A Comprehensive Review on Techniques for Isolating Phytochemicals from Plant Sources

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Abstract

The pharmaceutical industry acknowledges plants for their extensive structural diversity and their diverse range of pharmacological actions. Phytochemicals are the substances found in plants that are biologically active. The biologically active substances that are present in plant tissues, including pulps, roots, barks, leaves, flowers, and seeds, are known as phytochemicals. The preparation and collecting of plants, the extraction of active ingredients, and the qualitative examination of the phytochemicals contained in the plant sample are the main topics of this review. Direct medicinal agents are obtained from these phytochemicals. These days, a large variety of technologies with various extraction techniques are accessible. Therefore, this review aims to describe and compare the most widely used approaches based on their background, benefits, and drawbacks in order to facilitate the evaluation process. Therefore, in order to aid in assessing the appropriateness and financial viability of the methods, this review attempts to characterize and contrast the most widely used approaches according to their tenets, advantages, and disadvantages. They act as a foundational raw material for the development of increasingly intricate semi-synthetic chemical compounds.

Key words: Phytochemical Variation, Medicinal plants, Conventional technique, Novel emerging technique

1. Introduction

Phytochemicals are the naturally occurring substances that are present in plants. These days, due to their numerous medicinal uses, these phytochemicals are gaining more recognition. In the fight against a number of illnesses, such as cancer, rheumatoid arthritis, and asthma, phytochemicals are crucial. Unlike pharmaceutical compounds, these phytochemicals have no adverse effects. Another name for phytochemicals is "man friendly medicines" because they treat illnesses without posing a risk to humans. Medicinal plants are currently seen as being very important because of their special qualities, since they are a major source of therapeutic phytochemicals that may be developed into new medications. The majority of phytochemicals derived from plants, including flavonoids and phenolics, have been shown to improve health and prevent cancer [1]. Thousands of phytochemicals were discovered to be advantageous and to exhibit biological activity, including analgesic, wound-healing, anticancer, antibacterial, and antioxidant properties. This paper primarily focused on analytical methodologies, encompassing extraction techniques, as well as the identification and analysis of bioactive compounds found in plant extracts using a variety of techniques that involve the use of chromatographic techniques and certain detection methods [2]. Natural products are becoming more and more important because of their safe qualities and wide range of uses in industries including flavor, food, medicine, and scent. Moreover, stringent regulatory regulations and "green consumerism" have made it possible to use more phytochemicals. Every phytochemical has a unique stability and solubility due to its unique nature. It can be attributed

to distinct metabolic processes. Many variables, including environmental and metabolic factors, influence the production of phytochemicals. Plants are a valuable resource for bioactive compounds used in therapeutic research. Isolated bioactive molecules are used as a model to produce biologically active compounds and as building blocks for drug synthesis in the laboratory. To preserve the activities of known constituents and to maximize their concentration, phytochemical processing of raw plant materials is fundamentally necessary. In the process of phytochemically processing plant materials to identify their bioactive components, extraction is a crucial step. The choice of an appropriate extraction method is crucial for the standardization of herbal products because it is used to extract the desired soluble components while eliminating unnecessary ones with the help of solvents.

2. Fresh vs. dried samples

Studies on medicinal plants use both fresh and dried samples. Considering the amount of time required for experimental design, dried samples are typically preferred. Since fresh samples are brittle and have a tendency to deteriorate more quickly than dried samples, Suleiman et al. restrict the time between harvest and experimental work to a maximum of three hours in order to preserve sample freshness. There was no discernible difference in total phenolics between fresh and dried Moringa oliefera leaves, but the dried sample had a higher flavonoid content. [3].

Extraction of phytochemicals from various plant materials with novel extraction methods

The first step in the study of medicinal plants is to prepare plant samples so that the biomolecules in the plants are retained before extraction. Fresh or dried plant material, including leaves, barks, roots, fruits, and flowers, can be used to extract plant samples. The preservation of phytochemicals in the finished extracts is also influenced by additional plant material pre-preparation techniques like grinding and drying. Since different solvents are used under different extraction conditions, like temperature and time, plant extraction is an empirical exercise. It is crucial to separate the bioactive components from co-extraversive compounds as they are extracted from the plants. The extracted compounds are further fractionated according to their molecular size, polarity, or acidity. The most common extraction techniques have been covered. The extraction process is a crucial part of the plant analysis because it separates the necessary chemical components needed to investigate the plants additional pharmacological activities. The process of extraction involves employing particular solvents to separate the active phytoconstituents from the plant through a common protocol. Decoction, maceration, percolation, Soxhlet extraction (also known as hot continuous extraction), microwave assisted extraction, counter current extraction, ultrasonic extraction (also known as sonication), supercritical fluid extraction, accelerated solvent extraction, and distillation techniques either steam or water are a few of the frequently used techniques in extraction processes. The extraction processes involve the use of solvents such as ethanol, methanol, water, chloroform, ether, and acetone. The phytoconstituents being extracted determines which solvents are used. Table 1 show the extraction of phytochemicals from various plant materials with novel extraction methods.

Table 1 Extraction of phytochemicals from various plant materials with novel extraction methods

Plants Name	Compounds	Method	Optimum Condition	Result Extraction yield	Reference
Ashwaghandha (Leaves)	Twelve free amino acids, flavonoids, glycosides, condensed tannins, and withanolides	Traditional heat reflux	The UV detection range is from 225 to 230 nm. Rate of flow (mL·min−1)	45	[12]
Ashwaghandha (Root)	Reducing sugars, volatile oils, steroids, and alkaloids	Traditional heat reflux		45	
		Soxhlet		9.08:9.51	
		Maceration		20.8	
		extraction of subcritical water		30.5:65.6	
		Ultrasonic-assisted extraction		2.85:9.74	
		Microwave-assisted extraction		10.01:11.39	
Ashwaghandha	Bioactive compounds**,** Total phenolic content in the extracts	Conventional heat reflux	EtOH/H_20, ratio % (70)	100 ± 0.00Cb	[13]
		Soxhlet	EtOH/H_2O, ratio % (100)	80.5 ± 1.24Cb	
		Microwave-assisted extraction	EtOH/H_2O, ratio % (70)	91.8 ± 1.20Cb	
		Ultrasonic-assisted extraction	EtOH/$H2_O$, ratio % (100)	157.61 ± 0.05Cb	
Vetiveria Lawsonii (Leaves)	Phytosterols, Cardiac Glycosides, Flavonoids, Alkaloids, and Steroids	Soxhlet Extraction	20 g, 200ml, 40^0C, 24hr	0.21±0.02 0.25±0.03 1.61±0.05 2.01±0.03 1.1±0.02 1.5±0.05	[14]
Vetiveria Lawsonii (Root)	Phytosterols, Cardiac Glycosides, Flavonoids, Alkaloids, and Steroids	Soxhlet Extraction	20 g, 200ml, 40^0C, 24hr	1.12±0.03 0.19±0.04 1.31±0.02 1.85±0.04 0.95±0.03 1.10±0.05	
Vetiveria (Root)	Isovalencenol, α- vetivone, and zizanoic acid, khusimol	Supercritical extraction	At 60 ◦C and 20 MPa, 2.23% (m/m), and 2.66% (m/m) with 5% (V/V) of ethanol cosolvent.	2.23% (m/m) while the addition of 5% (V/V) of ethanol cosolvent. increased the	[15]

				amount of oil extracted by 19%, khusimol (31.3%)	
	Isovalencenol, α- vetivone, and zizanoic acid, khusimol	Pressurized-liquid extraction (PLE) with n hexane	At 60 ◦C and 20 MPa, 2.23% (m/m), and 2.66% (m/m) with 5% (V/V) of ethanol cosolvent.	80% yield increased the amount of oil extracted by 19%, khusimol (31.3%)	
	isovalencenol, α- vetivone, and zizanoic acid, khusimol	UAE with n-hexane	(60 ◦C, 20 mL g−1, and 54%),15 min of extraction with a solvent volume five times smaller	70% yield increased the amount of oil extracted by 19%, khusimol (31.3%)	
Nagod	Saponins, tannins, steroids, flavonoids and glycosides were detected in acetone Silver Nanoparticles (AgNPs)	Cold percolation method	Six distinct solvents (water, acetone, methanol, petroleum ether, benzene, chloroform, and 10-300 nm in size) at 37°C for twenty-four hours	0.078 mg/mL	[16]
Vitex pseudo – negundo leaf flower	Trans - ferulic acid, Chlorogenic acid, Hes-peredin, p – Coumaric acid,	Allelopathy experiments HPLC Analysis	1ml ;0. 025 –0.4 mg / ml	235 ± 0.06	
Dalbergia sissoo (Shisham)	Terpenoids, alkaloids, glycosides, flavanols, tannins, saponins, and glycosides	Analgesic, anti-inflammatory, anthelmintic, antidiarrheal, molluscicidal, antinociceptive, antioxidant, antiulcer, antimicrobial, antidiabetic, osteogenic, ant spermatogenic, antisemite and neuroprotective activities	14 mm at doses of 0.386 mg/ml and 0.005 mg/ml, Respectively, Three different concentrations of Dalbergia sissoo	100 milligrams per milliliter of Dalbergia sissoo carbon tetrachloride extract, 25mg/ml, 50mg/ml, and 75mg/ml	[17]

Dalbergia sissoo	Flavones, isoflavones, quinines and coumarins	Biological activity	Dried for 15 to 20 days, refluxing for eight hours	Compound was obtained Rf value was found to be 0.72	[18]
Leaves of Caesalpinia Bonduc	N-triacont-5(Z),19(Z)-dienoic acid	Soxhlet apparatus	Individually over silica gel columns (1.6 m x 16 mm x 2 mm), pressure to get dark brown masses, 112.3 g, 137.2 g and 116.8 g	The leaves of Caesalpinia bonduc afforded a new fatty acid characterized as n-triacont-5(Z),19(Z)-dienoic acid	[19]
Allium Sativum	Total phenolic content (TPC) (mgGAE/g sample)	Microwave-Assisted Extraction (MAE)	Dried samples small size (0.1 mm) using Laboratory grinder, moisture content of the sample was 5.0 ± 0.5% at constant weight of 105^{0}C, extraction time from 2 to 6 min, irradiation time 7.62 min, power increases from 520 Watt (40%) to 910 Watt (70%), solid-liquid ratio 0.0055g/ml	28.62%	[20]
Allium sativum	Silver nanoparticles	Biosynthesis	Firstly, 5 g of A. sativum, extracted using 50 mL of deionized water for 24 h. Preparation of All solution	4.97816 g mg− 1 min− 1	[21]
Allium sativum	Alliin content	Soxhlet Extraction	10 g, ethanol–water (1:1), and 100% ethanol as solvents with a 2 h extraction time, 35–40 ◦C, 50% EtOH	1.96 g	
Allium cepa Red onion skin	Total phenolic, flavonoids and anthocyanins content	Conventional method	Solvent: 20%, 40%, 60% ethanol. Time: 30-, 60-, 120-, and 240-min. Temperature: 25 °C	757.38 mg GAE/g	[21]
Orange colored onion peels (Korea)	Total phenolics and flavonoids	Hot water	Solvent: Distilled water Temperature: 80 °C Time: 3 h	Extraction yield: 8.31% Total phenol: 120.60 mg GAE/g extraction	

Table 2 Extraction of phenolic compounds from plant materials with microwave assisted extraction methods

Red onion waste (Sibiu, Romania)	Anthocyanins, Flavonoids and tannins	UAE and conventional extraction	UAE: Solvent: 70% (V/V) ethanol solution Sonication: 150W power and 40 kHz frequency, and 70% ultrasonic amplitude Solid to solvent ratio: 30/1 and 20/1 ratio Time of extraction: 5, 10-, 15-, 20- and 25-min Temperature: 34.02-58.40 °C Conventional: Solvent: 70% (V/V) ethanol solution Temperature: 40 °C Solid to solvent ratio: 30/1 and 20/1 ratio Time: 30, 60, 90 min	UAE yielded better bioactive compounds than conventional extraction method	
Onion peel	Total phenolic compound	UAE	Sonication: 25, 33, and 45 kHz Power: 500 W Time: 1h. and 16 h. Temperature: 25 °C	Increased the extraction yield of phenolic chemical	[22]
Onion peel	Tannic acid	UAE using deep eutectic solvent (DES)	Solvent: Sonication: 700 W, 20 kHz, solid to solvent ratio: 1:10, DES ratio: 1:1(cholinechoride: urea), Duty cycle: 10%, Time of extraction:3 h Temperature: 60 °C	1705.80 µg/g	[23]
Curry Leaves	Total polyphenol content	Batch Extraction	Methanol-water solvent compositions (0%, 50%, and 100% v/v) and temperatures (313, 323, and 333 K) for the particle size of 0.425 mm and solid-to-solvent ratio of 1: 40 (g/mL), 7 hr	79.34 mgGAE/L	[24]
Curry Leaves	Total polyphenol content	Ultrasound-Assisted Extraction	20 kHz frequency and 250 W power, sonicated for 1 h using 50% (v/v) methanol-water solvent concentration and samples were withdrawn at fixed time interval, filtered, and analyzed.	65.68%	
Tamarind seeds	Twelve different fatty acids	Supercritical Fluid Extraction (SFE)	Parameters Operating conditions Sample size 8 g, Extraction, time 45 min Solvent, flowrate 4 mL/min, Pressure 3000, 5000 and 7000 psi, Temperature 40 C, 60 C and 80 C	Arachidic acid (35.2 mol%) followed by a-linolenic acid (17.4 mol%)	[25]

Tamarind	Total phenolic content	Soxhlet Extraction	10 grams of powdered leaf material to Soxhlet extraction for a period of 36 hours with 350ml of ethanol as solvent at 50ºC.	26.67%	[26]
Tamarind	Total phenolic content	Microwave Assisted Extraction (MAE)	10 grams of the leaves was in a microwave oven (CATA-R) working at an 800W irradiation power and 2450MHz frequency. MAE was done using ethanol as solvent at a temperature of 50°C for a period of 5mins	64.45%	[26]

Material/ Part/ Microwave oven	**Component**	**Parameters**	**Optimized Parameters**	**Results**	**Remarks**	**Reference**
Eucalyptus Robusta / Swamp mahogany leaves/ Domestic MW (1200 W, Sharp Carousel, Japan)	Total phenolic compounds	Irradiation time = 1 - 3 min, Microwave power = 480 – 720 W, Sample/ solvent = 2 - 8 g/100 mL, Solvent = water	Irradiation time = 3 min, Power = 600 W, Sample/ solvent = 2 g/100 mL	TPC = 58.4 mg GAE/g, TFC = 19.15 mg RE/g, Proanthocyanins = 6.23 mg CAE/g, ABTS = 74.95 mg TE/g, DPPH = 67.95 mg TE/g, CUPRAC = 143.70 mg TE/g	Sample to solvent ratio has the highest effect, Followed by power and irradiation time. RSM (BBD) was used for design of experiment.	[27]
Rubus fruticosus/ Blackberry fruit/ Domestic MW (1080W, HRSECL, China)	Blackberry anthocyanins (BBAC)	Microwave power = 200, 450, 550 W, Solvent concentration = 20-60 %, Liquid to solid ratio = 15 - 25 g/mL, Time = 1 to 3 min	Power = 469 W, Solvent concentration = 52 %, Liquid to solid ratio = 25 g/mL, Time = 4 min	Extraction yield = 2.18 mg/g, ABTS assay = 32.18 mM TEAC per g, DPPH assay = 27.18 mM TEAC per g	Results obtained from MAE were better than ethanol extract. Variable scavenging activity was observed at different level of extraction. RSM (BBD) was used for design of experiment.	[28]
Cajanus cajan/ Pigeon pea leaves/ Commercial MW (900 W, NEOS, Milestone, Italy)	Essential oil	Extraction time = 20, 30, 40 min, Microwave power = 300, 500, 700 W, % Humidity = 60, 70, 80	Extraction time = 44 min, Microwave power = 660 W, % Humidity = 68	Extraction yield = 0.33 (%, w/w), Rich sesquiterpenes = 72.89 %, MIC/MBC values: B. subtilis = 1.06 mg/mL and 2.12 mg/mL, P. acnes = 0.13 mg/mL and 0.26 mg/mL	RSM (CCD) was used for optimization. Results were compared with HD. MAE had better MIC/MBC value for gram positive bacteria and extraction time was reduced from	[29]

					5h to 44	

Table 3 Extraction of phenolic compounds from plant materials with ultrasound assisted extraction methods

Sr. No	Material	Components	Parameter	Optimized parameter	Results	Reference
1	Potato peels	Ascorbic acid, Caffeic acid, Ferulic acid	Time of extraction, power of microwave, and concentration of solvent	Concentration of solvent and microwave power	1.44mg, Caffeic acid 1.33 mg and Ferulic acid	[30]
2	Caraway seeds	Malonyl-daidzin, Malonyl- genistin, Acetyl-daidzin, Acetyl-genistin	Sample size, Extraction time, Solvent quantity, Temperature	Extraction time	0.5 kg of sample extracted by 250mL of 50% EtOH at 50 C for 20min	[31]
3	Basil	methyl cinnamate, linalool, eucalyptol, β - cubebene, cadinol	Microwave power, Extraction time, Amount of water	Time, Amount of water	high level of time (30 min) and the low level of water(400mL) resulted to be the highest (>0.45%) yield	[32]
4	Lotus leaf	N-nornuciferine, O-nornuciferine, and Nuciferine	Microwave power, Time, Solid liquid ratio	Solid-liquid ratio	higher extraction efficiency (0.9–43.7% enhanced) and reduced extraction time (from 2 h to 2 min	[33]
5	Black Pepper	β- aryophyllene, β-Bisabolol, d-Limonene,	Time, Solvent, Temp.	Time	The yield of essential oil increased from 0.9 to 1.8% in black pepper	[34]
6	Cardamom	β-Myrcene, 1-8 Cineole, α-Terpinyl acetate, α-Pinene	Time, Solvent, Temp.	Time	The yield of essential oil increased from 1.9 to 2.5%	[35]

7	Coriander	Carvacrol, Thymol, Eucalyptol	Microwave power, time	Time	Compared to the conventional solvent extraction, high percent extraction of thymol at reduced extraction time was obtained with MAE.	[36]
8	Coca Leaves	Cocaine, Benzoylecgonine	Time, Microwave power, Particle size, Solvent	-	particle size of plant material, 90– 150 micrometers; Solvent/volume, 5 ml methanol; radiation time,30 s; microwave power 125 W	[37]
9	Curcuma longa	Curcuminoid	Time, Solvent concentration	Time	The extraction process was optimized at 20% PLgiving60% extraction of curcuminoids with 75% purity within 1 min	[38]

3.2 Cold extraction method:

The various plant parts were dried in a controlled environment at 50–60 °C, and the resulting powder was subsequently extracted using a variety of solvents. After weighing the dried powder and adding it to a conical flask with the appropriate solvents, shake the flask for thirty minutes at room temperature every twenty-four hours for a period of seven days. In the end, the extract is vacuum-filtered through Whatman filter paper and allowed to dry at room temperature in a watch glass dish. Record the weight of every dish both before and after the extracts have dried. Determine the extract's weight based on the difference. [4]. Table 2 show the extraction of phenolic compounds from plant materials with microwave assisted extraction methods.

3.3 Solvent extraction method

Solvent extraction has recently made use of the Universal Extraction System (Buchi). Various plant parts, powdered and dried, are placed in a glass thimble and extracted using different solvents. Each extract undergoes ten cycles of this process, which lowers the temperature to slightly below the solvents' respective boiling points. To ascertain whether phytoconstituents are present, the resultant solvent extract is filtered, concentrated in a vacuum concentrator, and utilized [4].

3.4 Supercritical fluid extraction (SFE)

Gases, typically CO2, are used in supercritical fluid extraction (SFE), which compresses the gas into a dense liquid. The material to be extracted is then placed inside a cylinder and this liquid is pushed through it. Subsequently, the liquid containing the extract is pumped into a chamber designed to separate the extract from the gas and recover the gas for future use. Pressure and temperature changes allow for the manipulation and adjustment of CO_2 solvent properties. One benefit of SFE is that it completely evaporates CO_2, leaving no solvent residues behind [5].

3.5 Microwave-assisted extraction (MAE)

It combines traditional solvent extraction with microwave extraction, and is simply called microwave extraction. Microwave-assisted extraction is the process of heating the solvents and plant tissue with a microwave to increase the extraction's kinetics [6]. The tiny, microscopic traces of moisture found in plant cells are the target of heating in dried plant material. Due to the microwave effect, this moisture inside the plant cell heats up, causing evaporation and applying extreme pressure to the cell wall. The pressure pushes against the cell wall from within, causing it to rupture. As a result, the ruptured cells release active constituents, which increases the phytoconstituent yield [7, 8]. Figure 1 shows the various extraction techniques. At a magnetron frequency of 2.45 GHz and a microwave power of 800 W, home microwave ovens (Electrolux EMM 2005, Hungary) were used for the microwave extractions. In order to avoid the solvent from overheating and evaporating, microwaves were run in pulse mode and kept cool with ice water. Pretest results were used to determine 40 seconds on and 20 seconds off, then 20 seconds on and 20 seconds off (until the time was up (10 min) [39].

Figure. 1: Various techniques for extraction a) Solvent extraction; b) cold percolation; c) supercritical fluid extraction; and d) extraction with microwave assistance

3.5 Ultrasound-assisted extraction (UAE) or Sonication Extraction.

Ultrasound frequencies ranging from 20 kHz to 2000 kHz are used in UAE. The permeability of cell walls and surface contact between solvents and samples are enhanced by the mechanical effect of ultrasonic cavitation's acoustic cavitation. When materials are exposed to ultrasound, their physical and chemical characteristics change and the plant cell wall is disrupted, which promotes compound release and improves the mass transport of solvents into the plant cells [9]. The process is a straightforward, reasonably priced technology that can be applied to phytochemical extraction on a small or large scale. Power ultrasound (3.5 W cm^2, 20 kHz) generated by a generator (Weber ULC 400

Premium Ultrasonic Generator, Germany) was used to perform the ultrasound-assisted extraction (UAE) with a timer-controlled treatment duration of thirty minutes. The 10 g plant materials sample was added to a flask along with the solvent that had been previously prepared. The temperature was kept at about 25 ºC using an icy water bath to stabilize the heat distribution throughout the treatments [39].

4. Identification of phytochemicals

Different types of bioactive compounds with varying polarities are found in plant extracts; however, the process of identifying and characterizing these compounds still faces significant difficulties in separating them. It is standard procedure to isolate these bioactive compounds using various separation techniques, which should be applied to produce pure compounds. These techniques include TLC, HPTLC, paper chromatography, column chromatography, gas chromatography, OPLC, and HPLC. The structure and biological activity of the pure compounds are then ascertained [10]. Table 3 show the extraction of phenolic compounds from plant materials with novel extraction techniques.

Discussion

The types of solvents used in the procedures (MAE, UAE) have a significant impact on all the methods that use them. The biologically active compounds in the poplar type propolis at ratio (1:10 w: v) were not significantly affected by the solvent volume used using the three methods (MAE and UAE), indicating that using solvents at higher ratios is not necessary [9]. Nevertheless, the results are restricted to the evaluation of total yield, flavonoid content, and phenolic content as benchmarks. Vongsak et al. have proposed that maceration is a more cost-effective, practical, and applicable method for small and medium-sized businesses (SMEs) than other contemporary extraction techniques.
Comparing the maceration technique also referred to as the "Green method"—with MAE and UAE, however, reveals a significant problem with chemical waste [3]. The efficacy of these crude extracts using nano-encapsulated processing in Centella asiatica showed to have similar efficacy as those that were purified, despite the fact that all of these extraction methods produced crude extracts that contained a mixture of metabolites [11]. This specific fact implies that, provided appropriate preparation and extraction are carried out, additional, labor-intensive, and complex extraction and purification steps may not be required. Adequate circumstances for every extraction technique are equally crucial.

Conclusion

The presence of bioactive compounds in plant material still poses challenges for extraction, identification, and determination because these compounds are multi-component mixtures. To isolate the bioactive compound, the majority of them practically need to be purified using a combination of multiple chromatographic techniques and other purification methods. In the study of medicinal plants, every stage of the extraction process from pre-extraction to extraction is crucial. The efficiency and phytochemical components of the final extractions were impacted by the sample preparation, such as grinding and drying, which ultimately had an impact on the final extracts. It is clear from this that no single extraction technique is best; rather, each extraction process is specific to a given plant. The selection of appropriate methods can be guided by previously optimized methods.

Conflict of Interest

The authors declare no conflict of interest.

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