Asian Plant Research Journal

Volume 11, Issue 4, Page 13-34, 2023; Article no.APRJ.101150
ISSN: 2581-9992

Phytochemical Screening for Medicinal Plants: Guide for Extraction Methods

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/APRJ/2023/v11i4216

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/101150

Review Article

Received: 04/04/2023
Accepted: 06/06/2023
Published: 16/06/2023

ABSTRACT

Sri Lankans have relied on traditional medicine to meet their primary health needs since the beginning of time. This island is blessed with an enormous number of medicinal plants, which play a very important role in traditional medicine. However, the lengthy documented history of traditional medicine is still composed of medicinal plants, which are not scientifically proven to have the mentioned abilities or activities. To obtain scientifically sound information from this documented history of traditional medicine, extraction of the biologically active compounds from these medicinal plants is very important. Also, to maintain the accuracy of results obtained from *in vitro* and *in vivo* assays, it is important to consider the pre-extraction procedures as well as the evaporation and storage conditions of the extract. There are several extraction methods accessible in Sri Lanka. This research aims to review the pre-extraction preparation, extraction methods, evaporation techniques, and storage conditions of the plant extract. This review highlights that the

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reliability of phytochemical screening results is affected by the identification and authentication of the plant, pre-extraction procedures, menstruum utilized, method of extraction, and storage conditions.

Keywords: Sri Lanka; extraction methods; phytochemical screening; secondary metabolites; bio-active compounds; maceration; medicinal plants; identification and authentication.

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1. INTRODUCTION

Traditional medicine has a long history in Sri Lanka, and medicinal plants play a significant role. Various illnesses and conditions are treated with medicinal plants in Sri Lanka [1-7]. Sri Lanka is an island surrounded by the Indian Ocean. In ancient times this island nation was known to be ruled by King Ravana, who was the author of many books of traditional medicine like Arkaprakasya, Kumarathanthraya, Udisha Thanthraya, and Nadivignanaya. During his reign, King Ravana represented Sri Lanka at a medical symposium at the base of the Himalayas in India, according to the Ramayana [8]. Sri Lanka is blessed with an enormous number of medicinal plants, with 1430 species representing 181 families and 838 genera. There are 174 endemic species discovered so far in Sri Lanka [8,9]. In 1988, British ecologist Norman Myers identified Sri Lanka as a biodiversity hotspot based on the specie's endemism and degree of threat [10]. Folklore medicine continues to meet the foremost health-related needs of 60% to 70% of Sri Lankans in rural regions. Sri Lankans have a well-documented and lengthy history of traditional medicine practice [1,9]. Medicinal plants are found to be prominent in phytoconstituents and secondary metabolites which play a major role in exhibiting many biological potencies such as anti-bacterial, anti-cancer, anti-diabetic, anti-fungal, anti-inflammatory, antioxidant, and antiparasitic activities. Secondary metabolites like alkaloids, essential oils, phenols, quinones, resins, saponins, steroids, terpenes, and tannins have shown various biological activities both *in vitro* and *in vivo* over the years. These secondary metabolites can be present in any plant part such as bark, flowers, fruits, leaves, rhizomes, roots, seeds, stem, and tubers [11,12]. Medicinal plants are one of the plentiful bioresources of medicinal drugs. Most current drugs involved in routine medications were originally extracted from plants, and now they are produced synthetically [13]. According to pharmaceutical sciences, Extraction is the separation of medicinally vital portions of selected plant or animal organs with the use of selective solvents and the most appropriate standard extraction procedure. Extracts of herbal plants can be utilized directly to treat specific illnesses and disorders, or they may need to be further processed before attaining therapeutic potential. There are many techniques used for the extraction purpose. Aqueous-alcoholic extraction by fermentation, counter-current extraction, decoction, digestion, continuous hot extraction (Soxhlet), infusion, maceration, microwave-assisted extraction, percolation, phytonic extraction (with hydrofluorocarbon solvents), supercritical fluid extraction, and ultrasound extraction (sonication) are some of them [14]. There can be many factors that contribute to the medicinal value of a plant. During the extraction of herbal plants, the active phytoconstituents of the plant or secondary metabolites like alkaloids, flavonoids, glycosides, saponins, steroids, and terpenes can be separated from the inert components of the plant [15].

The nature of the plant substance, the type of solvent being used, the pH of the solvent being utilized, the temperature of the system, and the solvent-to-sample ratio all influence the selection of the most suitable extraction method. It is also determined by how the final products will be utilized [16,17]. Accurate identification and authentication, appropriate and well-timed collection of plant material, and pre-extraction procedures also have a greater influence on the end product [18]. Numerous studies have proved that the storage condition of plant extracts has a major impact on their bioactivity. Therefore, considering the storage conditions are also very important after the extraction of medicinal plants [19,20]. Usually, extraction of medicinal plants includes the following steps: collection of the plant material, identification and authentication of the selected plant, size depletion or homogenization, extraction, filtration, concentration, drying, and reconstitution. This study aims at reviewing different types of extraction methods for plants.

2. COMMONLY USED MEDICINAL PLANTS IN TRADITIONAL MEDICINE

Table 1. This table indicates some medicinal plants, their family names, local names, and their medicinal uses [8]

Scientific name	Family name	Local name	Medicinal usage
Justicia adhathoda L.	Aanthaceae	Adhathoda	Treatment for Asthma Tuberculosis, Coughs, Catarrh, Tonsillitis
Annona cherimola	Annonaceae	Anoda	Treatment for Hemorrhoids and Sciatica
Eryngium foetidum	Apiaceae	Andu	Treatment for Snake bites, Skin Diseases, Mucosal diseases, Diabetes mellitus, Epilepsy Convulsions, Spasms, and Stomach disorders
Terminalia chebula Retz.	Combretaceae	Aralu	Treatment for abdominal disorders, digestive disorders, Cold, Coughs, Catarrh, and worm diseases
Ficus racemosa L.	Moraceae	Aththikka	Treatment for Wounds, Swellings, Skin diseases, and Diabetes mellitus
Ipomoea pescaprae	Convolvulaceae	Binthamburu	Treatment for Diarrhea, Rheumatism and Sprains
Terminalia belirica (Gaeern.) Roxb.	Combretaceae	Bulu	Treatment for Swellings, Digestive system disorders, Diarrhea, Vomiting, Urinary calculi, Skin diseases, Coughs, Asthma, Nervous system diseases, Dropsy, Piles, and Rheumatism
Tagetus patula	Asteraceae	Daspethiya	Treatment for Wounds and Skin diseases
Punica granatum L.	Punicaceae	Delum	Treatment for-Eye infections, Dysentery Heart diseases, Worms diseases, Coughs Asthma and Cold and Fevers
Ricinus communi	Euphorbiaceae	Endaru	Treatment for Arthritis Nervous system diseases, Worm diseases, Hemorrhoids, and Dysmenorrhea
Dillenia retusa	Dilleniaceae	Godapara	Treatment for dandruff
Centella asiatica	Apiaceae	Gotukola	Eye diseases, ear diseases, Catarrh, Mucosal diseases, Lactation diseases, Epilepsy, Nervous system disorders, and Paralysis
Alpinia calcarata Roscoe	Zingiberaceae	Heen araththa	Treatment for Rheumatism, Pain Hoarseness of voice, and snake bites
Clitoria ternatea	Fabaceae	Katarolu	Treatment for Anasarca, Diseases of the bladder and urethra, Ascites, Dyspepsia, Liver disorders, Enlargement of abdominal viscera, and Swollen joints

Scientific name	Family name	Local name	Medicinal usage
Salacia reticulata	Hippocrateaceae	Kothala Himbatu	Treatment for Diabetes mellitus and Renal stones
Asparagus falcatus L.	Asparagaceae	Maha hathavariya	Treatment for Diarrhoea, Dysentery, and kidney diseases
Phyllanthus emblica	Euphorbiaceae	Nelli	Treatment for Diabetes mellitus, Burning sensation, Skin diseases, Abdominal diseases Headaches, Hemorrhoids, Coughs, Asthma, and Tuberculosis
Cassia auriculata	Fabaceae	Ranawara	Treatment for-Skin diseases, Excessive bleeding, Excessive thirst, Dysentery, Diabetes mellitus
Hemidesmus indicus	Periplocaceae	Iramusu	Treatment for Skin diseases, Coughs, and Asthma

3. PHYTOCHEMICALS OF MEDICINAL PLANTS

A phytochemical is a broad term that refers to a variety of compounds produced naturally in plants. They are classified into six major types based on their chemical structure and properties. Carbohydrates, lipids, phenols, terpenoids, Alkaloids, and other nitrogen-containing compounds are among them. There are different subcategories within each category based on the biosynthetic origins [21].

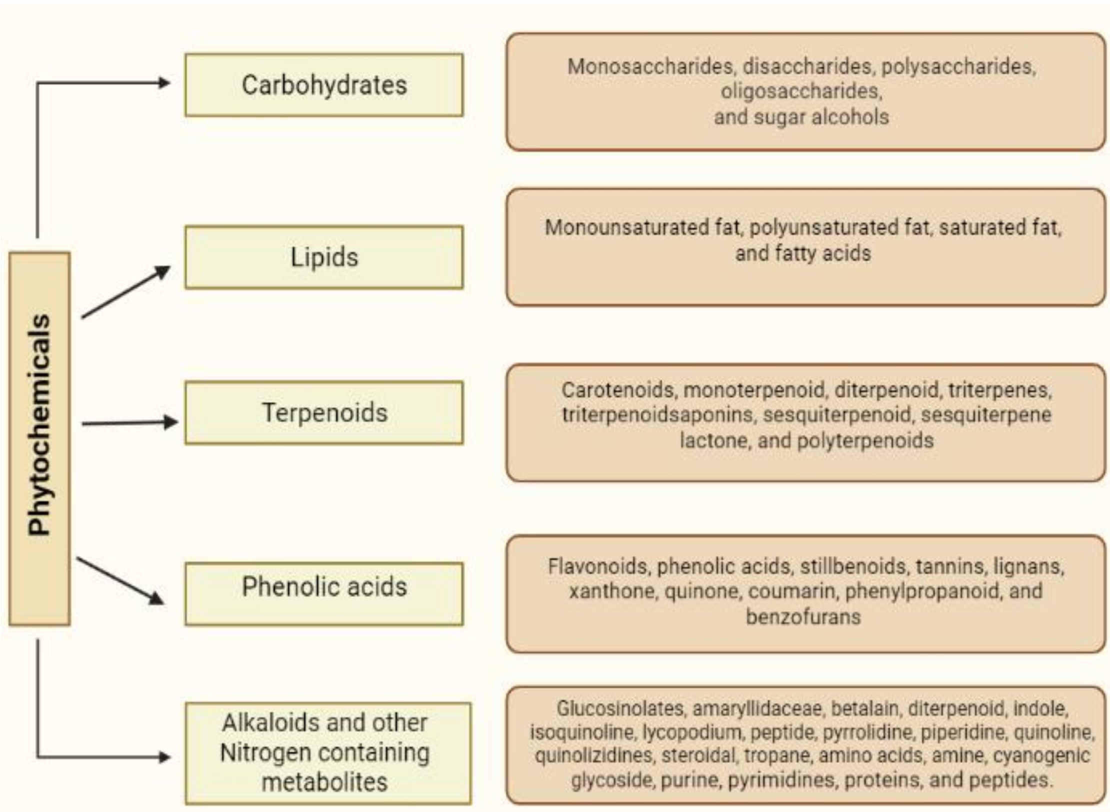

Fig. 1. Phytochemicals of plants and their subcategories, adapted from [22]

Phytochemicals are mainly classified into Primary and Secondary metabolites. Carbohydrates and lipids can be identified as the primary metabolites while Secondary metabolites in plants can be named nitrogen-containing compounds, phenolic compounds, and terpenes [23, 24].

Phytochemical screening is a process also called primary and secondary screening. Organs of medicinal plants such as roots, leaves, stems, seeds, and bark are known to be rich in these phytoconstituents. To find these phytoconstituents, extracts of plant materials are tested for phytochemicals either by phytochemical screening or quantitative analysis of phytochemicals. Standard procedures published within the scientific community can be used to analyze phytoconstituents [25].

4. IDENTIFICATION AND AUTHENTICATION OF THE PLANT

Plant authentication is the process of confirming the authenticity of plant material. After the selection of the plant for extraction, it should be identified accurately according to organoleptic properties such as color, odor, taste, and morphological characteristics such as appearance, shape, and size of the plant, before collection of the plant sample [26]. Then the plant material will be collected, prepared, packaged, and stored for the authentication process. In the Sri Lankan setting, according to the national herbarium guidelines the plant material should be dried and pressed for a week. Then the identification and authentication of the plant material are performed by a scientific officer with the use of the voucher specimen. In Sri Lanka, the authentication of plants can be done by the National Herbarium in Royal Botanical Garden, Peradeniya, and Bandaranaike Memorial Ayurveda Research Centre in Nawinna, Maharagama [27]. Some literature reveals that the authentication process is also done by a senior professor or Botanist/ Taxonomist [28,29,30].

5. PRE-TREATMENT PROCESSES OF PLANT MATERIALS

Several processes are involved in the pre-extraction preparation of the plant material, and it is very important to preserve bioactive components inside the plant extract before extraction. The initial step is the pre-washing of the sample collected, and it is very important when using organs of plants like roots, rhizomes, tubers, etc. [31]. The extraction process can be done using both fresh and dried samples. However, in many instances dried samples are preferred because it is difficult to maintain the freshness of the sample and fresh samples are delicate and are prone to deteriorate more quickly. It is also very cumbersome to design the experimental setting within a few hours of the sample collection [18,32].

Fresh vs dried samples used for extraction:

1. A study conducted in Nigeria revealed that extracts produced by raw and dried leaves of *Carica papaya* show different antimicrobial potentials. Dried leaf extract shows significant antimicrobial activity against both Gram-positive and Gram-negative bacteria while fresh leaf extract shows significant antimicrobial activity against only Gram-negative bacteria [33].
2. A study conducted in Poland revealed the total phenolic count, scavenging activity, and ferric-reducing antioxidant power (FRAP) of dried and fresh extracts of aerial parts of *Coriandrum sativum* L., *Levisticum officinale* Koch., and leaves of *Plectranthus amboinicus*. Results revealed that dried extracts of the herbs exhibited higher total phenolic content and greater antioxidant activity compared with the fresh extracts [34].
3. A study was conducted in South Africa to reveal the phytochemical content and antioxidant properties of dried and fresh extracts of rhizomes of *Tulbaghia violacea* Harv. Results revealed that fresh methanolic extract contains higher flavonoids, flavanols, phenolics, tannin, and proanthocyanidin concentrations and it also exhibited higher antioxidant activity compared with the dried extract [35].

After prewashing, excess water should be blotted off with a clean cloth otherwise fungi and other microorganisms can grow in the plant material during drying. Fresh samples are most often allowed to dry to obtain a constant weight. There are several methods used in drying plant materials for the preparation of extracts namely, air-drying, freeze-drying, microwave-drying, and oven-drying.

Air-drying: The time needed for air drying can vary from several days to months or even beyond a year depending on the plant species and the plant material being dried (e.g., bark, leaves, seeds, and tubers). In this method, plant materials are allowed to air-dry in a dry, shaded, and well-ventilated place at ambient temperature. Air drying does not utilize high temperatures, thereby enabling the preservation of heat-labile components inside the plant. This technique is widely used in the extraction of medicinal plants in Sri Lanka, but it consumes a lot of time and is susceptible to contaminations in fluctuating temperature conditions, compared with other drying methods like freeze-drying, microwave-drying, and oven-drying [18,36].

Microwave-drying: Microwave-drying method utilizes microwaves as electromagnetic radiation. The microwaves can penetrate the plant material, promoting water heating inside the sample. This creates a significantly higher difference in the vapor pressure between the center of the plant and the surface. This pressure difference allows the moisture in the plant material to be removed very quickly. Therefore, microwave drying is considered to be faster, and more consistent. It uses less energy than traditional hot air drying. However, at the same time promote the degradation of phytoconstituents [18].

Oven-drying: This is another pre-extraction procedure that employees heat to remove moisture from plant materials. It is considered one of the simplest and quick drying techniques that can simultaneously preserve phytochemicals. Some studies have revealed that this method is a very effective technique to obtain optimum results [18].

E.g.: The maximum antioxidant activity in *Cosmos caudatus* extracts was obtained by oven-drying at 44.5°C for 4 hours while using 80% methanol, while a similar result was obtained by optimizing 80% methanol extracts at 44.12°C for 4.05 hours [37].

However, this drying technique is always not the best choice when there are heat-sensitive phytochemicals. Eg: drying *Orthosiphon stamineus* did not significantly affect its antioxidant activity, but it did have an impact on its bioactive phytochemicals, such as sinensetin and rosmarinic acid content, suggesting that the compounds are temperature-sensitive [38].

Freeze-drying: Another form of product drying is freeze-drying, and this method utilizes the principle of sublimation. This process is also known as lyophilization or molecular drying. Sublimation can be defined as the direct transfer of a substance from the solid phase to the gas phase without passing through the transitional liquid phase. In the ice sublimation process, product drying is accomplished by the removal of water from the frozen material [18,36,39].

Before extraction, and after the drying process, plant material is subjected to shredding and grinding. The particle size influences the degree of extraction; when the particle size is small, the surface area becomes high resulting in a greater area of exposure among the plant sample and respective solvents. This results in a higher extraction yield. Shredding produces coarse particles, whereas powdered specimens have more homogeneity levels and finer particles. This results in greater interaction of solvents with the plant material. Past research has demonstrated that when the particle size is less than 0.5 mm, it can give a higher yield of extraction of active components [40]. For the homogenization process of plant material, traditional mortar and pestle, and laboratory blenders are frequently used in routine laboratory setups [18].

6. SOLVENTS FOR EXTRACTION

It is important to select the most appropriate solvent (also known as the menstruum) during an extraction process. It is determined by the plant species, which plant part is extracted, the makeup of biologically active compounds, and the accessibility of the menstruum. To extract polar components, a polar solvent such as ethanol, methanol, or water can be used. Non-polar components should be extracted using non-polar menstruum (e.g., dichloromethane and hexane) [15,17,41]. Usually, two solvents that are capable of being mixed are used during liquid-liquid extraction (e.g.: water-dichloromethane, water-ether, and water-hexane). Water is involved in all combinations because of its high polarity and the possibility of being mixed with organic solvents. It is important that the compound which needs to be extracted should dissolve in the organic solvent but not in the water for a successful separation [42]. Furthermore, menstruum is classified according to the polarity, with n-hexane having the lowest polarity while water has the highest polarity of them all [15,17,41].

Below are some different extraction solvents listed in increasing polarity order [15,43].

Table 2. Polarities of different extraction solvents

Solvents	Polarity
n-Hexane	0.009
Petroleum ether	0.117
Diethyl ether	0.117
Ethyl acetate	0.228
Chloroform	0.259
Dichloromethane	0.309
Acetone	0.355
n-Butanol	0.586
Ethanol	0.654
Methanol	0.762
Water	1.000

7. PROPERTIES OF SOLVENTS USED IN THE EXTRACTION

Alcohol: This is a polar substance that can be mixed with water and is capable of extracting polar constituents from plant materials [43,44]. Self-preservation of the plant extract in concentrations above 20%, non-toxic properties at low concentrations, and only a minimal amount of heat required to concentrate can be listed as the advantages of using alcohol as a solvent. There are also some disadvantages such as being flammable, evaporative, and failing to dissolve waxy substances, gums, or fats [43,44].

Chloroform: This solvent is nonpolar and used in the extraction of compounds like flavonoids, fats, oils, and terpenoids. Being colorless, odorless, soluble in alcohols, and easily absorbed and metabolized by the body are the advantages of chloroform. Disadvantages are, it is both sedative and carcinogenic [17,44,45].

Dichloromethane: This is a solvent that is widely involved in the extraction of bioactive compounds from medicinal plants because it can extract both nonpolar as well as polar compounds [46]. Dichloromethane is widely used in the extraction of caffeine as it is found to be more soluble in dichloromethane (140 mg/ml) than in water (22 mg/ml) [47]. This solvent has many advantages. Some of them are, it is not miscible in water and therefore it has the capacity to dissolve a scope of organic compounds. When these properties are combined with its volatility, it makes dichloromethane a very effective solvent in many industries. Along with these advantages, there are many disadvantages too. Very high volatility and stability, ability to cause CO poisoning, neurotoxicity, and carcinogenicity are some of them [48].

Ether: Can be identified as a solvent that is nonpolar and capable of being utilized to extract components such as fatty acids, terpenoids, alkaloids, and coumarins. Ether has some benefits, including the ability to mix with water, having a low boiling point, and the absence of taste. It is also a substance that is extremely stable and is not influenced by metals, acids, or bases. Its disadvantages include its high flammability and volatility [17,44,45].

Hexane: Considering its ease of recovery, low boiling point (63–69 °C), and exceptional dissolving properties, hexane has been frequently utilized to extract oil from oil seeds [17]. Hexane gets released into the environment over the procedure of extraction and recovery steps, where it can interact with pollutants to produce ozone and photo-chemicals [49]. Hexane is found to be soluble in neural lipids which can have a significant impact on the nervous system when inhaled by individuals [15]. Thus, to replace n-hexane without compromising oil yield, researchers are looking for alternatives that are safer for the environment, human health, and safety. Green solvents have thus offered an effective replacement for the extraction of oil.

Green Solvent (ionic liquid): This is a one-of-a-kind menstruum that has a high polarity and is steady against high temperatures (e.g.: 3,000℃). It has the ability to mix with water and other polar solvents, making it ideal for polar compound extraction. The benefits of green solvents can be listed as follows, it is ideal for microwave-assisted extraction, non-flammable, have high polarity, and are useful for liquid-liquid extraction. The disadvantages are, not ideal to create tinctures [50].

Water: It can be nominated as the solvent with the highest polarity among other solvents and is utilized to extract diverse polar compounds. The advantages of using water as a solvent are, it dissolves many different substances, is inexpensive, has nonflammability, is nontoxic, and has high polarity. Disadvantages are, it encourages bacterial and fungal growth, could result in hydrolysis, and needs a lot of heat to concentrate the extract [43,44].

8. FACTORS THAT NEED TO BE CONSIDERED IN THE SELECTION OF SOLVENTS FOR EXTRACTION

Numerous factors have to be considered while selecting a solvent for extraction.

Boiling Temperature: The boiling temperature of the solvent should be minimum to prevent degradation by heat.

The cost should be as affordable as possible.

Safety: Non-toxicity and non-flammability are the preferred characteristics of a perfect extraction solvent.

Selectivity: The solvent used for extraction should have a high selectivity, which is the capability to separate biologically active compounds from inert constituents in plant material.

Reactivity: The reactivity of the solvent has to be very low so it doesn't react with the extract.

Recovery: It should be possible to separate the solvent from the extract quickly.

Viscosity: It's ideal for a solvent to have a low viscosity to facilitate easy penetration [17,43,51].

9. COMMONLY USED EXTRACTION METHODS

9.1 Maceration

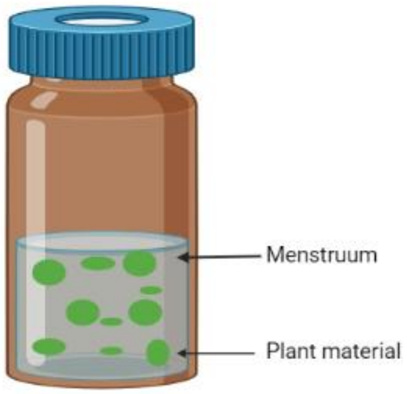

Fig. 2. Extraction by maceration method, adapted from [52]

In this routinely used extraction method, an airtight vessel (preferably amber in color) is used. Then the vessel is packed with plant material such as barks, flowers, leaves, roots, seeds, and tubers which are coarsely powdered. Menstruum, which is the solvent used in extraction, is subsequently poured over the plant material until being fully covered. Then the vessel is tightly sealed and stored for 3-7 days with continuous agitation regularly. The days kept for maceration depend on the type of plant material being used and the method of evaporation chosen. After the extraction process, the mixture is filtered using filter paper or a muslin cloth before evaporation. Then, the micelle can be evaporated with the help of an oven or a water bath to isolate it from the extraction solvent. This method is considered very suitable for heat-stable plant materials. Based on the requirement, the sample-to-solvent ratio can vary from 1:4, 1:5, and 1:10 [16,17,18,42,49,53].

9.2 Infusion

This process is more similar to maceration. Here, finely powdered plant material is used for the extraction. Hot or cold menstruum is then added over the finely powdered plant material and the mixture is stored for a short time. Infusion is considered more appropriate for the extraction of readily soluble biologically active compounds. Also, it is found to be a very suitable technique to obtain fresh extracts before use. Here, the sample-to-solvent ratio is generally 1:4 or 1:16 which depends on the requirement [16,17,18,42].

9.3 Digestion

Digestion is a type of maceration where low heat is applied during the extraction procedure. This slightly warm temperature does not change the plant material's active elements. This results in more effective usage of the menstruum. In this method, we introduced the plant parts to be extracted into a container where the pre-heated menstruum is present. The sample-solvent mixture is kept for 30 minutes to 24 hours with intermittent shaking. A temperature between 35-40℃ is used commonly. Sometimes, this can go to a maximum of 50℃. This extraction method is very useful to extract poorly soluble components present in tougher parts of plants [45].

9.4 Decoction

This is a continuous hot extraction technique where the powdered crude plant material is boiled with a specific amount of water for a period of specific time (about 15 minutes). Heat is applied throughout the procedure to speed up the extraction. This method is ideal when extracting water-soluble and thermally stable compounds from plant materials. In this process, the most commonly used sample-to-solvent ratios are 1:4 or 1:16 [16,17,18,42].

9.5 Percolation

A percolator is a narrow cone-shaped container made of glass that has openings on both sides. The finely powdered plant material will be added to a clean vessel followed by adding a higher quantity of menstruum to soak the powder. This mixture is then stored for a certain time (4 hours). This mixture is then introduced to the percolator (during this procedure, the lower end has to be closed). Now, the system will be kept standing for 24 hours. After 24 hours, the solvent used for extraction is added from the upper end to flow down until the plant material is saturated with the solvent. The stop cork is opened at the lower end while adding menstruum from the top. The liquid is then collected from the bottom. This procedure is carried out with the help of gravitational force which helps to push down the solvent through the plant material. The pouring of the menstruum should end when the amount of menstruum reaches around 75% of the total preparation. Finally, the collected micelle will be filtered and concentrated [17,18,42] (Figs. 3, 4).

9.6 Soxhlet Extraction

The Soxhlet extraction method is a liquid-solid extraction method. It is a very effective extraction technique in instances where the substances to be extracted have a restricted solubility in the menstruum, while having insoluble impurities. Continuous heat is applied in this procedure. Here, a glass instrument known as the Soxhlet extractor is utilized. This instrument comes with a solvent flask, a siphon tube, a condenser tube, and many other parts. The plant material which needs to be extracted is introduced to a part known as the thimble, which is a porous bag. First, the solvent flask should be filled with the menstruum. Then heat is applied from the bottom of the solvent flask. This results in the production of solvent vapor. This vapor will go up the distillation tube, into the main chamber, and up into the condenser where it will condense and drip down. As the solvent fills the main chamber, some of the desired compounds in the solid sample will be dissolved. Eventually, the chamber will be almost full of the solvent. When this happens, the siphon tube will empty the main chamber while transferring the solvent back to the solvent flask, promoting the process to start over again. With each subsequent extraction, more of the target components gets dissolved, leaving the insoluble contaminants in the thimble. This is how compounds of interest are removed from a sample (Fig. 5).

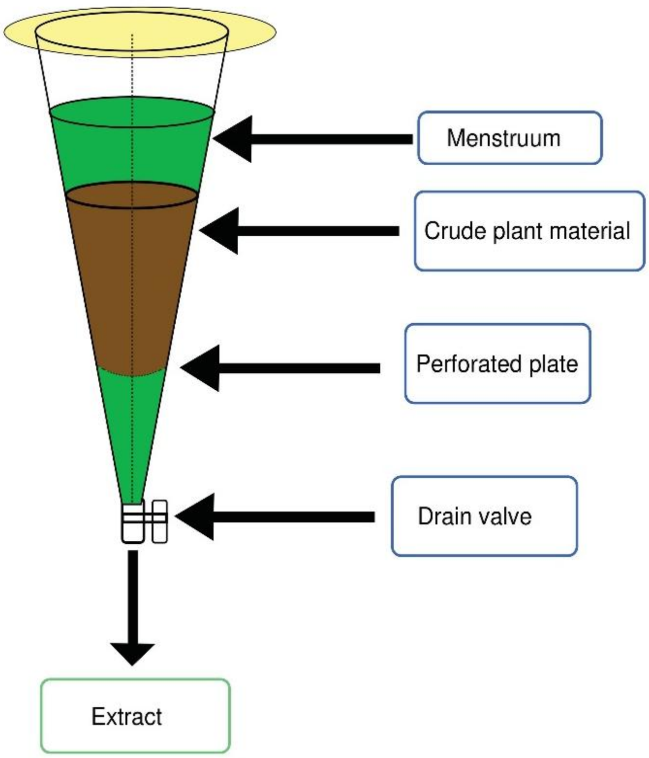

Fig. 3. A diagram of the percolation extraction, adapted from [54]

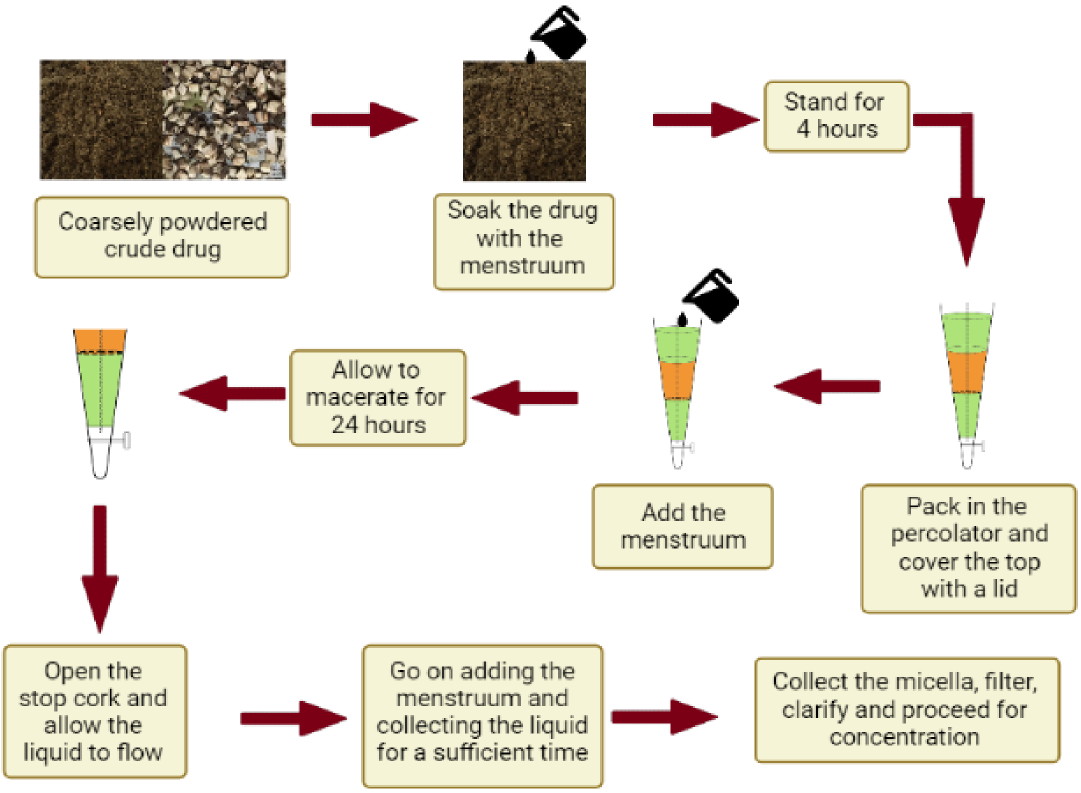

Fig. 4. The process of percolation, adapted from [54]

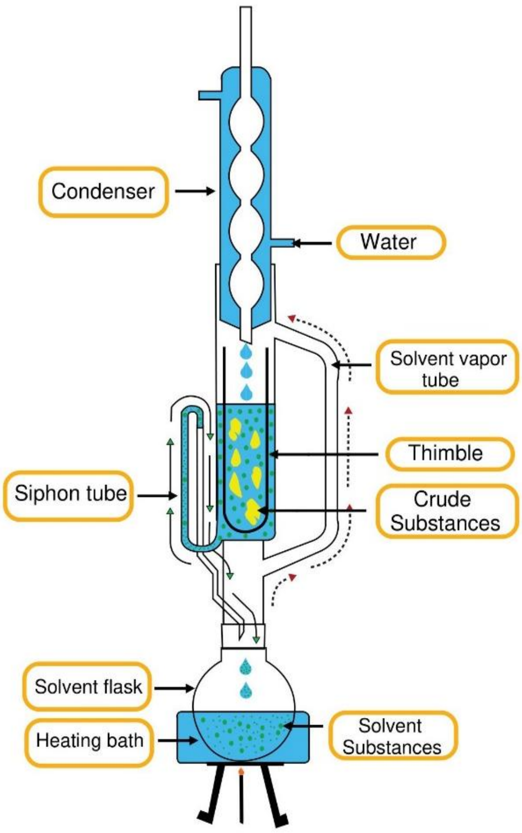

Fig. 5. A diagram of the Soxhlet extraction instrument, adapted from [55]

The advantages of this method are,

It can be utilized for the extraction of large numbers of drugs with low volumes of solvents. This is valid for plant material that can withstand high heat. It does not require filtration.

The disadvantages of this method are,

Requires a large amount of heat.

Impossibility of regular shaking and not being suitable for thermolabile materials [16,17,18,42, 49,56,57,58].

9.7 Microwave-Assisted Extraction

Microwave-assisted extraction is an advanced extraction technique used in the extraction of herbal medicines. It involves the mechanism of dipole rotation and ionic transfer to displace charged ions from the solvent and drug materials. This technique employs electromagnetic radiation having frequencies ranging from 300 MHz to 300 GHz and wavelengths ranging from 1 cm to 1 m. This approach uses microwave radiation to bombard a substance that absorbs electromagnetic energy and converts it into thermal energy. Due to the heat generated, it facilitates the solvent to penetrate the plant material (Fig. 6).

This method requires a minimum quantity of solvents and time for extraction. It also can give a higher output. However, being suitable only for flavonoids and phenolic compounds and the possibility of degrading tannins and anthocyanins due to the high heat can be some downsides of this method [16,41,49,50].

9.8 Sonication (Ultrasound-Assisted Extraction)

In this extraction technique, ultrasound frequencies are used that are ranging from 20 KHz to 2000 KHz. They can disturb the plant cell walls and thereby increase the surface area of plant material to facilitate the penetration of solvents. Subsequently, this facilitates the release of biologically active compounds (Fig. 7).

The advantages of this method are,

This method can be used for small sample amounts. It gives us a high yield while reducing extraction time and the quantity of menstruum.

Even though this method is useful in some incidents such as the extraction of rauwolfia roots, involving this process on a large scale can be a disadvantage because of the high cost. Also, the high ultrasound energy can sometimes degrade the phytochemicals due to the production of free radicals [17,18,41,45].

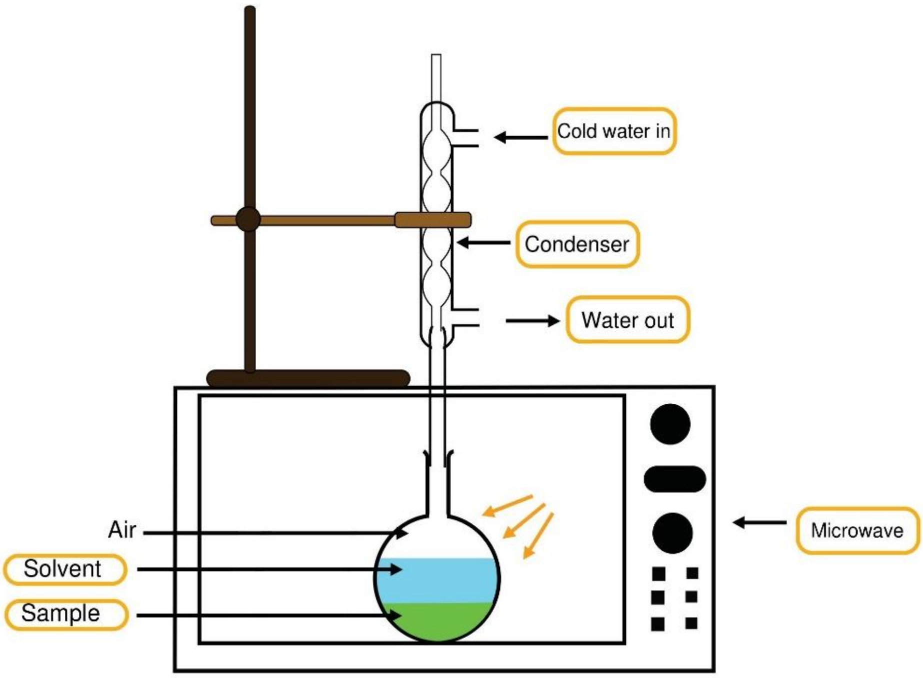

Fig. 6. A diagram of Microwave-assisted extraction, adapted from [59]

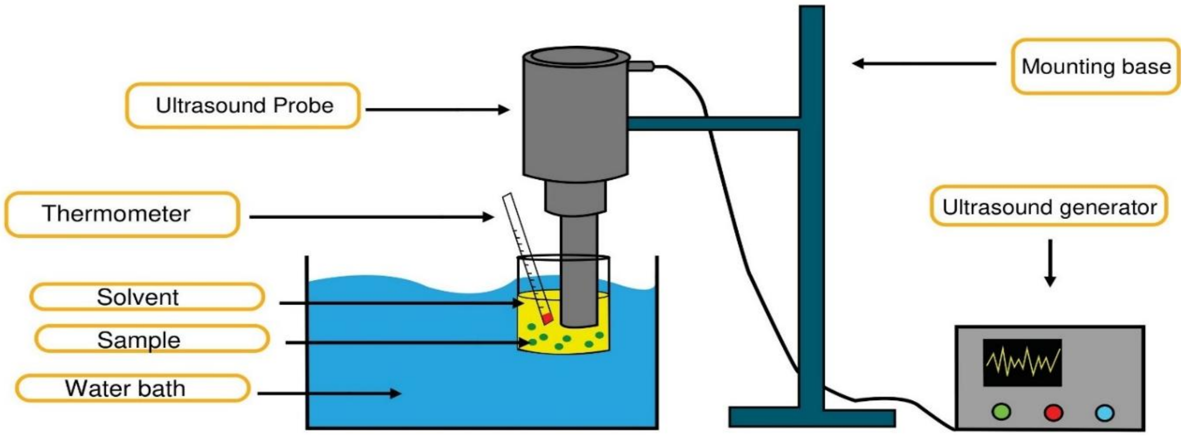

Fig. 7. A diagram of extraction by sonication, adapted from [60]

9.9 Aqueous-Alcoholic Extraction by Fermentation

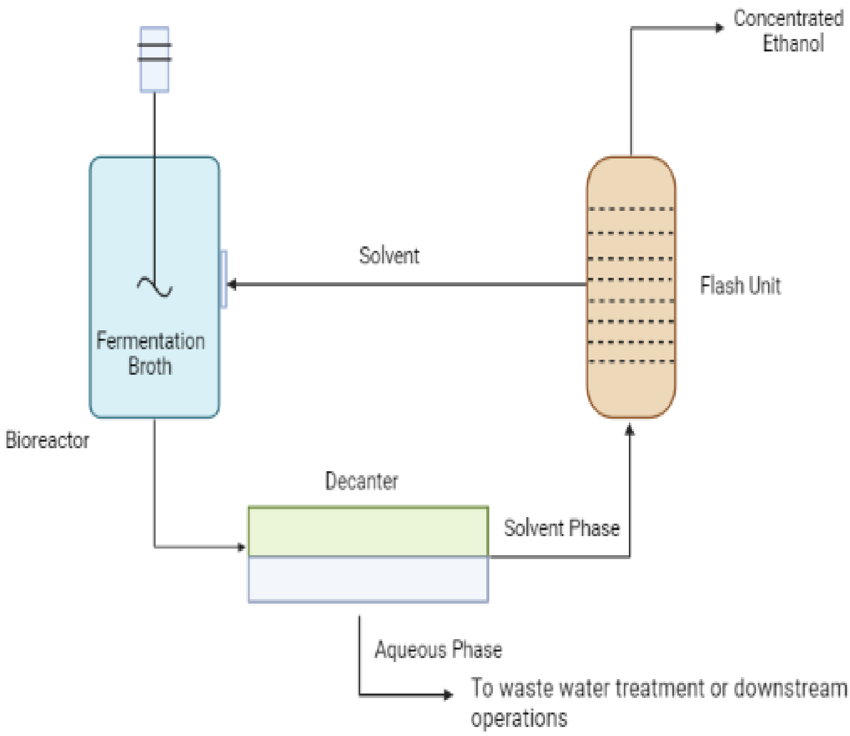

Fig. 8. A diagram of extraction by aqueous-alcoholic fermentation, adapted from [61]

To extract the active ingredients, some Ayurveda medicinal preparations (such as ãsava and arista) use fermentation as a process. For the extraction of active components from the plant, the crude drug must be soaked for a designated amount of time, either as a powder or a decoction (kasaya). During this time the drug mixture will undergo fermentation and produce alcohol. This action will enable the active compounds present in our plant to be extracted. However, this extraction technique is not yet standardized in Ayurveda, but when considering the extraordinary level of improvement in fermentation technology, it can be used to produce herbal drug extracts [18].

9.10 Counter-Current Extraction

In this method, to create a fine slurry, the wet material is ground using a toothed disc disintegrator. The material to be extracted is allowed to move in a single direction inside a

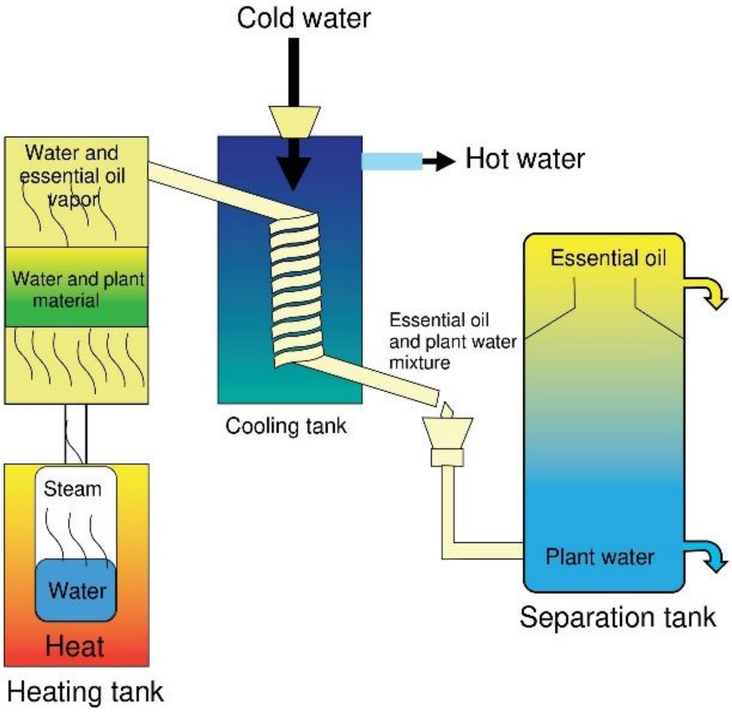

Fig. 9. A diagram of counter-current extraction, adapted from [62]

cylindrical extractor. The plant material will contact the menstruum. The more the plant material moves, the extracts become more concentrated. When the solvents, material quantities, and their rate of flowing is optimized, full extraction is attainable. This procedure is considered very quick and efficient, raising fewer adverse effects from high temperatures. Then, the extract which is sufficiently concentrated exits the extractor at one end while the marc, which is now free of solvents, exits at the other end [14].

This method comes with many advantages.

When compared with methods like maceration, decoction, and percolation, this method requires less volume of solvent. As the extraction happens at room temperature, the thermolabile compounds will be preserved by heat. Again, as the grinding happens in wet conditions, the thermal energy generated during grinding will be transferred to water thereby, protecting the thermolabile compounds [14].

9.11 Superficial Fluid Extraction

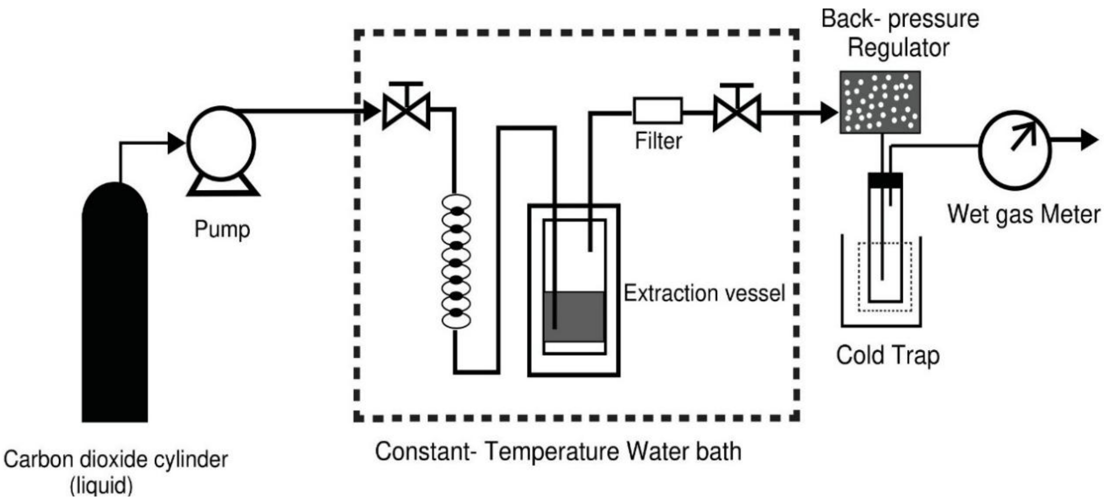

Fig. 10. Schematic diagram of extraction by superficial fluid, adapted from [63]

Superficial fluid extraction (SFE) is an advanced extraction technique that utilizes supercritical fluid as the menstruum. Even though it can also be done with liquids, extraction is typically done from a solid matrix. SFE is utilized to remove unwanted compounds or collect wanted compounds from a sample mixture. Carbon dioxide (CO_2) is the most commonly used supercritical fluid but sometimes, alcohols like methanol and ethanol can also be used. This procedure of extraction, in contrast to other methods, leaves no solvent residue behind [64].

This method comes with great advantages.

SFE can be named as a replacement for liquid extraction where it involves hexane or dichloromethane as the solvent. The extract and matrix will always contain a small quantity of residual solvent; therefore, their use will always have some negative effects on the environment. Contrarily, CO_2 is simple to remove by simply lowering the pressure, virtually undetectable, and environmentally safe. Therefore, this method helps in the improvement of the environment with a reduced number of contaminations.

Also, this method gives pure products with high speed and recovery [64].

9.12 Reflux Extraction Method

The reflux method is a popular extraction method in Sri Lanka, and studies show that it can increase extraction yield whilst lowering the amount of solvent utilized. Reflux extracting refers to a solid-liquid extraction method at a uniform temperature with replicated solvent evaporation as well as condensation for a set period of time without loss of solvent. The technique is commonly utilized in the herbal industry since it is effective, simple to operate, and affordable [66,67].

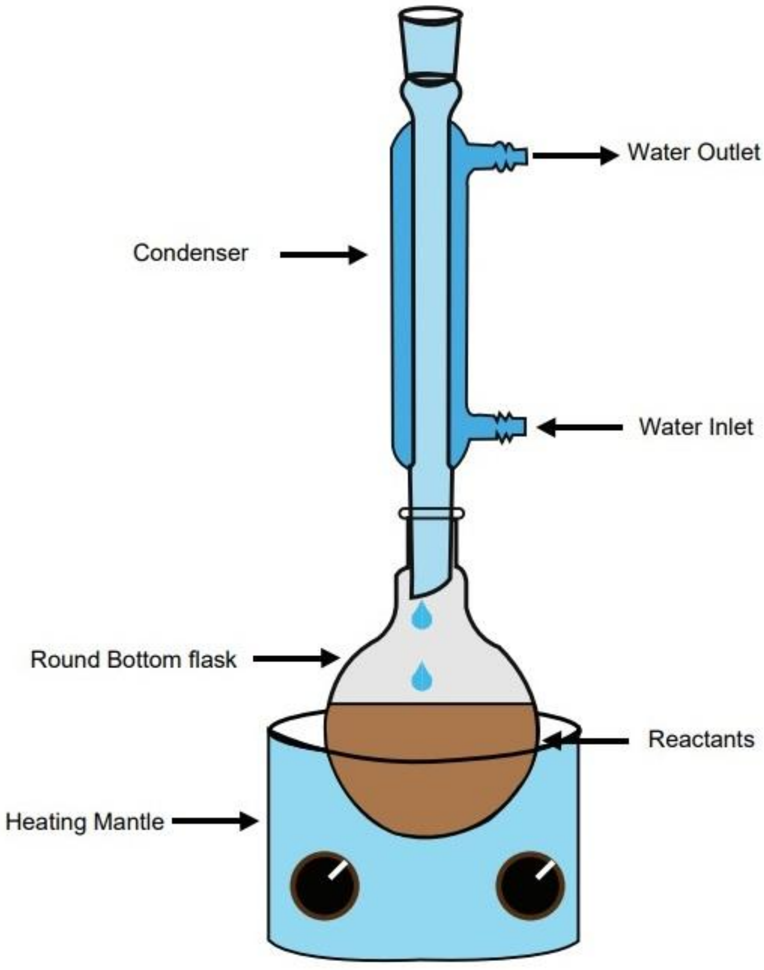

Fig. 11. A schematic diagram of the heat reflux extraction method, adapted from [65]

10. EVAPORATION OF PLANT EXTRACTS

After extracting a plant material, it has to be evaporated to obtain a dry extract. This can help to maintain the viability of the constituents in the extract.

Several evaporation methods are used such as,

Evaporation by air at room temperature underneath a hood for twenty-four hours in the light.

Evaporation using a rotary vacuum evaporator under reduced pressure. (The temperature and time can change according to the type of solution).

Evaporating inside an oven in the dark (The temperature and time can change according to the type of solution) [68,69].

11. THE ROTARY VACUUM EVAPORATOR

This instrument was invented by Lyman C. Craig. It is a device used in chemical laboratories to effectively evaporate solvents from samples under low pressure [71]. The rotary vacuum evaporator is a lab instrument frequently used for sample extraction, distillation, and purification. This instrument is particularly helpful for laboratory work and research in a variety of disciplines including biotechnology, biology, chemistry, and pharmaceuticals. This instrument comes with several parts that are equally important while having different functions. The condenser, rotating flask (solvent flask), collecting flask, and water bath can be identified as some of the important parts.

The solvent flask, sometimes referred to as a rotating flask, is used to hold the material that needs to be extracted or purified (for instance, crude plant extract). As it rotates during the procedure, this flask is known as a rotating flask. By removing air, the vacuum pump produces a vacuum inside the flask. As a result, there is a decrease in pressure inside the rotating flask. The boiling point of solvents is significantly reduced due to the lowering of the pressure inside the flask, which enhances the evaporation and separation process at low temperatures in just a short amount of time without affecting the sample's composition. The temperature of the water bath and the speed of rotation is set before starting the process. During the procedure, the solvent (menstruum) starts to evaporate while leaving the sample in the flask. The condenser is used to cool and collect the evaporated samples after they have been passed through.

Below are listed the two well-known rotating evaporators.

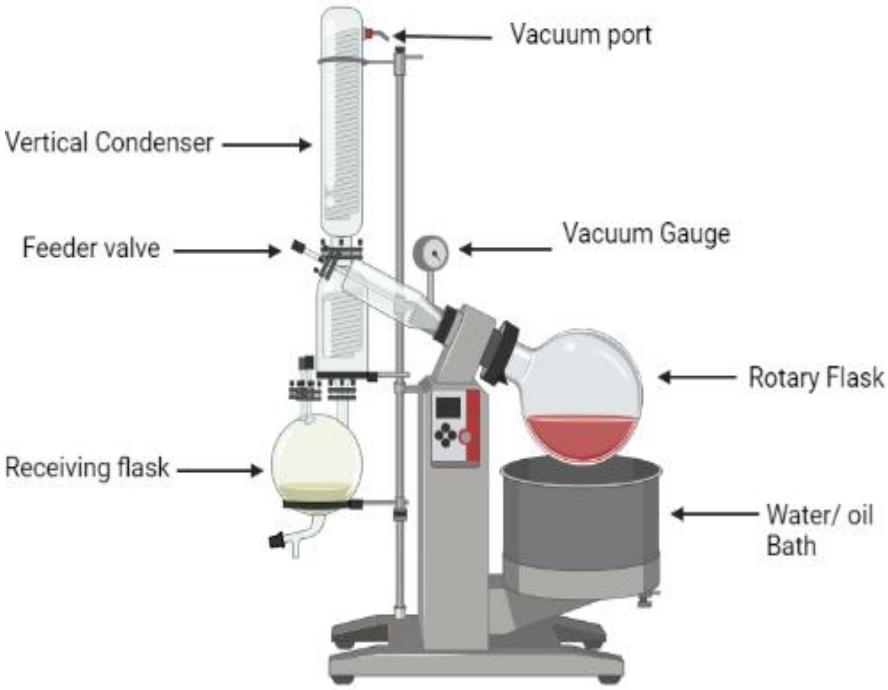

Fig. 12. A labeled diagram of the rotary vacuum evaporator, adapted from [70]

Vertical rotary evaporators: These are frequently utilized in research or chemistry labs. These are primarily utilized for samples with small volumes. The recovery of solvents and the extraction of chemicals (natural products) from the crude extract are its two main purposes.

Horizontal rotary evaporators: These are typically used in big industries like those that produce chemicals, medicines, food, etc. These are primarily utilized for massive amounts of samples.

The advantages of using this instrument are listed below.

Distillation is carried out at a low temperature because evaporation happens under reduced pressure. It is therefore very helpful for compounds that are sensitive to heat.

Since the rate of evaporation is quick, separation can be completed quickly [70].

All solvents do not have a common boiling point. Therefore, the pressure inside the solvent flask should be changed accordingly. The Buchi 20/40/60 rule for rotary evaporators describes how the temperature and vacuum pressure inside the rotary vacuum evaporator should be adjusted according to the type of solvent. According to this rule,

The water bath temperature should be set to 60^0C (It should not exceed this temperature). The temperature of the cooling water should be below 20^0C.

The required vacuum for a solvent boiling point of 40^0C, 30^0C, and 50^0C can be adjusted using Table 3 below [72].

Table 3. This table gives information on how to change the vacuum pressure inside the rotary vacuum evaporator at different boiling points [72]

Solvent	Vacuum [mbar] for bp of 30°C	Vacuum [mbar] for bp of 40°C	Vacuum [mbar] for bp of 50°C
Acetic acid	26	44	72
Acetone	370	556	815
Acetonitrile	153	226	315
n-Amyl alcohol, n-pentanol	6	11	20
n-Butanol	14	25	44
tert-Butanol	78	130	231
Chlorobenzene	22	36	56
Chloroform	332	474	665
Cyclohexane	154	235	347
Dichloromethane	699	Atm. press.	Atm. press.
Diethyl Ether	838	Atm. press.	Atm. press.
Di isopropyl Ether	251	375	545
Dioxane	68	107	165
Dimethylformamide (DMF)	6	11	17
Ethanol	97	175	276
Ethyl Acetate	153	240	366
Heptane	77	120	183
Hexane	264	335	525
Isopropyl alcohol	78	137	231
Isoamyl alcohol	9	14	29
Methanol	218	337	607
Pentane	834	Atm. press.	Atm. press.
n-Propanol	37	67	115
Pentachloro ethane	8	13	21
Tetrachloromethane	179	271	398
Tetrahydrofuran (THF)	234	357	539
Toluene	48	77	118
Trichloroethylene	119	183	275
Water	42	72	120
Xylene	15	25	40

12. STORAGE CONDITIONS FOR PLANT EXTRACTS

There are many different ideas on how to store plant extracts. Ideal storage of plant extracts is very important to preserve the enzyme activities, biological properties, phytochemical constituents, and various other plant characteristics. Proper storage will prevent microbial growth and preserve the plant extracts. Several studies have investigated the importance of ideal storage conditions having a great effect on a plant's biological activities [73,74].

Any kind of extract should be dried using a rotary evaporator or cephalization (if aqueous extraction is used). After that, you can keep the extracts in a refrigerator for more than two years by sealing the container tightly [75,76].

"The type of active compounds you're looking for will have a big impact on how long you should store your medicinal plant extract. Some substances, like alkaloids, are stable, but others are not. I believe that inside plants, particularly those that have been freshly lyophilized or freeze-dried using liquid nitrogen, is the best environment for storing natural compounds. Additionally, I advise keeping the dried plant sample in a cool, dark environment. Compared to storing the extract, it is preferable" says Majid Azizi, The Academy of Sciences of the Islamic Republic of Iran [77].

"Extract of the plant can be kept at 4°C for as long as it is in dry condition (i.e., the sample has been completely dried using a rotavapor or freeze dryer after extraction). To prevent degradation, it is best to keep your extract or solution at -20°C or lower. When performing a bioassay, make a fresh extract solution" says Narayan D Chaurasiy, Southern Research Institute [78].

"It does rely heavily on the active ingredients, but the solvent is also important. The best course of action is to get your analysis as soon as you can, especially if you're thinking about getting antioxidant tests. The activity is not always maintained when stored at 4℃. It would be best to store it at -20℃, at the very least, if there are essential oil compounds present. For instance, matricin concentration can remain constant at -20°C for more than 6 months, but at 4–8°C, it can drop to half or even less of its initial value. To have a good activity, you must take the chemical compound's stability into account" says Oana Cioanca, Universitatea de Medicina si Farmacie Grigore T. Popa Iasi, Faculty of Pharmacy [79].

13. CONCLUSION

Phytoconstituents present in medicinal plants are responsible for exhibiting various biological properties. Phytochemical screening is important in finding those responsible phytoconstituents. The reliability of phytochemical screening results is affected by the identification and authentication of the plant, pre-extraction procedures, menstruum utilized, method of extraction, and storage conditions. The method of extraction used varies according to the type of plant material, the drying method utilized, and the financial support for the study. Storage conditions are very important in phytochemical screening since the sample obtained can deteriorate with time if they are not kept in optimal conditions.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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