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Conventional and novel techniques of phytochemical extraction

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Abstract
Phytochemicals are an ancient medicine that can treat several diseases and have made their position at the top in medicine since the ancient era. Phytochemicals are one of the major disease-curing agents that attract a population towards itself. Many Novel extraction techniques have been developed with ancient conventional ones. Some methods need dried powdered material for extraction, and some direct fresh material for boiling. Soxhlet extraction technique at present also works in laboratories to extract phytochemicals, Cold Aqueous Percolation, decoction, and maceration are some other conventional methods. Apart from this, some novel extraction techniques like; Microwave-assisted extraction (MAE) which involves the use of the microwave for extraction, Ultrasonication Assisted Extraction (UAE), Super-critical Fluid Extraction (SFE), and Pressurized liquid extraction (PLE). This review article discusses about phytochemicals and different extraction techniques, their method of performance, advantages, requirements, benefits, and speciality of techniques.

Keywords: Phytochemicals, extraction, conventional, novel

Introduction

The term phytochemical is derived from the Greek word *phyton*, means plant, and chemical; which means plant-origin chemicals i.e. Phytochemicals. Plants are natural factories for the production of Phytochemicals (Iqra Akhtar *et al*. 2019) [9] which have Biological activity and play a role in the growth of plants and the defence of plants against competitors or pathogens (different microorganisms) like fungi, bacteria, viruses, protozoans, nematode, etc. (Mayuri *et al*. 2023) [14] With biotic stress tolerance, phytochemicals also provide abiotic stress tolerance to plants (Ramkumar, *et al*., 2021) [20] such as UV light, temperature, light intensity, cold, drought, during adaptation in a particular ecosystem, etc.

Because of a great variety of phytochemicals or Secondary metabolites Herbal drugs are prepared from plants and used to cure many diseases since ancient times; as saponins, tannins, flavonoids, phenolic compounds, lignins, alkaloids and glycosides, and many others. (Zoliansanga, 2022) [25] Phenolic compounds act as a potent antioxidant and for instance, medicinal plants are rich reservoirs of phenolic compounds. (Jyotsna, 2023) [12]

By providing food, shelter, and clothing, nature fulfils various needs of human beings. There are no medicines or drugs in the primitive world, humans find and derive their cure from naturally available sources like Plants in their surroundings. These led to an exploration of natural products with trial and error. Hence humans depend on nature to survive and thrive for meeting its various needs. (Zoliansanga, 2022) [25] Medicinal plants are the richest bio-resource of Drugs of traditional medicine, modern medicine, folk medicine, pharmaceutical and chemical entities for synthetic drugs. World Health Organization (WHO) promotes herbal remedies in national health care programs because they are easily available at low cost and they are safe hence people have faith in them. (Amita & Shalini, 2014)

Ayurveda has used many herbs possibly as early as 1900 BC. Existing medicinal system in India such as Unani, Homeopathy, Naturopathy, Sidha, etc. utilizes plants in health care and they are commonly used medicine systems. (Bishan Singh & Khushboo, 2014) [7] *Phyto* therapy involves plant-based therapeutic agents." Which are." based on a long history of their traditional use and benefits. Ethnopharmacology aims to increase our relation with traditional herbal medicines, their working principles, preparation, and use effectively for the well-being of humans. (Jyotsna, 2023) [12]

So many extraction techniques have been developed and are working in various industries, laboratories, and clinics to isolate phytochemicals or secondary metabolites from plant parts—the main focus of this paper on Extraction Techniques.

The extraction techniques of phytochemicals

India has a great text enlisting "Methods of preparation." and uses various plant-originated medicines. A variety of extraction methods have been developed to obtain Secondary metabolites from plant parts in high yield so that they can be used as drugs for different diseases (Mariamma, 2012) [13]. Plant extraction is the process of separating, active plant materials, secondary metabolites, or pharmacologically active compounds by using appropriate solvents and standard extraction procedures. There are so many extraction methods such as; digestion, maceration, infusion, percolation, decoction, superficial extraction, soxhlet extraction, etc. (Abdullahi & Mainul, 2020) [3]

Techniques such as High-performance thin layer chromatography (HPTLC), Thin-layer chromatography (TLC), gas chromatography (GC), and paper chromatography (PC) are used for the purification of secondary metabolites from extracts. (Abdullahi & Mainul, 2020) [3]

Collection of plants

For the extraction of secondary metabolite from plants firstly it needs its collection. By giving self-field visits or by purchasing from somewhere we can collect the study

material, and by studying its well-known present phytochemical or secondary metabolites we can choose it for extraction. If extraction is on any specific part of a plant then there will be no need for a collection of the whole plant. For example, if the extraction is for leaves only then there will be no need for a shoot, flower, or fruit collection.

While collecting a plant sample make sure the land allows removal of plant or natural specimens. Firstly choose the plant that you want for your study, then identify its parts such as, fruit, flower, leaves, stem, and roots, and collect it. Try to avoid the collection of infected plant parts as it can spread diseases.

Make sure that before starting the study and extraction of material you have properly authenticated it.

Plant material

Natural constituents of a plant can be derived from any part of the plant like leaves, flowers, roots, bark, fruits, and seeds, i.e. all parts of the plant may contain phytochemicals. (Amita & Shalini, 2014)

Solvents used for active component extraction

For the separation of the plant's secondary metabolites, the selection of a solvent is one of the major steps. The factors affecting the choice of solvent are the rate of extraction, diversity of different compounds extracted, (Mariamma, 2012) [13] quality of phytochemicals, their chemical properties, etc. Secondary metabolites are generally miscible in nonpolar solvents. Solvent for extraction can be; Water, Ethanol, Methanol, Chloroform, Ether, and Acetone. (Amita & Shalini, 2014)

Conventional extraction methods

A wide range of techniques are available for the extraction of active components (phytochemicals) and essential oils from plant parts. There are conventional extraction methods which are;

1. Cold aqueous percolation
2. Hot aqueous extraction (decoction)
3. Hydro distillation
4. Soxhlet extraction
5. Maceration

1. Cold Aqueous Percolation

Cold Aqueous Percolation involves the use of water for up to 24 hours with powdered plant material, complete immersion will be done of powdered plant material in water, which enables the water-soluble ingredients of the plant to attain equilibrium in the water. (Mariamma, 2012) [13] Percolation operation is very easy, thermally unstable components can be effectively extracted through this method. (Wang W. Y. *et al* 2020) [24]

Fig 1: Percolator

2. Hot Aqueous Extraction (Decoction)

Decoctions of plant material are prepared by, allowing the mixture to boil until the volume of water is reduced to one-fourth its original volume and filter. This facilitates the ingredients present in the plant material to be extracted. (Mariamma, 2012) [13]

3. Hydrodistillation

Essential oils, aromatic compounds, and volatile compounds are generally isolated from plant material by hydrodistillation. (Mariamma, 2012) [13] A specific amount of water or menstruum is added to the container followed by either boiling or interposing of direct steam to plant material. This introduction of boiled menstruum leads to important factors in removing the bioactive compounds of plant tissue. (Jyoti S. P. 2022)

Fig 2: Hydrodistillator

Steps of hydrodistillation

Add water to the plant material

↓

Steam to plant material then oil will free from oil glands

↓

The mixture of water and oil

↓

Indirect Cooling of mixture

↓

From the condenser, distillate flows into the separator

↓

The oil extract is separate from water

4. Soxhlet extraction

Soxhlet extraction was invented by Dr Franz Von Soxhlet 140 years ago for the extraction of milk fats. (Muqsit P. & Zeynep A. 2021) The advantage of this method is that just one batch of solvent can be recycled, instead of utilizing many portions of warm solvent being passed through the sample, and this method cannot be used for thermo-labile compounds, as prolonged heating may lead to the degradation of compounds (Amita & Shalini, 2014) Organic solvents are generally selected along with acidic or basic residues. (Muqsit P. & Zeynep A. 2021)

The advantage of using the Soxhlet extractor is that it forms a long-term extraction state as the sample material is repeatedly in contact with the new extractant. (Pufeng Qin, *et al*., 2023) [18]

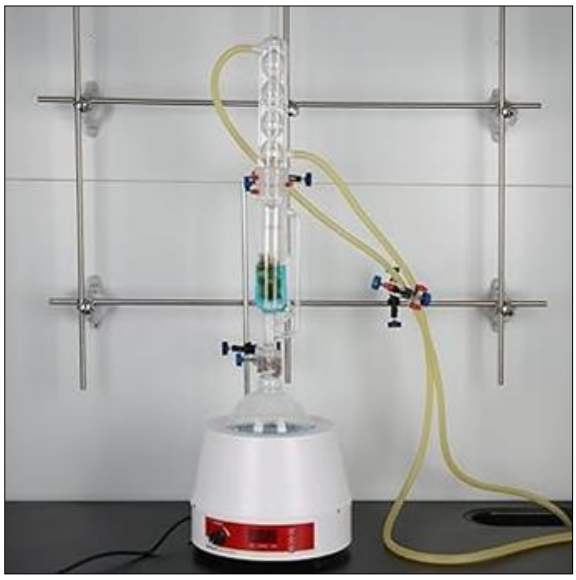

Fig 3: Soxhlet

Steps of soxhlet extraction

Pack the powdered material in a pocket of filter paper and put it in a Thimble.

↓

Pour the solvent into a distillation flask, and the vapour will generate and pass through the thimble containing the material to be extracted. (Pufeng Qin, *et al*., 2023) [18]

↓

Maintain the temperature, the solvent should not overheat because it leads to the flow of solvent into the thimble

↓

When the liquid reaches the overflow level in the thimble, it will fall back into the distillation flask.

↓

Repeat the cycles several times, for example up to 7 to 8 cycles will require up to 24 hours.

↓

The distillation flask carries the extracted solutes into the bulk liquid, and separation of solute from solvent takes place in the distillation flask.

↓

Then solute is left in the flask and fresh solvent vapours pass back into the solid bed of sample material.

After extraction, the solvent can be removed, typically through a rotatory evaporator, yielding the extracted compound.

The non-soluble portion (solid) remains in the thimble and is usually discarded

5. Maceration

Maceration is one of the ancient and simplest extraction techniques, it is conducted at room temperature. (P. Subramanian & C. Anandharamakrishnan, 2023) [17]

Steps of Maceration

Add coarsely powdered plant material to the stoppered container

Add solvent and place at room temperature 37^0c

Dissolution of phytochemicals in a solvent

Extraction in a drop-wise manner (Darshan D. and Nalin P. et. al. 2017)

The main advantage of this technique includes a simple process, low installation cost, and low maintenance cost. (Amita & Shalini, 2014) But this method requires a few hours to a few days for extraction.

Novel extraction methods

The traditional methods of extraction require large amounts of solvents and are time-consuming, like, maceration requires 2-7 hours at least for complete extraction. (Iqra Akhtar et. al. 2019) [9] The novel extraction methods such as; Ultra sonication-assisted extraction, Microwave-assisted extraction, Supercritical fluid extraction, and Pressurized liquid extraction (PLE). (Mariamma, 2012) [13]

1. Ultra sonication assisted extraction (UAE)

One of the fasted extraction processes compared to traditional ones like; maceration, percolation, soxhlet extraction, etc. UAE uses high-frequency sound waves to create minute pores in the cell walls of plant cells and thereby release phytochemicals from the material. The method is beneficial for the extraction of essential oils, and bioactive phytochemicals such as campothecin, pyrethrins, cardiac glycosides, menthol, etc.

Fig 4: Ultrasonicator

Steps of UAE

Drying of plant sample and grinding into powder form

Selection of solvent (ethanol, methanol, etc)

An ultrasound generator is used to generate the ultrasound effect.

To Control temperature, use a water bath.

Ultrasonic probe immersed directly into the solvent containing the sample

Extraction (Nur Amirah A. R. Z. *et al*. 2020)

The ultrasonic device with a bubble implosion will induce a cavitation effect which helps in the extraction process. (Nur Amirah A. R. Z. *et al*. 2020)

The technique of sonication generates a lot of heat so various heat-labile compounds such as proteins require a cooling step to keep them cold during sonication. (Mariamma, 2012) [13] The advantages of UAE include low solvent, energy consumption, and time. (Qing-Wen Z, *et al*. 2018)

2. Microwave-assisted extraction (MAE)

The use of microwaves for the extraction of constituents (Secondary metabolites) from plant material has shown great research interest and potential. Microwaves are nonionizing electromagnetic waves with frequencies between 300 MHz to 300 GHz, (Mariamma, 2012) [13] made up of two perpendicular oscillating fields which are used as energy and also information carriers. (Iqra Akhtar et. al. 2019) [9]

Fig 5: Microwave-assisted extraction instrument

Generate heat by interacting with organic components in the plant matrix following the ionic conduction and dipole rotation mechanisms, polar compounds such as water. (Iqra Akhtar et. al. 2019) [9]

The transfers of heat and mass are in the same direction in MAE, because of this synergistic effect is generated which accelerates extraction and its yield. (Qing-Wen Z, *et.al.* 2018) There are two different types of microwave instruments in use; Open-vessel system and Closed-vessel system.

There are four major components of both open-vessel and closed-vessel systems:

1. **Magnetron:** Microwave generator that generates microwave energy.
2. **Wave guide:** used to propagate the microwaves from the source to the microwave cavity.
3. **Applicator:** where the sample is placed.
4. **Circulator:** will allow the microwave to move only in the forward direction (Iqra Akhtar et. al. 2019) [9]

Closed vessel system

- It is the most common procedure in microwave-assisted extraction. In this extraction is done under controlled temperature and pressure
- To obtain extract under drastic conditions, for acid mineralization closed vessel systems are generally preferred.
- Teflon cells are used, any solvent can be chosen and the pressure inside the vessel depends upon the vapor pressure of the selected solvent.
- The interior temperature is directly proportional to the power level used and hence can be controlled. (Iqra Akhtar et. al. 2019) [9]

Open vessel system

- Under atmospheric pressure, an open extracting vessel is used
- A solvent is heated and refluxed back through a condenser like soxhlet extraction, a cellulose cartridge can also be used to carry plant samples as it helps to avoid an extra step of filtration.
- Quartz cells are used, which depend solely on boiling points of solvents for heating purposes and work under atmospheric pressure.
- Open vessel systems are also considered safer in solvent handling and can be used to extract larger quantities of plant samples. (Iqra Akhtar et. al. 2019) [9]

3. Supercritical fluid extraction (SFE)

In Supercritical Fluid Extraction or SFE, a substance is in its supercritical fluid state i.e. when both the temperature and pressure equal or exceed the critical point, for example, 31°C and 73 atm for carbon dioxide (Mariamma, 2012) [13] and mainly carbon dioxide is used as it is an ideal solvent with low viscosity, high volatility, and high diffusion rate. Because of its nonpolar property, it is inefficient for the extraction of polar solutes. As has a low critical temperature hence easily applicable to thermally labile compounds. (Jihye Kim et. al. 2012) [10]

This characteristic of supercritical fluids provides the ideal condition for extracting phytochemicals with a high degree of recovery in a short period. (Mariamma, 2012) [13] Extraction solvent becomes a gas after extraction and the analytes are conveniently concentrated in the collecting medium this is the major advantage of SFE over a liquid-based method. (S.J. Lehotay & F.J. Schenck, 2000) [21]

Other extraction techniques require so many steps during extraction but in SFE, all operations are performed in one instrument, and parameters from one step to another are independent of each other. (Serban C. & Victor, 2002) [22]

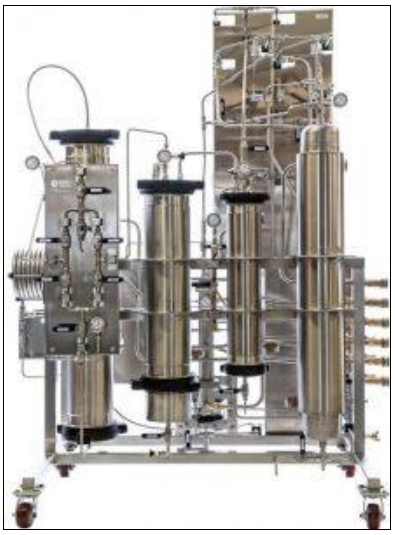

Fig 6: Supercritical fluid extraction instrument

Steps of SFE

Withdraw liquid CO2 from the gas cylinder and mix it with an appropriate modifier

Introduce them in an extraction chamber at specific temperature and pressure

Through restrictor the fluid-containing extract move from the rest of the sample

One side of the restrictor is at high extracting pressure while the other side is usually at ambient pressure

Fluid expands at the point of expansion, supercritical fluid becomes gas, and solute drops off

Collect the released solutes (solid, liquid, gas) on trapping material that can be adsorbing solid (silica, charcoal, tenax) or liquid

Transfer the trap in a solution, the solvent must bechosen, dissolve the solute deposited there and deliver a solution to the processed sample. (Serban C. & Victor, 2002) [22]

4. Pressurized liquid extraction (PLE)

Pressurized liquid extraction (PLE) has also been described as accelerated solvent extraction, enhanced solvent extraction, accelerated fluid extraction, high-pressure solvent extraction, or pressurized fluid extraction. (Qing-Wen Z, *et.al*. 2018) It says that Pressurized liquid extraction is a "green" technology for the extraction of nutrients from foods and herbal plants. (Arwa & Charlotta, 2011)

PLE applies high pressure in extraction. High pressure keeps solvents in a liquid state above their boiling point resulting in a high solubility and high diffusion rate of lipid solutes in the solvent, and a high penetration of the solvent in the matrix. PLE decreases the extraction time, requires low solvent, and has better repeatability compared to other methods. (Qing-Wen Z, *et al*. 2018)

Fig 7: pressurized liquid extraction instrument

Discussion

So many extraction techniques for phytochemicals are known and work for the isolation of secondary metabolites, and active biological components within plant parts. Conventional extraction methods are very easy to work, cheap, and don't require expensive, complicated equipment and operations for example, Soxhlet extraction (Pufeng Qin, *et al*., 2023) [18], percolation, and Hydro distillation. We can also perform them at home for our personal needs as medicine like for extracting oil from seeds and fruit, drying and grinding of material then boiling in the water or solvent we can get an extract. However, the continuous boiling and heating of the material may destroy thermo-labile compounds (Jyoti Sankar Prusty, 2022) [11] despite this, conventional methods require a high amount of solvent and time (Wang W. Y. *et.al*., 2020) [24] if we need techniques with fast extraction, best yield, and low solvent consumption then we can go for Novel extraction techniques.

Nowadays at the industrial level, laboratories for the preparation of drugs or medicines for fast and quality extraction peoples moving towards Novel extraction techniques. These methods give very fast results and also the best quality extracts, require a low amount of solvent and time during extraction (Arwa & Charlotta, 2011) for example, SFE gives a high extraction yield and has better fractionation capability (A.P. Sanchez-Camargo et. al., 2014), etc. Novel extraction techniques are good for solvent and time requirements and give the best yield, with this, novel methods also have some disadvantages, high cost of automated instruments (S.J. Lehotay & F.J. Schenck, 2000) [21] and requiring expertise to handle them. UAE presents a problem while isolating a DNA or large amount of Protein due to the shearing process.

We can conclude that as per our requirements and plant material, we can choose the technique or method. Conventional as well as Novel extraction techniques both are good in their way of working and requirements. If we afford high-cost instruments and experience, we can choose Novel extraction techniques.

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