, respectively. In addition, more potent antioxidant activity was observed in the extracts after ATPS (He et al., 2016).

The ATPS has been combined with the UAE and MAE methods for extracting and purifying bioactive ingredients in natural products, thereby improving the extraction and purification processes (Yuan et al., 2022; Guo et al., 2015; Lin et al., 2019a; Lin et al., 2019b). ATPS is an efficient approach based on non-toxic phase-forming components and water as green extractants. Using ATPE, target compounds and undesired impurities are enriched in the top and/or bottom phases, respectively. With this process, extraction, concentration, and purification can be accomplished in one step, and its scalability makes it ideal for industrial processes.

Dynamic Countercurrent Extraction

Dynamic countercurrent extraction (or Multi-stage countercurrent extraction), through the application of several extraction units to achieve extraction, percolation, dynamic and countercurrent technology into one can be arbitrarily selected between 25~100°C to extract natural products of new technology. The almond hull contains significant levels (37% dry weight) of sugars that can be extracted for industrial use (sucrose, glucose, and fructose). Used countercurrent extraction of soluble sugars from almond hulls found sugar recovery efficiency was 88%. A high conversion rate of 86%

was achieved with the concentrated sugar syrup fermentation into ethanol (Holtman et al., 2015).

Multi-stage countercurrent extraction (MSCE) of resveratrol from peanut sprouts was investigated based on alkaline extraction and acid precipitation technology. Under optimal conditions, MSCE was compared to single-pot extraction (SPE). MSCE proved a cost-effective, time-saving, and energy-saving extraction method (Zhang et al., 2015).

Steam Explosion (SE)

Steam explosion extraction is the use of steam to heat natural products to 180~235°C and maintain the pressure for a few seconds to a few minutes, the steam is released instantly when the pressure is reduced instantly, secondary steam is generated, the gas expands rapidly, the structure of a solid substance is destroyed under the action of mechanical forces release components.

A study was conducted on the extraction of citrus peel essential oil by SE. The optimum essential oil yield by the SE was obtained: At 170°C, 8 bars in 240 seconds. It took 8 times less time than hydro-distillation. Hydro-distillation and SE processes yielded 77% and 100%, respectively, of limonene, a major favorable component (Golmohammadi et al., 2018).

Extraction of oil from *Camellia oleifera* Abel. by SE. The highest oil yield was 86.56% when SE pretreatment was performed at 1.6 MPa for 30 seconds. An electron microscope scan revealed that SE pretreatment effectively released oil from camellia seeds by destroying their cell structures. For this reason, SE can be an effective method of removing camellia seed oil (Zhang et al., 2019).

Because SE technology requires only high temperature and high-pressure steam, no chemical additives, and lower pollution, it is widely used in the modification of Dietary fiber (DF) (Ma et al., 2022). Furthermore, SE treatment can dissolve the connection between cellulose and functional components in materials and modify DF to enhance the corresponding functional properties.

Flash Extraction

Flash extraction method, also known as tissue crushing extraction method, through high-speed rotating shear force and hyperkinetic molecular percolation technology, natural products are cut into particles in a few seconds to a few minutes to efficiently extract the active ingredients.

Using a rapid flash extraction method (3.3-min extraction with 53% ethanol at a solvent-sample ratio of 34mL/g) for the antioxidant composition of *N.sibirica* fruit, Twenty-seven compounds of the extract were recognized, among them, four

compounds were reported for the first time (Song et al., 2019).

Pulsed Electric Field (PEF)

A pulsed electric field (PEF) is a technology that uses brief pulses of a powerful electric field to process cells. Using an external electric field to activate the membrane of plant cells results in pore formation, which facilitates the release of compounds from the cells. Such as extraction of extra virgin olive oil (EVOO) (Martínez-Beamonte et al., 2022), sugar extraction from carrots (Dastango et al., 2020), polyacetylene extraction from carrot slices (Dastango et al., 2020; Aguiló-Aguayo). A moderate voltage (<10 kV/cm) and low specific energy (<10 kJ/kg) can permeate cell membranes in this manner, thus, it is a non-thermal and energy-efficient technique. Using pulsed electric fields as an alternative pre-treatment for drying to enhance polyphenol extraction from fresh tea leaves (Liu et al., 2019)

Phytochemical Analysis

Qualitative Analysis of Primary Metabolites

Test for Carbohydrates

a) Benedict's test: An equal volume (0.5ml) of the filtrate was combined with Benedict's reagent and heated in a water bath for about two minutes. Red precipitate indicates the presence of sugar.

b) Molisch's test: Take 2 drops of alcoholic solution, α -naphthol, add to 2 ml sample, shake well, and add to mixture. Add a few drops of conc. H_2SO_4 along the side of the test tube. If sugars are present, a violet ring will appear.

Test for Starch

Take 0.01g of iodine and 0.075 g of potassium iodide, add 5 ml of distilled water, and take this solution to about 2-3 ml of the extract. Starch formation results in a blue color.

Test for Proteins

a) Biuret test: Take 1 drop of 2% copper sulfate solution to 2ml of filtrate, then take 95% ethanol(1ml) and excess KOH, pink color indicates protein presence.

b) Take 2ml of extract and 2ml of water and about 0.5% of conc. HNO_3 to mix, yellow color indicates the presence of the protein.

c) Take 2ml of extract and 2ml of Miller's reagent and heat, the white precipitate turns red to confirm that proteins are present.

Test for Amino Acids

a) Mix 1ml of extract and a few drops of ninhydrin reagent (10mg of ninhydrin in 200ml of acetone), the purple color indicates the amino acids presence.

b) Take 2ml of extract and a few drops of nitric acid (added along the sides of the tube), the yellow color indicates the presence of proteins and amino acids.

Test for Fatty Acids

Take 1ml of extract and 5ml of ether to mix, and take extracts to evaporate on a dried filter paper, transparency indicates fatty oils presence.

Test of Resins

Precipitation test: Extracted 0.2 g of an extract with 15 ml of 95% ethanol extracted, then poured the alcoholic extract into a beaker with 20 ml of distilled water. Add a few ml of acetic anhydride and 1 ml conc. H_2SO_4 to 1 ml of extract, the appearance of orange to yellow color indicates the presence of resins.

Test of Fixed Oils and Fats

a) Spot test: a small quantity of the extract was pressed between 2 filter papers. Emergence spots mean oils.

b) Saponification test: Take a few drops of phenolphthalein and a few drops of 0.5N alcoholic KOH to the extract, then heat the mixture for 2 hours, partial neutralization of alkali or soap formation indicates the presence of fixed oils and fats.

Gums and Mucilage

Adding 2 ml of absolute ethanol to 1 ml of extract with constant stirring produces a white or cloudy precipitate that indicates gums or mucilage.

Carboxylic Acids

a) Add a pinch of sodium bicarbonate to 1ml of extract, effervescence indicates carboxylic acid presence.

b) The alcoholic extract(2ml) was diluted in warm water and filtered. Following the test with litmus paper and methyl orange, the filtrate appeared blue.

Qualitative Analysis of Secondary Metabolites

Test for Anthraquinones

Add a few ml of conc. H_2SO_4 and 1 ml of diluted ammonia to 5 ml of extract. It is confirmed by rose pink that anthraquinones are present.

Test for Quinones

Add alcoholic KOH to 1ml of extract, then colors ranging from red to blue indicate quinones.

Test for Alkaloids

- a) Mayer's test: Add 2 drops of Mayer's reagent to a few ml of filtrate, the appearance of a creamy or white precipitate shows the presence of alkaloids.
- b) Wagner's test (iodine–potassium iodine reagent): Add a few drops of Wagner's reagent to 1 ml of extract. The appearance of reddish–brown precipitate indicates the presence of alkaloids.
- c) Add 2 ml of HCl and 1 ml of Dragendorff's reagent to 5 ml of extract, the appearance of an orange or red precipitate shows the presence of alkaloids.

Test for Glycosides

- a) Borntrager's test: Add 3ml of chloroform to 2ml of filtrate and shake. A 10% ammonia solution was added to the chloroform layer after it was separated. A pink color indicates glycosides are present.
- b) Extracts(5ml) were hydrolyzed with concentrated HCl(5ml) and boiled over boiling water for a few hours. In 2ml of water, a small amount of alcoholic extract and 10% NaOH were dissolved, yellow indicates glycoside presence.
- c) Add 0.4 ml of glacial acetic acid containing traces of ferric chloride and 0.5 ml of conc. H₂SO₄ to 2ml of extract. The appearance of blue color is positive for glycosides.

Test for Cardiac Glycosides (Keller-Killani Test)

Add 2 ml of glacial acetic acid, a drop of ferric chloride solution, and 1 ml conc. H₂SO₄ to 5ml of solvent extract. In the interface, a brown ring indicates the presence of cardenolides deoxy sugars. Under the brown circle, a violet ring may appear, while a green ring may appear just gradually towards the acetic acid layer.

Test for Phenol

- a) Gelatine test: Add 2 ml of 1% solution of gelatin (containing 10% of NaCl) to 5 ml of extract. The production of a white precipitate indicates the presence of phenol.
- b) Lead acetate test: Add 3 ml of 10% lead acetate solution to 5 ml of extract and mix gently. The appearance of a bulky white precipitate indicates the presence of phenols.

Test for Polyphenols

- a) Add 10ml of ethanol to the 3ml of extracts and warm in a water bath for 15 minutes, then add a few drops of ferric cyanide (freshly prepared) to this solution, the appearance of blue–green color indicates the presence of polyphenols.
- b) Add a few drops of 5% solution of lead acetate to 1 ml of extract. The production of yellow precipitate indicates the presence of polyphenols.

- c) Add 3 ml of 0.1% gelatine solution to the 5 ml ethanolic extract. The production of precipitate indicates the presence of polyphenols.

Test for Flavonoids

- a) Add 10% ammonia solution to the aqueous solution of the extracts and is heated. The formation of yellow fluorescence indicates the presence of flavonoids.
- b) Add 10% of lead acetate to 1 ml of extract, the appearance of a yellow precipitate indicates the presence of flavonoids.
- c) Add concentrated H₂SO₄ to the extract, the production of orange color indicates the presence of flavonoids.
- d) Add 5 ml of dilute ammonia to the plant extract and shake well. Separation of the aqueous portion and add concentrated H₂SO₄. The formation of yellow color indicates the presence of flavonoids.

Test for Phytosterols

- a) Add 2 ml of acetic anhydride and 1 or 2 drops of concentrated H₂SO₄ (added along the sides)to the extract, there is an array of color changes when phytosterols are present.
- b) Extracts were refluxed with alcoholic KOH, and saponification occurred. After ether dilution, the layer evaporated, and phytosterols were detected in the residue. We dissolved it in diluted acetic acid and added a few drops of concentrated H₂SO₄. The formation of bluish-green color indicates the presence of phytosterols.

Test for Saponins

Add a few ml of distilled water to 0.5 mg of extract and vigorously shake it. The formation of frothing indicates the presence of saponins. After the above reactions, take the froth and add some olive oil while vigorously shaking, then observe for the formation of an emulsion.

Test for Terpenoids (Salkowski Test)

Add 1 ml of chloroform and 1.5 ml of concentrated H₂SO₄(added along the sides of the tube) to 3 ml of the extract, the appearance of reddish-brown color in the interface indicates the presence of terpenoids.

Test for Leucoanthocyanins

Take 5ml of Isoamyl alcohol to 5ml of extract dissolved in water, the red formation of the upper layer indicates the presence of leucoanthocyanins.

Test for Anthocyanins

Take 2N HCl and then ammonia to 2ml of aqueous extract, when pink-red becomes blue-violet, anthocyanins are present.

Test for Coumarins

Take 3 ml of 10% aqueous solution of NaOH to 2 ml of the extract, the appearance of yellow color is positive for coumarins.

Test for Emodins

Add 2ml of NH₃OH and 3ml of benzene to 5ml of extract, the formation of red color indicates the presence of emodins.

Qualitative Analysis of Vitamins**Test for Vitamin A**

250 mg of powdered sample is dissolved in 5ml of chloroform and filtered, then take 5ml of antimony trichloride solution to this filtrate. A transient blue color indicates vitamin A presence.

Test for Vitamin C

The 1ml sample was diluted with 5 ml of distilled water, add a drop of 5% sodium nitroprusside, and 2 ml of NaOH, then add few drops of HCl, as soon as the yellow color turns blue, it indicates the presence of vitamin-C.

Test for Vitamin D

500 mg of the powdered extract is dissolved in 10 ml of chloroform and filtered, added 10 ml of antimony trichloride, the formation of pinkish-red color indicates the presence of vitamin-D.

Test for Vitamin E

The sample was extracted with ethanol and filtered (500 mg in 10 ml), taking a few drops of 0.1% ferric chloride and 1 ml of 0.25% of 2'-2'dipyridyl to 1 ml of the filtrate. The bright-red color was formed with a white background.

Qualitative and Quantitative Analysis

In the face of unknown chemical composition, we need to use scientific instruments for chemical composition analysis, which can help us both qualitative and quantitative. It is convenient, fast, and high precision. Modern chemistry research is inseparable from the aid of precision scientific instruments. Now we introduce commonly used qualitative and quantitative chemical instruments.

Spectroscopy Analysis

Spectroscopy has the characteristics of simple operation, high sensitivity, good accuracy, and wide application range, etc. It is the primary analytical means for the study of plant chemical constituents, such as ultraviolet, visible spectrophotometry (UV-Vis), fluorescence spectrophotometry (FS), near-infrared spectrophotometry (NIR), atomic absorption

spectrophotometry (AES), and so on. Spectral analysis is widely used in the identification and content determination of sure chemical components.

Ultraviolet-Visible Spectrophotometry (UV-Vis)

Ultraviolet-visible spectrophotometry (UV-Vis) is an analytical method based on the selective absorption properties of substances. UV spectroscopy measures attenuation after a light beam passes through a sample or reflects off a sample surface. Ultraviolet radiation is energetic enough to promote outer electrons to higher energy levels, this results from the transition between the electronic energy levels. So UV spectroscopy is usually applied to molecules or inorganic complexes in solution. Qualitative applications require at least some of the UV-VIS spectra to characterize optical and electronic properties. Analyte concentration (quantitative analysis) can be determined using Beer-Lambert's law by measuring absorbance at some wavelength.

Infrared Spectroscopy (IR)

Infrared absorption spectroscopy measures the wavelength and intensity of the absorption of mid-infrared light by a sample. Light in the mid-infrared spectrum can be energetic enough to cause molecular vibrations to reach higher energy levels. Many IR absorption bands are characterized by the wavelength of specific types of chemical bonds, making IR spectroscopy most useful for qualitative analysis of organic and organometallic compounds. IR spectroscopy is used to determine the functional group present in the sample and as a tool to determine the newly synthesized molecule.

Fluorescence Spectrophotometry (FS)

Fluorescence spectrophotometry (FS) is an analytical method for qualitative or quantitative analysis of substances by using their fluorescence characteristics. The generation of material fluorescence is caused by the absorption of excited light by the substance molecules in the ground state under normal conditions into an excited state. These molecules in the excited state are unstable, and part of the energy is released in the form of light in the process of returning to the ground state, thus producing fluorescence. The same substance should have the same excitation spectrum and fluorescence spectrum, and the shape and position of the excitation spectrum and fluorescence spectrum of the unknown substance can be compared with that of the standard substance. When the excitation light intensity and other conditions are fixed, the emitted light intensity is proportional to the concentration of the substance in a certain concentration range, which can be used for quantitative research of the substance.

Atomic Absorption Spectrometry (AAS)

Atomic absorption spectrophotometry (AAS) is a spectral method based on the difference in absorption intensity of characteristic electric radiation of gaseous atoms. The measurement object of atomic absorption spectrophotometry is the metallic element and some non-metallic elements in the atomic state. When the characteristic spectral line emitted by the element is tested through the atomic vapor generated by the atomization of the test product, it is absorbed by the ground state atom of the element to be tested in the vapor. The content of the element to be measured in the test product is obtained by measuring the degree of weakening of the radiation light intensity. Qualitative and quantitative analysis was carried out compared with control products.

Chromatography

The chromatographic method is a separation analysis method, because of the difference in the physical and chemical properties of each component in the mixture, the distribution of each component in the fixed phase and the mobile phase is different, so each component moves at different speeds to achieve the purpose of separation. The common chromatographic methods are thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), gas chromatography (GC), etc.

Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) is a method for comparative analysis of simple mixtures. After the sample and the control product were sampled on the thin layer, the location of each spot movement was compared, and the retardation factor (R_f) was calculated. Then the substance can be qualitatively identified according to the spot position and the R_f value of the reference product. Even quantitative measurement.

High-Performance Thin Layer Chromatography (HPTLC)

This method is an upgrade of TLC. This type of planer chromatography uses high-performance layers with detection and an advanced workstation to acquire sample components. By reducing the thickness of the layer, HPTLC is a more advanced method for qualitative, quantitative, and micro-preparative chromatography.

High-Performance Liquid Chromatography (HPLC)

HPLC is also known as High- Pressure Liquid Chromatography. Separation takes place based on the interaction between solid particles of the column and the solvent in the mobile phase. Before analytes pass

through the detector, they must be eluted through the column at pressures up to 400 bars. High-performance liquid chromatography is suitable for compounds with high thermal stability. High-performance liquid chromatography provides both quantitative and qualitative analysis in one operation.

Ultra-High Performance Liquid Chromatography (UHPLC)

Ultra high-performance liquid chromatography (UHPLC) is an upgraded version of HPLC that uses higher pressures in the analysis system and can use smaller diameter columns to achieve analytical results in a shorter period of time. To save time.

Gas Chromatography(GC)

Gas chromatography is used to analyze volatile compounds. Unlike liquid chromatography, the gas phase is a mobile phase. The sample to be tested becomes a gas at high temperature and enters the mobile phase (commonly used helium, argon, etc.). Separation is based on the rate at which chemicals migrate into liquids. Chemicals with a higher percentage will migrate faster in liquids. The method is widely used in phytochemical analysis, both qualitatively and quantitatively.

Chromatographic Combination Technology

With the advantages of high throughput, sensitivity, and accuracy, chromatography coupled with mass spectrometry has become one of the most suitable techniques applied in phytochemistry.

Mass Spectrometry (MS)

Mass spectrometry (MS) is an analytical method to measure the ratio of ions to charge to mass (charge-mass ratio), from which the chemical formula of a particle can be deduced. The molecular weight of the sample can be determined from MS Spectrum. The technology has commonly been analyzed by mass spectrometry techniques coupled to a chromatographic separation (GC-MS or LC-MS, gas or liquid chromatography coupled to mass spectrometry). The development of mass spectrometry (MS) technology has enabled comprehensive analysis and rapid identification of complex phytochemical components and may be able to satisfy the needs of the industry for the analysis of medicinal plants.

Gas chromatography-mass spectrometry (GC-MS) is the earliest chromatography-mass spectrometry technique. The technology combines the high separation capability of gas chromatography and the high detection capability of mass spectrometry to complete the separation, identification, and quantification in one step. In the

analysis of volatile drugs, the content and properties of volatile components can be avoided in the multi-step detection analysis, which has a strong specificity for the analysis of volatile drugs. The method has high sensitivity, large peak capacity, large information content, and a good repeatability of the obtained mass spectrum fragments. Therefore, the GC-MS combined instrument has a huge mass spectrum library and has an absolute advantage in the rapid identification of chemical structures.

Liquid-phase mass spectrometry (LC-MS) combines the advantages of the high separation ability of liquid chromatography and the qualitative ability of mass spectrometry, the combination of the two makes the liquid phase can be qualitative without comparing according to standards, and mass spectrometry can be analyzed without purification into monomers. This combination of optimization makes the analysis fast, sensitive, and specific. Compared with GC-MS, LC-MS samples are applicable to a wider range, not only limited to the analysis of volatile components with weak polarity but also suitable for the analysis of strongly polar and thermally unstable compounds.

Nuclear Magnetic Resonance Spectroscopy (NMR)

Using simple one-dimensional techniques, nuclear magnetic resonance spectroscopy reveals the physical, chemical, and biological properties of matter. Molecules with more complex structures are determined using two-dimensional methods. To determine the structure of proteins, these techniques are replacing X-ray crystallography. A time-domain NMR spectroscopic technique is used to study molecular dynamics in solutions. A solid's molecular structure can be determined by solid-state NMR spectroscopy. The ^{13}C -NMR method is used to determine what type of carbon is present in a compound. Using ^1H -NMR, you can determine the types of hydrogen in a compound and how the hydrogen atoms are connected. Liquid chromatography-nuclear magnetic resonance (LC-NMR) technology: It integrates the separation of liquid chromatography and the identification of the structure of the spectrum and perfectly solves the problem of separating complex mixtures. NMR is the main method for structural identification. With the development of HPLC-NMR technology, it has been realized that the structure of the effective chemical components of the mixture can be determined directly by using multiple chromatography online technologies. It does not need to be purified and then analyzed and has the advantage of fast online data analysis and data acquisition.

X-Ray Crystallography

By using X-rays diffracted by crystals, X-ray crystallography is an experimental technique. The wavelength of X-rays (in the Angstrom range of 10-8) is sufficient to scatter electron clouds of comparable sizes. Reconstruction can be completed by supplementing diffraction experiments or extracting additional phase information from diffraction data. Gradually building a molecular structure into the experimental electron density is refined by comparing it to the data, resulting in a very accurate molecular structure.

Conclusion

It is becoming increasingly clear that medicinal plants have empirical therapeutic efficacy, as well as being a vast natural compound pool that can be used to discover and develop new drugs. Medicinal plants' efficacy, safety, and quality are primarily determined by analyzing their plant phytochemicals in a comprehensive manner. Extraction and analysis of phytochemical components are essential techniques for determining the active principles of the known biological activities exhibited by plants and are key to assessing their medicinal potential. The system is also helpful for performing targeted isolations of compounds and performing more precise investigations. The techniques mentioned in this paper are important for the extraction and qualitative and quantitative analysis of phytochemicals

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Conflicts of Interest

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