

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Extraction of Bioactive Compounds from Medicinal Plants and Herbs

Fongang Fotsing Yannick Stéphane, Bankeu Kezetas Jean Jules, Gaber El-Saber Batiha, Iftikhar Ali and Lenta Ndjakou Bruno

Abstract

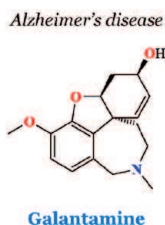
Human beings have relied on herbs and medicinal plants as sources of food and remedy from time immemorial. Bioactive compounds from plants are currently the subject of much research interest, but their extraction as part of phytochemical and/or biological investigations present specific challenges. Herbalists or scientists have developed many protocols of extraction of bioactive ingredients to ensure the effectiveness and the efficacy of crude drugs that were used to get relief from sickness. With the advent of new leads from plants such as morphine, quinine, taxol, artemisinin, and alkaloids from *Voacanga* species, a lot of attention is paid to the mode of extraction of active phytochemicals to limit the cost linked to the synthesis and isolation. Thus, the extraction of active compounds from plants needs appropriate extraction methods and techniques that provide bioactive ingredients-rich extracts and fractions. The extraction procedures, therefore, play a critical role in the yield, the nature of phytochemical content, etc. This chapter aims to present, describe, and compare extraction procedures of bioactive compounds from herbs and medicinal plants.

Keywords: Herbs, Medicinal plants, Plants extracts, Extraction, Bioactive ingredients, Phytoconstituents, Secondary metabolites, Phytochemicals

1. Introduction

With the increasing demand for herbal medicinal products, nutraceuticals, and natural products for primary healthcare worldwide, medicinal plant extract manufacturers and essential oil producers have started using the most appropriate extraction techniques. Different methods are used to produce extracts and essential oil of defined quality with the least variations.

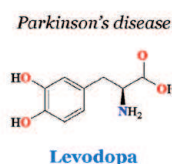
Herbs and medicinal plants have been used for centuries as source of a wide variety of biologically active compounds. The plant crude material or its pure compounds are extensively used to treat diverse ailments by generations of indigenous practitioners [1, 2]. They are currently the subject of much research interest, but their extraction as part of phytochemical and biological investigations presents specific challenges that must be addressed throughout the solvent extraction [3]. Natural products provide unlimited opportunities for new drug discovery because of the unmatched availability of chemical diversity [4]. Thanks to two drugs



Galantamine is an alkaloid in the Amaryllidaceae family widely used to treat Alzheimer's Disease (AD). Since its approval for clinical use in 2001, its effectiveness has been attested in numerous clinical trials. Galantamine is an effective well-tolerated symptomatic treatment which improves cognition, function and activities of daily living in the short term (up to 6 months) in patients with mild to moderate AD [9,10].



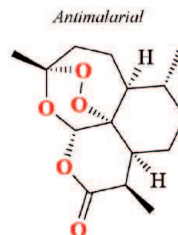
Vicia faba L.



Parkinson's disease (PD) is a common neurodegenerative disease typified by a movement disorder consisting of bradykinesia, rest tremor, rigidity, and postural instability [12]. Levodopa, the precursor of dopamine, was first developed to treat PD in the 1960s and continues to be the most-effective therapeutic agent for PD in 2020 [D]. Treatment of PD involves pharmacologic approaches (typically with levodopa preparations prescribed with or without other medications) and non-pharmacologic approaches (such as exercise and physical, occupational, and speech therapies) [12].



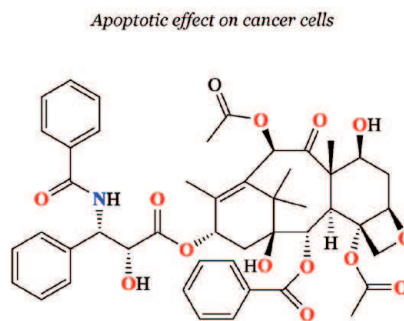
Artemisia annua



The 2015 Nobel Prize in Physiology or Medicine was awarded to Professor Youyou Tu for her critical contributions to the discovery of artemisinin. Artemisinin has saved millions of lives and represents one of the significant contributions of China to global health [E]. Artemisinin was isolated from the leaves of a Chinese medicinal plant, *Artemisia annua*. The use of artemisinin-based combination therapy (ACT) with a competent partner drug and having multiple ACT as first-line treatment choice sustained high levels of effectiveness [14].



Taxus brevifolia
(pacific yew or Western yew)



Paclitaxel, sold under the brand name Taxol (isolated from the bark of *Taxus brevifolia*) among others, is a chemotherapy medication used to treat a number of types of cancer. This includes ovarian cancer, esophageal cancer, breast cancer, lung cancer, Kaposi sarcoma, cervical cancer, and pancreatic cancer. It is given by injection into a vein. Taxol is classified as a "plant alkaloid," a "taxane" and an "antimicrotubule agent."

Figure 1.
Chemical structures of a few important bioactive compounds isolated from plants.

derived from alkaloids of Madagascar's rosy periwinkle (*Catharanthus roseus*), the likelihood of remission for a child who has leukemia increased by 85 percent between 1960 and 1997 [5, 6]. New compounds, such as one recently discovered in a plant in Madagascar, are likely to provide novel antibiotics and help to curb the epidemic of antibiotic-resistant diseases [7].

Natural products are currently of considerable significance due to their unique attributes as a significant source of therapeutic phytochemicals and their efficacy, safety, and minimal side effects [2, 8]. Bioactive compounds in plants include alkaloids, terpenoids, coumarins, flavonoids, nitrogen-containing compounds, organo-sulfur compounds, phenolics, etc. A wide spectrum of bioactivities is exhibited by these compounds such as anti-inflammatory, immunostimulatory, anticancer, antioxidant, antimicrobial, etc.

Research on medicinal plants is particularly important as that on conventional drugs due to the beneficial phytochemicals from plants and the shift towards natural products in pharmaceutical and cosmeceutical industries. Chemical structures of a few essential bioactive compounds isolated from plants are presented in **Figure 1** [9–14].

Extraction of the bioactive constituents from plants has always been challenging for researchers [15]. As the target compounds may be non-polar to polar and thermally labile, the suitability of the extraction methods must be considered. The study on medicinal plants starts with extraction procedures that play a critical role in the extraction outcomes and the consequent assays.

Hence, this chapter aims to provide an overview of the process of plant extraction, describe, and compare extraction methods based on their principle, the effect of solvent on extraction procedures, strength, limitations, and economic feasibility, with their advantages and disadvantages. This chapter shall also emphasize the common problems encountered and methods for reducing or eliminating these problems. Since millions of natural products derived from plants are known, only selected groups and compounds are presented.

2. Medicinal plants and herbs

The term “medicinal” as applied to a plant indicates that it contains a substance or substances which modulate beneficially the physiology of sick mammals, and man has used it for healthful purpose [16]. Medicinal plants were described by Farnsworth and Soejarto as: “all higher plants with medicinal effects that relate to health, or which are proven as drugs by Western standards, or which contain constituents that are defined as hits.” [17].

Medicinal plant (MP) refers to any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors of the synthesis of valuable drugs. A whole plant or plant parts may be medicinally active [18–22]. Medicinal plants (MPs) are becoming very important due to their uses mainly as a source of therapeutic compounds that may lead to novel drugs. MPs are plants that are used for healthcare purposes in both allopathic and traditional medicine systems. MPs cover various species used including condiments, food aromatic and cosmetics [23–26].

Herbs may be defined as the dried leaves of aromatic plants used to impart flavor and odor to foods with, sometimes, the addition of color. The leaves are commonly traded separately from the plant stems and leaf stalks [27].

Herbal medicine is referred to as medicinal preparations comprising active ingredients obtained from the herbal plant. The product can be made from the whole plant or any part. Preparations from by-product herbal plants such as oil, gum, and other secretions are also considered herbal medicines [18, 19, 22].

3. Primary and secondary metabolites

Metabolites are intermediate processes in nature and are small molecules. Primary metabolites are known vital or essential compounds and are directly involved in the average growth, development, and reproduction of plants [28]. Primary metabolites include cell constituents (e.g. carbohydrates, polysaccharides, amino acids, sugars, proteins, and lipids) and fermentation products (ethanol, acetic acid, citric acid, and lactic acid), and are mainly used during their growth and development stages [19, 22, 29, 30].

Secondary metabolites are not directly involved in those processes and usually have a function but are not that important for the organism (e.g. phenolic, steroids, lignans, etc.). They are found only in specific organisms or groups of organisms, and express of the individuality of species [19, 30, 31]. They are not necessarily produced under all conditions, and most often, the function of these compounds and their benefit to the organism is not yet known. Some are undoubtedly made for readily appreciated reasons, e.g., as toxic material providing defense against predators, as volatile attractants towards the same or other species, but it is logical to assume that all do not play some vital role for the well-being of the producer [27, 30]. Secondary metabolites are produced after the growing stage and are used to increase the ability of plants to survive and overcome their local challenges. Bioactive compounds are classified as terpenoids, alkaloids, nitrogen-containing compounds, organosulfur compounds, and phenolic compounds [29].

Bioactive compounds are reported to possess diverse bioactivities such as antioxidant, anticancer, antimalarial, antiulcer, antimicrobial, anti-inflammatory activity [32–36].

4. Bioactive compounds

The definition of bioactive compounds remained ambiguous and unclear for a long time. Very few references describe the term “bioactive”. It is composed of two words *bio-* and *-active*. In etymology *bio-* is from the Greek ($\beta\iota\omicron$ -) “bios” that means life while *-active* is derived from the Latin word “activus” that refers to dynamic, full of energy, with energy, or involved in activity [37–39]. The term “bioactive” is an alternative term for “biologically active” [40]. Hence, a bioactive compound is simply a substance with biological activity [41, 42].

A plant extract is a substance or an active substance with desirable properties removed from the tissues of a plant, frequently by treating it with a solvent, to be used for a particular purpose. The term “bioactive compounds” is generally referred to as biologically significant chemicals but not established as essential nutrients [43]. Bioactive compounds are essential (e.g., vitamins) and non-essential (e.g., polyphenols, alkaloids, etc.) compounds that occur in nature, are part of the food chain, and can affect human health [44]. They are derived from various natural sources such as plants, animals, microorganisms (e.g., fungi) and marine organisms (e.g., lichens) [2]. The amount of bioactive natural products in natural sources is always fairly low [45, 46]. Plant active compounds are usually contained inside plant matrixes. Active compounds are synthesized in small quantities and different concentrations in all plant organs or parts such as leaves, roots, barks, tubers, woods, gums or oleoresin exudations, fruits, figs, flowers, rhizomes, berries, twigs, as well as the whole plant. Further processes may be required after extraction to purify or isolate the desired compounds.

5. Fresh or dried plant materials

Fresh and dried samples are used and are reported in the literature in the preparation of medicinal remedies. Ideally, fresh plant tissues should be used for phytochemical analysis, and the material should be plunged into boiling alcohol within minutes of its collection. Alternatively, plants may be dried before extraction [47]. In most reported cases, dried materials are preferred considering their long conservation time compared to fresh samples. Furthermore, fresh specimens are fragile and tend to deteriorate faster than dried ones. Phytoconstituents such as Essential Oils (EOs) are found in fewer dried samples than in fresh samples. In case of fresh plant material extraction using organic solvents such as methanol or ethanol, is required to deactivate enzymes present in the plant sample. The extractive might contain a substantial portion of water; hence it can be partitioned using specific immiscible organic solvents [3].

6. Drying procedures

Drying is the most common method to preserve the plant material from enzymatic degradation, such as hydrolysis of glucoside, etc. It should be dried as quickly as possible in the open room under primitive conditions at ambient room temperature with air circulation around the plant material to avoid heat and moisture [47]. However, they placed in shallow trays with good atmospheric air-up dryness either in the sunshine or in shade depending on nature of the indicated or identified constituents. However, direct sunlight is usually avoided to reduce the possibility of chemical reactions, responsible for forming of the artifact that may result from chemical transformations after exposure to ultraviolet radiation. Alternatively, plant materials should be dried under optimum temperature conditions between 40 and 50°C, or they can be dried in the oven if needed. Generally, plant material is dried at temperatures below 30°C to avoid the decomposition of thermolabile compounds [3]. Plants containing volatile or thermolabile components may be lyophilized (freeze-dried). In freeze-drying the frozen material is placed in an evacuated apparatus with a cold surface maintained at -60 to -80°C. Water vapors from the frozen material then pass rapidly to the cold surface to yield the dry material [8, 48].

7. Grinding or powdering plant materials

Lowering particle sizes increase surface contact between samples and extraction solvents and therefore, increase the yield rate and yield. Grinding resulted in coarse smaller samples, meanwhile, powdered samples gave a more homogenized and smaller particle, leading to better surface contact with solvents used for extraction. Before the extraction, pretreatments such as drying and grinding of plant materials are usually conducted to increase the extraction efficiency [48]. It is essential that the particles are of as uniform size as possible because larger particles take a longer time to complete the extraction process [49]. Usually, solvent molecules most contact the larger analytes, and particle size smaller than 05 mm is ideal for efficient extraction [8]. Conventional methods are usually used to reduce the particle size of dried plant samples viz. mortar and pestle or electric blenders and mills, etc.

8. Extraction techniques of actives compounds from plants and herbs

Extraction is separating the medicinally active mixture of many naturally active compounds usually contained inside plant materials (tissues) using selective solvents through the standard procedure [50]. It can also be defined as the treatment of the plant material with solvent, whereby the medicinally active constituents are dissolved and most of the inert matter remains undissolved. Thus, the purpose of all extraction is to separate the soluble plant metabolites, leaving behind the insoluble cellular marc known as residue [8]. The obtained product is a relatively complex mixture of metabolites, in liquid or semisolid state or (after removing water) in dried powder form, and are intended for oral and/or external uses. Extraction is based on the difference in solubility between the solute, other compounds in the matrix, and the solvent used to stabilize [29].

In general, there are three common type of extractions: liquid/solid, liquid/liquid and acid/base [51]. The extraction of these active compounds needs appropriate extraction methods that consider the plant parts used as starting material, the solvent used, extraction time, particle size and the stirring during extraction [52, 53]. Extraction methods include solvent extraction, distillation method, pressing, and sublimation according to the extraction principle. Solvent extraction is the most widely used method [47].

The solvent used, the plant part used as starting material and the extraction procedure are three basic parameters reported that influence the quality of an extract [15]. Proper extraction procure is the first step towards isolating and identifying the specific compounds in crude herbal material. It plays a significant and crucial role in the outcome. Successful extraction begins with careful selection and preparation of plant sample and thorough review of the appropriate literature for indications of which protocols are suitable for a particular class of compounds or plant species [3]. For instance, if the components are volatile or prone to degradation, they can first be frozen and homogenized with liquid nitrogen [29]. The extraction, in most cases, involves soaking the plant material in solvent for some specific time. Reported properties on an excellent extraction solvent include low toxicity, preservative action, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, and inability to cause the extract to be complex or dissociate.

The principle of solid–liquid extraction is that when a solid material comes in contact with the solvent, the soluble components in the solid material are dissolved in, and move to the solvent. In solvent extraction, the mass transfer of soluble ingredients to the solvent takes place in a concentration gradient. The mass transfer rate depends on the concentration of ingredients, until equilibrium is reached. After that, there will no longer be a mass transfer from plant material to the solvent. In addition, heating the solvent can also enhance the mass transfer because of better solubility.

Moreover, the concentration gradient changes if fresh solvent replace the solvent equilibrium with the plant material [50]. Properties required for an excellent extracting solvent (or a mixture of solvents) include removal, inert, non-toxic, free from plasticizers, not easily inflammable, and no or less chemical interaction [53]. The selection of solvent is therefore crucial for solvent extraction. Solubility, selectivity, cost, and safety should be taken into account in selecting solvent [47]. The factors affecting the choice of solvent are quality of phytochemicals to be extracted, rate of extraction, diversity of metabolites extracted, the toxicity of the solvent in the bioassay process, and the potential health hazard of the extractants and ease of subsequent handling of the extract. Obtaining maximum yield and the highest quality of the targeted compounds is the central goal of the extraction process [29]. Extraction methods are usually chosen per the properties of targeted active compounds, the water content of the

plant material, and the objectives of extraction. Initially, natural bioactive compounds are extracted using various extraction techniques, and their bioactivities are identified using *in vitro* and *in vivo* testing [45, 47]. A successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction. Since the extract will contain traces of residual solvent, the solvent should not interfere with the bioassay [15].

Various conventional (classical) and non-conventional (innovative) methods can extract plant materials. Variation in extraction procedures usually depends on key factors as extraction time, the temperature used, the particle size of tissues, the solvent-to-sample ratio, the pH of the solvent.

8.1 Classical and/or conventional techniques

The commonly employed extraction methods (long been used) are primarily based on liquid–solid extraction. They are ordinarily easy to operate and are based on heat and/or solvents with different polarities.

8.1.1 Maceration

This process is conducted by soaking the plant materials (coarse or powered) in a closed stoppered container in a solvent allowed to stand at room temperature for 2–3 days with frequent stirring to obtain plant extracts. A sealed extractor is used to avoid solvent evaporation at atmospheric pressure. The process is intended to soften and break the plant's cell walls to release the soluble phytoconstituents. The mixture is then pressed or strained by filtration or decantation after a specific time [8, 54]. Maceration is the simplest and still widely used procedure. The extraction procedure in this stationary process works on principle of molecular diffusion, which is a time-consuming process. Maceration ensures dispersal of the concentrated solution accumulation around the particles' surface and brings fresh solvent to the surface of particles for further extraction [46].

8.1.2 Digestion

This is a kind of maceration in which gentle heat is applied during the maceration extraction process. The temperature does not alter the active ingredients of plant material, so there is greater efficiency in the use of menstruum (solvent or mixture of solvent used for extraction). It is used when the moderately elevated temperature is not objectionable and the solvent efficiency of the menstruum is increased thereby [15]. The most used temperatures are between 35 and 40°C, although it can rise to no higher than 50°C. The plant part to be extracted is placed in a container with the pre-heated liquid to the indicated temperatures, is maintained for a period that may vary between half an hour to 24 hours, shaking the container regularly. This process is used for the herbal material or plant parts that contain poorly soluble substances or polyphenolic compounds [49].

8.1.3 Infusion

Infusion is a simple chemical process used to extract plant material that is volatile and dissolves readily or release its active ingredients easily in organic solvents [49]. Infusion and decoction use the same principle as maceration; both involve soaking the plant material in boiled or cold water which is then allowed to steep in the liquid. The maceration time for infusion is, however shorter. The liquid may then be separated and concentrated under a vacuum using a rotary evaporator.

Infusion finds its application in tea preparation and consumption prescribed in psychophysical asthenia, diarrhea, bronchitis, asthma, etc. In Tropical Africa, the infusion of the bark of *Prunus africana* (pygeum) is taken orally to increase the ease of urination and reduce inflammation and cholesterol deposits [30].

8.1.4 Lixiviation (elution)

The word “lixiviation” (comes from the Latin *lixivium*, “lessive”.) The extraction is carried out with cold or boiled, fresh and new solvent, always. Extraction of components is done using water as solvent.

8.1.5 Decoction

The current process involves boiling the plant material in water to obtain plant extracts. Heat is transferred through convection and conduction, and the choice of solvents will determine the type of compound extracted from the plant material [8]. The sample is boiled in a specified volume of water for a defined time (15 to 60 minutes.) It is then cooled, strained, filtered, and added enough water through the drug to obtain the desired volume. This method is suitable for extracting thermostable (that does not modify with temperature) and water soluble compounds, hard plant materials and commonly resulted in more oil-soluble compounds than maceration.

8.1.6 Tincture

It is the extraction of plant material in alcohol. Usually, the plant material (fresh) and ethyl alcohol are taken at the ratio of 1:5. Because of the alcohol content, the tinctures can be stored at room temperatures without decomposing [55].

8.1.7 Percolation

It is conducted by passing the boiled solvent through the plant material at a controlled and moderate rate (e.g. 5–7 drops per min) until the extraction is complete before evaporation. The concentrated plant extracts are commonly collected at the bottom of the vessel. To obtain a significant amount of extract, successive percolations can be performed by refilling the percolator with fresh solvent and pooling all extracts together. This procedure is mostly used to extract active compounds in the preparation of tinctures and fluid extracts. Its major disadvantage is that large volumes of solvents are required, and the procedure can be time-consuming and may require skilled persons [49].

8.1.8 Steam distillation and hydrodistillation

Steam and hydrodistillation methods are usually used to extract volatile compounds, including essential oil, insoluble in water, from various aromatic and medicinal plants. This is conducted by boiling the plant materials in water to obtain EOs after vapor condensation. Steam distillation occurs at a temperature lower than the boiling point of the ingredients. The method is useful for thermos-sensitive bioactive compounds e.g., natural aromatic compounds. The heat leads to breakage in the sample's pores and then enables the release of the target compound from a matrix. As Raoult's law states that while mixing two immiscible liquids, the boiling point will be reduced. Therefore, in the mixture of volatile compounds having a boiling point between 150 and 300°C and water having a boiling point at about 100°C (at atmospheric pressure), the mixture evaporation will be getting closer to that of the water [29, 56].

There are similarities between the hydrodistillation and the steam distillation principles. In brief, plant material is immersed in water or a proper solvent followed by heating to boiling under atmospheric pressure in the alembic. In a condenser, EO vapors and water undergo a liquefaction process, and EO separates from water/solvent after collection of the condensate in the decanter. The principle of extraction is based on isotropic distillation. Hydrodistillation with water immersion, direct vapor injection, and water immersion and vapor injection are the three main types of hydrodistillation. The distillation time depends on the plant material being processed [56].

8.1.9 Hot continuous extraction or Soxhlet extraction, soxhletation

In this method, finely ground sample is placed in a porous bag or “thimble” made from a strong filter paper or cellulose, set in the thimble chamber of the Soxhlet apparatus. The first Soxhlet apparatus was developed in 1879 by Franz von Soxhlet (**Figure 2**) [58]. Extraction solvents are heated in a round bottom flask, vaporized into the sample thimble, condensed in the condenser, and dripped back. When the liquid content reaches the siphon arm, the liquid content is emptied into the bottom flask again, and the process is continued [8]. The disadvantages include no possibility of stirring, and a large amount of solvent is required. This method is unsuitable for thermolabile compounds as prolonged exposure (long extraction time) to heat may lead to their degradation. It constitutes an official classical method used to determine different foods’ fat content [15, 29, 57].

Exposure to hazardous and flammable liquid organic solvents are the most noticed disadvantages in this method, and the high purity of extraction solvents needed may add to the cost. Also, shaking or stirring cannot be provided in the Soxhlet device to accelerate the process [57].

However, it requires a smaller quantity of solvent as compared to maceration. Besides, instead of many portions of warm solvent passing through the sample, just one batch of solvent is recycled. Other advantages of this technique include its simple operational mode, its applicability to a higher temperature that increases the kinetics process, its low capital cost, the absence of filtration, and the continuous contact of the solvent and the sample. It maintains a relatively high extraction temperature with heat from the distillation flask [29, 57, 59].

8.1.10 Serial exhaustive extraction

It is a standard extraction procedure that involves successive extraction with various solvents of increasing polarity from non-polar to polar ones. The aim is to ensure that a broad polarity range of compounds could be extracted [15].

8.1.11 Fermentation (aqueous-alcoholic extraction)

Some medicinal preparations adopt the technique of fermentation for extracting the active principles. The extraction procedure involves soaking the crude drug, either a powder or a decoction, for a specified period. Alcohol is generated *in situ* after fermentation occurs; this eases the extraction of the active components contained within the plant material. The alcohol hence generated additionally serves as preservative. Water should be boiled first, if the fermentation is to be performed in an earthen vessel. Wooden vats, porcelain jars, or metal vessels are used in place of earthen vessels in large-scale manufacturing. This method is not yet standardized [50].

Hydrodistillation and steam distillation, hydrolytic maceration followed by distillation, expression and effleurage (cold fat extraction) may be employed for

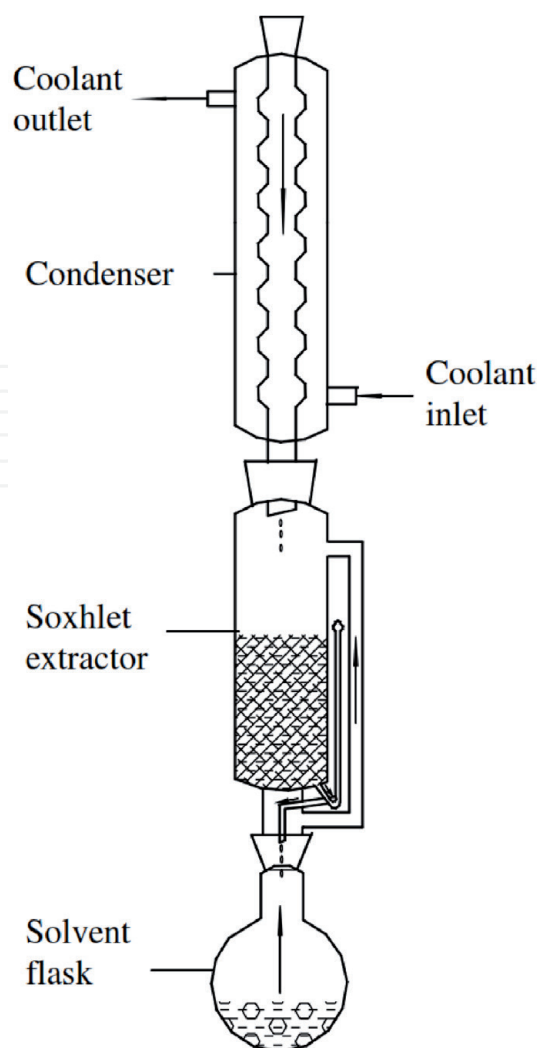


Figure 2.
Experimental Soxhlet extraction apparatus [57].

aromatic plants. Some of the latest extraction methods for aromatic plants include headspace trapping, solid phase micro extraction, protoplast extraction, micro distillation [15].

These techniques are the easiest and simplest methods. Despite the establishment of advanced extraction methods, the potential of conventional solid–liquid extractions is still being used to obtain active compounds from plants. These methods are criticized due to large solvent consumption and long extraction times that can destroy some metabolites. Solvents used in these techniques for soaking play a critical role. Many other advanced extraction methods that incorporate various technologies have been developed [8, 48].

8.2 Innovative (non-conventional) techniques

There is steady progress in the development of extraction technology in recent years. They are also known as advanced techniques with the most recently developed.

8.2.1 Microwave-assisted extraction (MAE)

Microwaves are part of the electromagnetic spectrum of light with a range of 300 MHz to 300 GHz, and wavelengths of these waves range from 1 cm^{-1} to 1 m^{-1} [60]. These waves are made up of two perpendicular oscillating fields which are used as energy and information carriers.

In this extraction process, the use of microwave energy results in faster heating. Due to the exposure of each molecule to the microwave field, its direct effects include, thermal gradients reduction, volume generation due to heat, equipment size reduction, because of the higher process rates, and thus increase in productivity, through better usage of the same equipment process volume [61]. MAE is a feasible green solvent extraction procedure as it uses water or alcohol at elevated temperature and controlled pressure conditions (**Figure 3**).

This procedure has demonstrated various benefits like ease to handle and understand steadiness. Many studies reported that MAE has higher yields and is significantly faster than conventional methods for extracting active substances from plant materials [48, 54, 62]. MAE can be presented as a potential alternative to the traditional solid-liquid extraction techniques. A few of the potential advantages are as follow:

- i. a lesser amount of solvent is required (few milliliters of solvent can be used);
- ii. shorter extraction time, from few seconds to few minutes (15–20 min);
- iii. improved extraction yield;
- iv. favorable for thermolabile constituents;
- v. heavy metals and pesticides residue which is present in the trace can be extracted from a few milligrams of plant sample;
- vi. during extraction, it provides a stirring, by which the mass transfer phenomenon is improved [54, 60, 62, 63].

MAE intensification needs special equipment to be functional, and electricity produces waves, leading to higher investments and higher operating costs than conventional methods [64]. Banar and collaborators extracted the bioactive compounds from *Urtica dioica* grown in Lebanon using conventional methods (maceration, reflux, Soxhlet, hydrodistillation, Ultrasound-Assisted Extraction (UAE) and

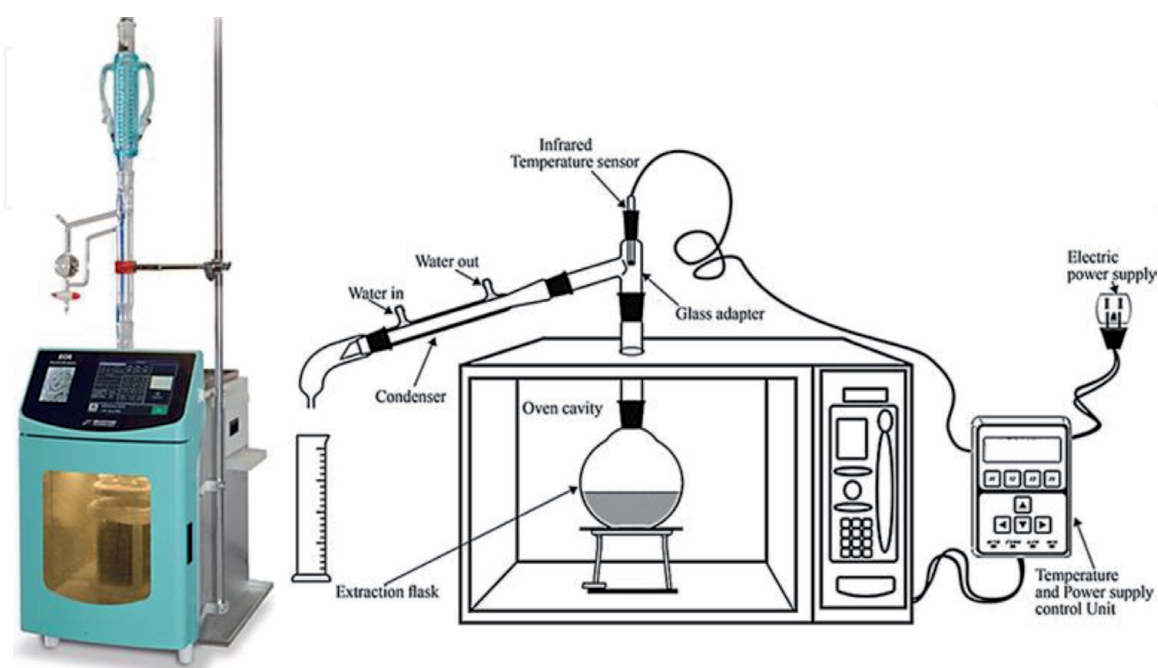


Figure 3.
Schematic representation of microwave-assisted extraction equipment [62].

Microwave-Assisted Extraction (MAE)) with different solvents. Their results revealed that MAE was the most effective technique. The extraction time was reduced, the lesser solvent was used and the amount of extracted compounds was increased [65].

8.2.2 Ultrasound-assisted extraction (UAE) or sonication extraction

This extraction method involves using ultrasound with frequencies ranging from 20 to 2000 KHz; this increases the permeability of cell walls and produce cavitation. Although the process is helpful in some cases, its large-scale application is limited due to its high cost. The most noticeable disadvantage of the procedure is the occasional but known deleterious effect of ultrasound energy on the active components of the medicinal plants through the formation of free radicals and consequently undesirable changes on the drug molecules [50]. The schematic representation of the equipment is given below (**Figure 4**).

Factors that affect the efficiency of UAE are extraction time, power, solvent, Liquid/Solid (L/S) ratio, plant material, frequency, amplitude, and intensity. UAE more advantageous than other advanced extraction methods and provided the best mass and heat transfer efficiency, lowest energy consumption and carbon emission. It was reported to yield high total phenolic content, antioxidant activity, or specific active compounds [62, 66].

8.2.3 Pressurized liquid extraction (PLE) or accelerated solvent extraction (ASE)

Pressurized liquid extraction (PLE) also known as pressurized fluid extraction (PFE), accelerated solvent extraction (ASE), and pressurized solvent extraction (PSE), or as enhanced solvent extraction system (ESE) [67].

Dionex Corporation introduced PLE in 1995 as an alternative to maceration, percolation, sonication, Soxhlet extraction, etc. It is an automated technique for extracting solid samples with liquid solvents (either aqueous or organic, single or mixtures) above their boiling point, combine high pressures (4–12 MPa) and moderate to high temperatures (50–300°C) [68]. When water is the extraction solvent,

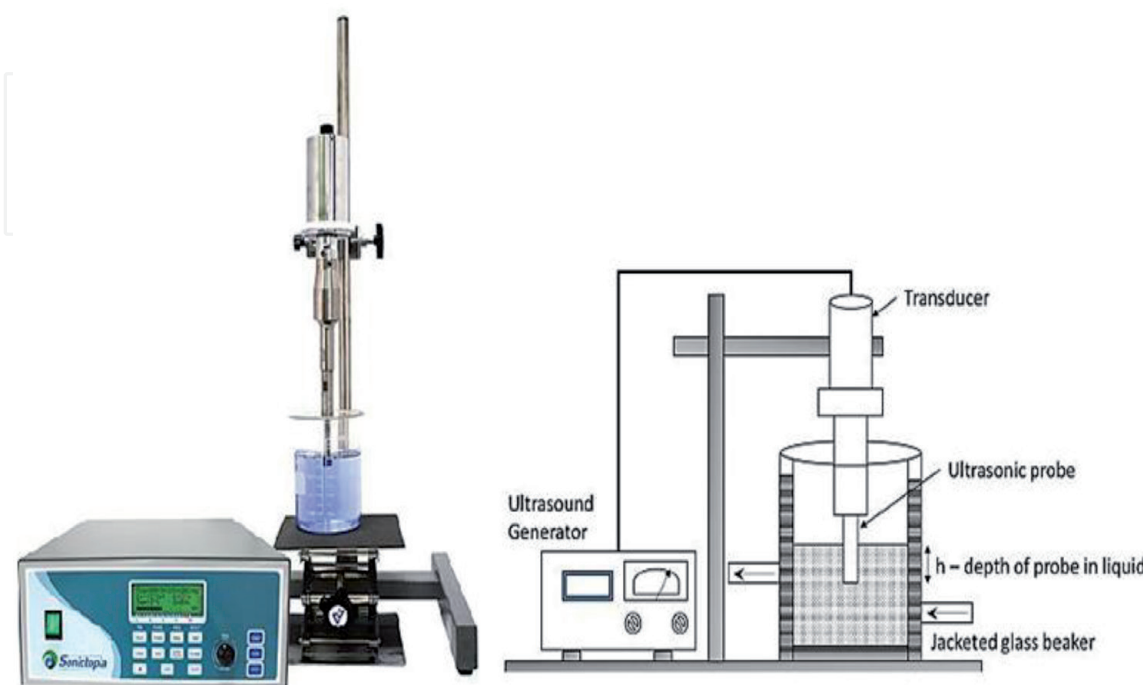


Figure 4.
Schematic representation of an ultrasound-assisted extraction equipment.

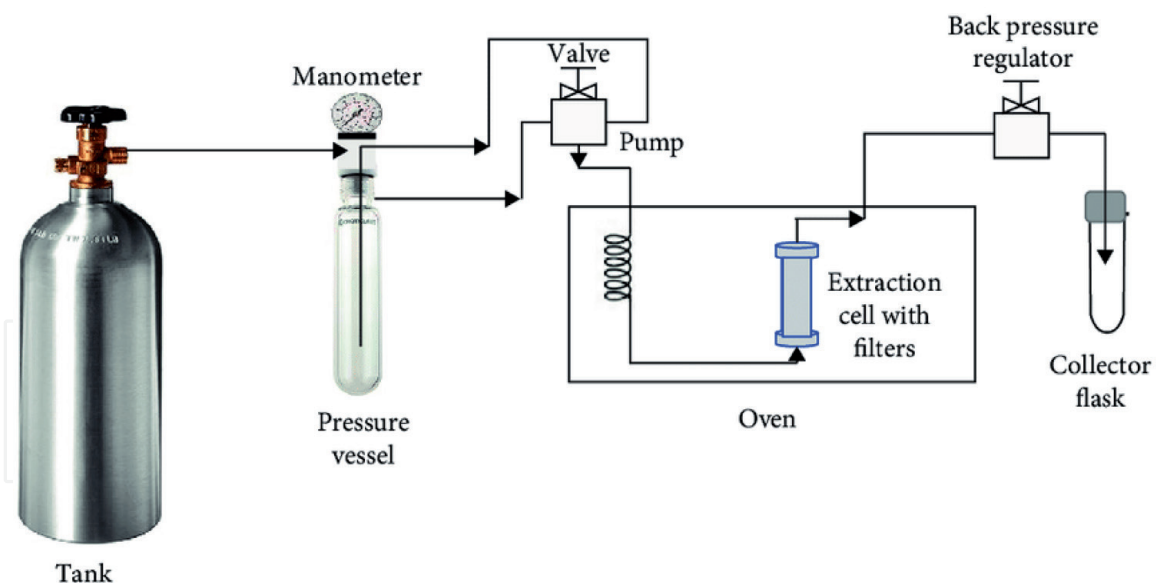


Figure 5.
 Scheme of pressurized liquid extraction equipment [68].

different terms are used to define the method, that includes hot water extraction (HWE), subcritical water extraction (SWE), high-temperature water extraction (HTWE), hot water extract pressurized (PHWE), liquid water extraction or superheated water extraction [67]. Sample size, solvent, pressure, temperature, pH, flow rate, extraction time are the standard parameters influencing the PLE process, with temperature and solvent type being the most significant ones [69–71].

In this process, for a short period of time (5–10 min), a cartridge in which the sample has been placed is filled with an extracting solvent and used to statically extract the sample under elevated temperature and pressure. To purge the sample extract from the extraction cell into a collector flask pressurized gas is used (**Figure 5**) [68].

To increase the efficiency of this extraction process, environmentally friendly liquid solvents are used at moderate to elevated temperature and pressure [72]. The increased temperature causes dramatic changes in the physical–chemical properties of water, enhances the analytes’ solubility, breaks matrix-analyte interactions achieving a higher diffusion rate, and accelerates the extraction process by increasing the diffusivity of the solvent. The increased pressure in contrast, keeps the solvent in a liquid state without boiling and forces the solvent to penetrate the matrix pores [55, 73–75].

The main advantages of this technique are: (i) faster extraction from 15 to 50 min, (ii) low quantity of solvents (15–40 mL), and no filtration is required. However, costly equipment and the need for a throughout optimization of variables to avoid a matrix-dependent efficiency are the main demerits [72–74].

8.2.4 Supercritical fluid extraction (SFE)

SFE is used for separating components from the matrix with the application of supercritical fluids as the extracting solvent (**Figure 6**) [30].

Using CO₂ as the extracting fluid has many advantages. Besides, its lower boiling point (31°C) and its critical pressure (74 bar). Moreover, carbon dioxide is abundant in nature, safe and inexpensive. But while carbon dioxide is the preferred fluid for SFE, it possesses several polarity limitations. When extracting polar solutes and when strong analyte-matrix interactions are present solvent polarity is crucial. Carbon dioxide fluid is usually mixed with organic solvents to alleviate the polarity limitations (**Figure 7**) [2].

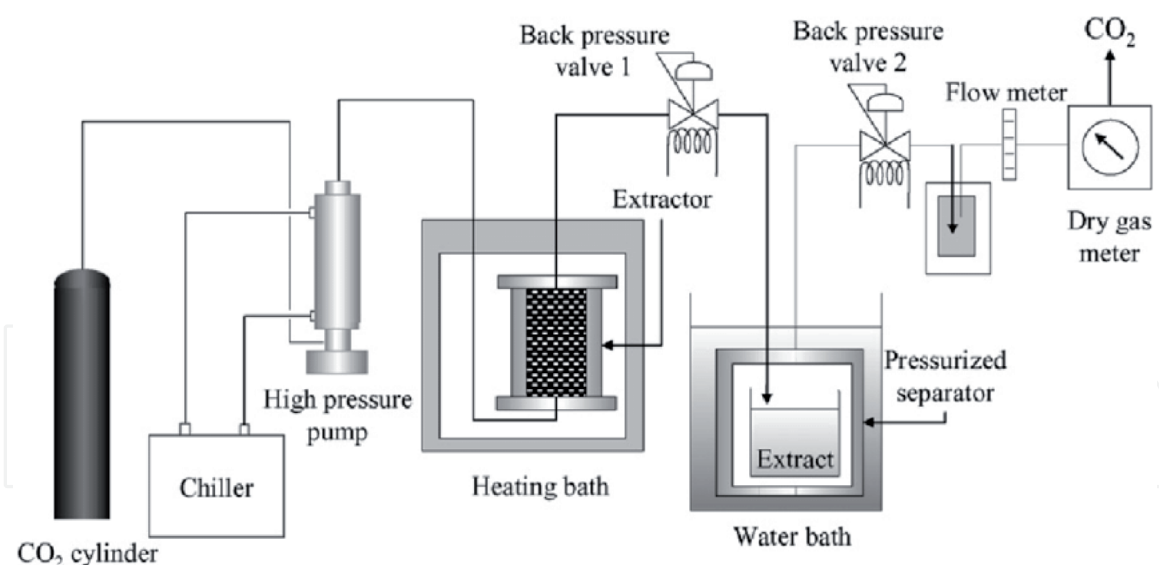


Figure 6.
Schematic diagram of supercritical fluid extraction (SFE) set-up [76].

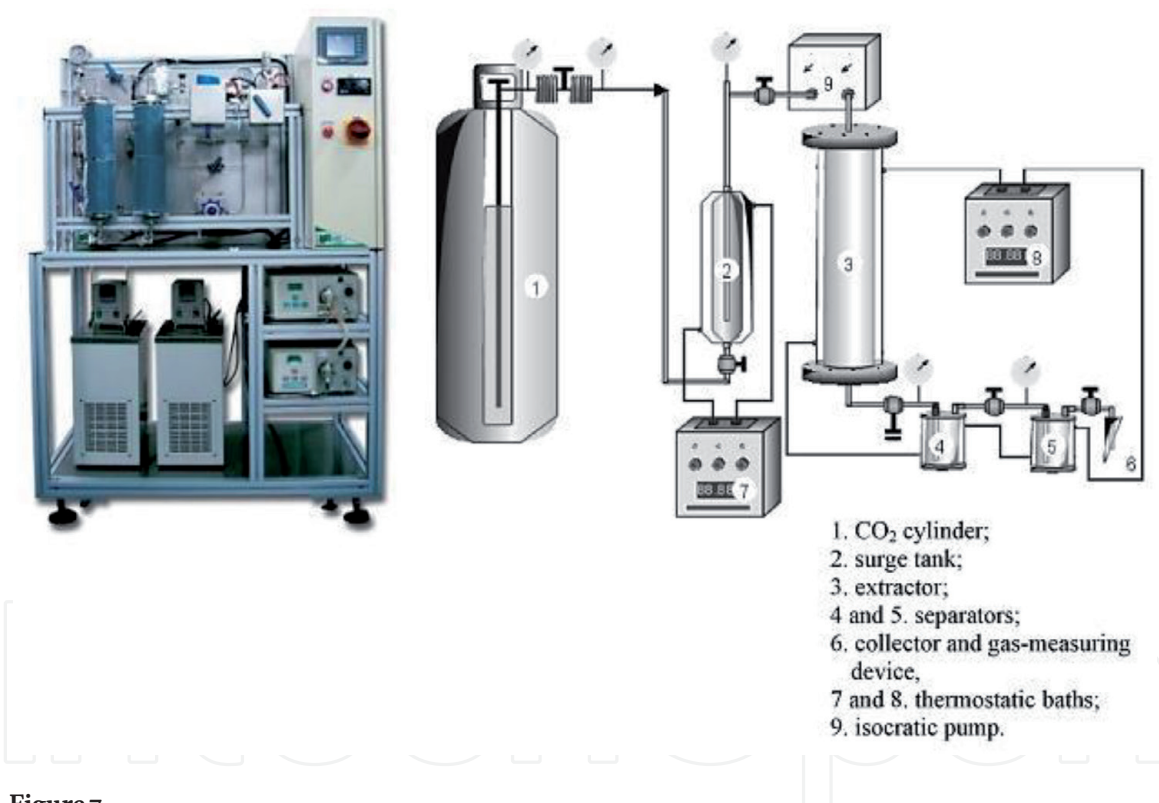


Figure 7.
Schematic representation of a supercritical fluid extraction (SFE) system [62].

- i. The SFE extraction procedure possesses distinct advantages:
- ii. the extraction of constituents is carried out at a low temperature, strictly avoiding damage from heat and some organic solvents. SFE offers gentle treatment for heat-sensitive material;
- iii. fragrances and aroma remain unchanged;
- iv. CO₂ is an inexpensive solvent;
- v. No solvent residues are left behind;

- vi. possibility of direct coupling with analytical chromatographic techniques such as gas chromatography (GC) or supercritical fluid chromatography (SFC);
- vii. environmentally friendly extraction procedure. CO₂ as the solvent does not cause environmental problems and is physiologically harmless, germicidal, and non-flammable.

Some specific disadvantages of this method are:

- i. high investment cost;
- ii. the use of high pressures leads to capital costs for the plant, and operating costs may also be high, so the number of commercial processes utilizing supercritical fluid extraction is relatively small, due mainly to the existence of more economical methods;
- iii. high polar substances (sugars, amino acids, inorganic salts, proteins, etc.) are soluble;
- iv. phase equilibrium of the solvent/solute system is complex and making design of extraction conditions is difficult.

SFE finds extensive application in extracting pesticides, environmental samples, foods and fragrances, essential oils, polymers, and natural products [50, 77]. Conde-Hernández and collaborators extracted the essential oil of rosemary (*Rosmarinus officinalis*) by S-CO₂ extraction, hydro distillation and steam distillation. They found that both yields of essential oil and antioxidant activity of SFC extract were higher than those from the other two methods [78, 79].

8.2.5 Pulsed electric field (PEF) extraction

Pulsed electric field extraction is a technique based on the exposure of vegetable matrix to an electrical potential. A transformer generates an electric pulse, increasing voltages from 140 or 220 V to 1000 V, or even greater than that (25000 V). A capacitor transforms this high voltage in a closed chamber with metallic electrodes. The general scheme of PEF equipment is presented in **Figure 8** [80].

This “cold” extraction assisted by PEF prevent the degradation of the cell and the extraction of components from the intracellular vacuoles [81]. It considerably increases the yield and decreases the time because it can increase mass transfer by destroying membrane structures during the extraction process.

Specific energy input, treatment temperature and field strength are considered among parameters that can influence the treatment efficacy of the PEF extraction. It is known as a non-thermal method which reduces the decomposition of the thermolabile components [47].

8.2.6 Enzyme-assisted extraction (EAE)

The EAE is an enzymatic pre-treatment that is carried out by the addition of specific hydrolyzing enzymes during the extraction step. In the cell membrane and cell wall structure, micelles are formed by macromolecules such as polysaccharides and protein. The coagulation and denaturation of proteins at high temperatures during extraction are the main barriers to extracting natural products. EAE enhance

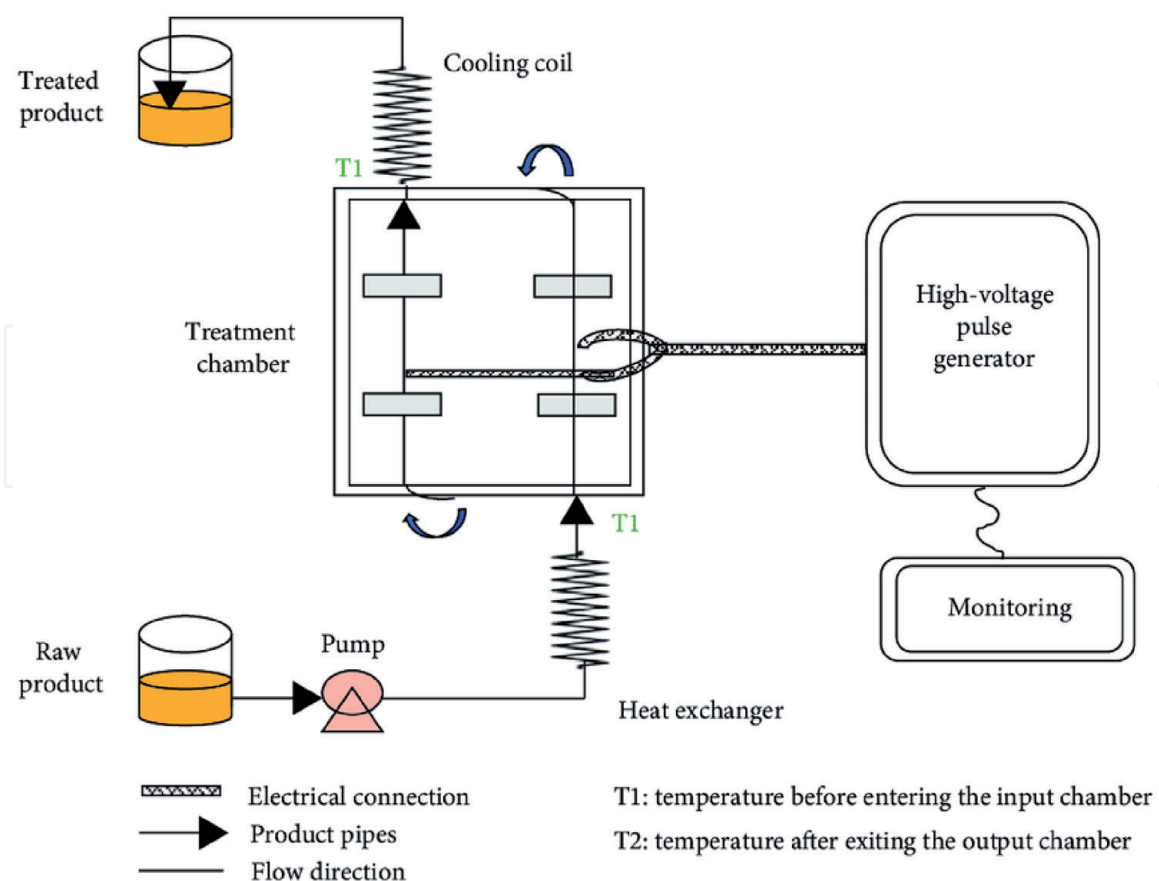


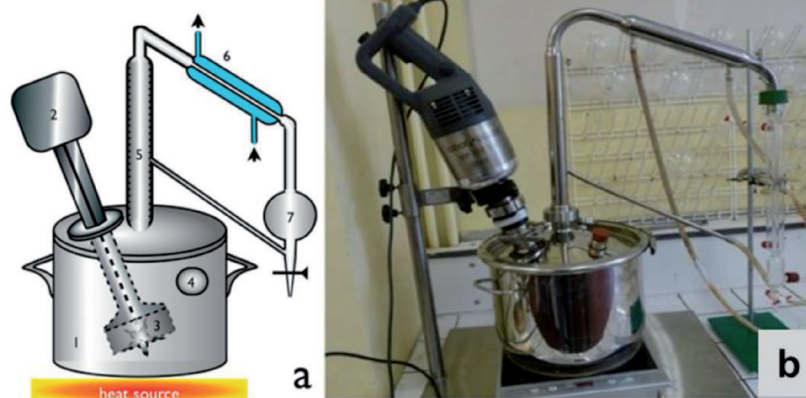
Figure 8.
General scheme of a PEF equipment process.

the extraction efficiency due to the hydrolytic action of the enzymes on the components of the cell wall and membrane and the macromolecules inside the cell, which facilitate the release of the natural products. Cellulose, α -amylase, and pectinase are hydrolyzing enzymes usually employed in EAE [47, 82]. This procedure is suitable for extracting various bioactive substances from plant matrices, but after filtration the obtained fraction is rich in small water-soluble molecules that include polyphenols and flavonoids [82].

8.2.7 Turbo-distillation extraction or turbo-extraction (turbolysis)

Turbo-distillation was patented in 1983 by Martel, and has been used in several companies as an industrial purpose for extracting EOs from hard matrixes (such as wood, bark, and seeds) [83]. The extraction process is similar to hydrodistillation with slight modifications [84]. The turbo-extraction or turbolysis is based on extraction with stirring and simultaneous reduction of particle size. Due to of high shearing force, cells disruption leads to rapid dissolution of the active constituents. It results in an extraction time of the order of minutes and the plant content is almost completely depleted [85]. Compare to hydrodistillation, turbo-distillation minimize extraction time and energy consumption and prevents the degradation of volatile constituents (**Figure 9**) [84].

In 2017, Martins and collaborators studied the turbo-extraction of stevioside and rebaudioside A from *Stevia rebaudiana* dried and powdered leaves. The extraction is carried out by applying a fractional factorial design that allowed the evaluation of the main effects of drug powder size, solvent to drug ratio by weight, temperature, stirring and time on the yield of these glycosides. Their work demonstrated that turbo-extraction was promising for *Stevia rebaudiana* glycosides extraction.



The vessel (1); the rotor (2); the turbo shredder (3); the thermometer (4); the distillation column (5); the condenser (6); the receiver-cum separator (7)

Figure 9.

Laboratory turbo-Clevenger: (a) schematic, (b) bench apparatus. The vessel (1); the rotor (2); the turbo shredder (3); the thermometer (4); the distillation column (5); the condenser (6); the receiver-cum separator (7) [46, 84].

It stimulated new research on the purification of these extracts, which became an exciting source of income for developing countries such as India and Brazil [86]. Perino and collaborators showed that the essential oil extracted by turbodistillation in 30 minutes were quantitatively (yield and kinetics profile) and qualitatively (aromatic profile) similar to those obtained using conventional hydrodistillation in 3 hours. They concluded that this process, which gave a reduced extraction time, was perfectly adapted to the extraction of hard matrixes [84]. It can be advantageous over dynamic maceration.

8.2.8 Counter-current extraction (CCE)

In this procedure, the wet raw material is pulverized to produce a fine slurry. The target material is moved in one direction (usually as a fine slurry) within a cylindrical extractor where it comes in contact with extracting solvent. Further, the starting material moves making more concentrated extract. Thus, complete extraction is possible when the amounts of material and the flow rate of solvent are optimized the complete extraction is possible. The process is extremely efficient, takes little time and poses no danger when high temperature is applied. Lastly, the extracts come out sufficiently concentrated at one end of the extractor, while the residue falls on the other end [50]. This extraction procedure has great advantages:

- i. compared to other methods such as maceration, decoction, percolation a unit amount of the plant material can be extracted with a much smaller volume of solvent;
- ii. CCE is usually performed at room temperature, which avoids the thermolabile constituents from being exposed to heat which is used in most other techniques;
- iii. Since the drug is pulverized under wet conditions, the heat generated during comminution is neutralized by water. This once more avoids the thermal degradation of components from heat exposure;
- iv. Compare to continuous hot extraction, CCE is rated to be more efficient and effective.

8.2.9 Solid-phase extraction (SPE)

Solid-phase extraction (SPE) is a sample preparation technology using chromatographic packing material, solid particle, commonly found in a cartridge-type device, to chemically separate the different components. Samples are almost constantly in the liquid state (although special applications can be run with some samples in the gas phase). In this method, the dissolved or suspended compounds in a liquid mixture are separated from other compounds depending on their physical and chemical properties. The technically correct name for this technology is “Liquid–Solid Phase Extraction”, since the chromatographic particles are solid and the sample is in the liquid state [87].

SPE has many benefits, but four significant benefits deserve special attention:

- i. simplification of complex sample matrix along with compound purification;
- ii. reduce ion suppression or enhancement in MS applications;
- iii. capability to fractionate sample matrix to analyze compounds by class;
- iv. trace concentration (enrichment) of very low-level compounds.

This rapid, economical and sensitive technique uses different types of cartridges and disks, with various sorbents, where the solute molecules are preferentially attached over the stationary phase.

8.2.10 High-voltage-assisted extraction

The principle of this equipment is similar to PEF, with the difference that electrical discharge is made through a small point. For this, a needle electrode is used from which the release is made in a plate ground electrode.

These methods are known as greener methods, are often better than conventional ones in terms of high yields, high selectivity, lower solvent consumption and shorter extraction time. They are also found to be environmentally ecofriendly since energy, and organic solvent consumption are reduced. The combination of extraction methods to obtain high purity extracts or high overall yields are described in the literature [40, 88–90]. Its main advantage is the operability in continuous mode, which is very important from an industrial and economic point of view [80].

8.2.11 Phytonics process

A new solvent-based on hydrofluorocarbon-134a and a new technology to optimize its remarkable properties in the extraction of plant material offer significant environmental advantages and health and safety benefits over traditional processes to produce advanced quality natural fragrant oil, flavors and biological extracts.

The technology known as “phytonics process” was developed and patented by Advanced Phytonics Limited (Manchester, UK). Fragrant components of EOs and biological or phytopharmacological extracts that can be used straightly without additional chemical or physical treatment are the products frequently extracted by this process. The properties of the new generation of fluorocarbon solvents have been applied to the extraction of plant material. The core of the solvent is 1,1,2,2-tetrafluoroethane, better known as hydrofluorocarbon-134a (HFC-134a) with a boiling point of – 25°C; a vapor pressure of 5.6 bar at ambient temperature. It

is flammable and non-toxic. This product was developed as a replacement for chlorofluorocarbons and more importantly, it does not deplete the ozone layer. By most standards this is a poor solvent that is unable to break up (dissolve) plant waste.

The process is advantageous because the solvents can be customized: by using modified solvents with HFC-134a, the process can be made highly selective in extracting a specific class of phytoconstituents. Likewise, to withdraw a broader spectrum of constituents other modified solvents can be employed. The biological products obtained by this process contain extremely low residual solvent. Residuals are constantly below the levels of detection and are fewer than 20 parts per billion. Therefore, selected solvents have minimal potential reaction effects on the botanical material, and are neither acidic nor alkaline. At the end of each production cycle, the processing plant is sealed so that solvents are constantly recycled and totally recovered. Electricity is the unique utility required to perform these systems and, even then, they consume little energy. There is no scope for the escape of the solvents, and even if some solvents come to escape, they pose no threat to the ozone layer because they do not contain chlorine. The waste product (biomass) from these plants is dry and “ecofriendly” to handle.

As the benefits of this procedure, we have the following:

- i. the phytonic process is soft and its products are never damaged by exposure to temperatures over ambient because relatively low temperatures are employed;
- ii. vacuum stripping is necessary which, in other processes, leads to the loss of precious volatiles;
- iii. the process is performed completely at neutral pH, and in without oxygen, the products never suffer acid hydrolysis damage or oxidation;
- iv. the procedure is extremely selective, and offer a choice of operating conditions end products;
- v. it requires a minimum amount of electrical energy;
- vi. it is less threatening to the environment;
- vii. no harmful emission in the atmosphere and the subsequent waste products (spent biomass) are inoffensive and pose no effluent disposal problems;
- viii. the solvents employed are neither toxic, nor flammable, or ozone-depleting;
- ix. the solvents are entirely recycled within the system.

In biotechnology, the utilization of the phytonics process is frequently employed to extract (e.g., for the production of antibiotics), herbal drug, food, EOs and flavor industries, and pharmacologically active products. It is particularly used to produce top-quality pharmaceutical-grade extracts, pharmacologically active intermediates, antibiotic extracts, and phytopharmaceuticals. However, the fact that it is used in all these areas prevents its use in other areas. The technique is being used to extract high-quality essential oils, oleoresins, natural food colors, flavors and aromatic oils from all types of plant material. The technique is also used in refining crude products obtained from other extraction processes. It provides extraction without wax or other contaminants. It helps in the removal of many biocides from contaminated biomass [50].

8.3 Liquid–Liquid extraction (partitioning)

Upon extraction of the solids and release of desired organics into the extraction solvent, the most common next step is a liquid–liquid extraction, taking advantage of mixing two (or sometimes three or even more that can establish two phases) non miscible solvents, for example, water and ether. The standard rule of thumb is that polar compounds go into polar solvents (e.g., amino acids, sugars, and proteins remain in water). To the contrary, the nonpolar components usually remain in the organic phase (e.g., steroids, terpenoids, waxes, and carotenoids are typically extracted into a solvent such as ethyl acetate).

It is important to minimize interference from compounds that may coextract with the target compounds during the extraction of plant material by conventional or by advanced methods. It is also needed to avoid contamination of the extract and to prevent decomposition of important metabolites or artifact formation as a result of extraction conditions or solvent impurities [3]. Regardless of the extracting procedure employed, the resulting solution should be filtered to withdraw whatever particulate matter. Due to the accompanying increased risk of formation of artifact and decomposition or isomerization of extract components plant extract should not be stored in the solvent for a long time at room temperature or in sunlight because [3].

9. Extraction of specific metabolites

The chemical investigation profile of a plant extract, fractionation of a crude extract is suitable to isolate the major classes of compounds from each other before further chromatographic analysis. One procedure based on varying polarity that might be used on an alkaloids-containing plant is indicated in **Figure 10**. The type and quantity of components to be separate into different fractions will, vary from plant to plant. Such procedure can be modified when labile substances are investigated [47].

9.1 Extraction of essential oils (EOs)

Essential oils (EOs) are concentrated aromatic hydrophobic oily volatile liquids characterized by a strong odor and produced by all plant organs [91]. They are obtained from raw material by several extraction techniques such as water or steam distillation, hydrodiffusion, solvent extraction, Soxhlet extraction, expression under pressure or cold pressing method, also known as scarification method, microwave-assisted extraction, microwave hydrodiffusion and gravity, supercritical fluid or subcritical water extractions. The best extraction method to use depends on the ease of evaporating (volatility) and the hydrophilicity or hydrophobicity (polarity) of the desired components [92–96]. However, the three most commonly applied techniques to extract EOs are Soxhlet, hydrodistillation, and SFE [97]. The extraction method chosen significantly affects the chemical composition of EOs [91]. Benmoussa and collaborators have recently found that the microwave hydrodiffusion and gravity (MHG) appeared like a rapid process, a green technology, and a desirable alternative protocol to enhance both the quality and the quantity of the EOs extracted from medicinal and aromatic plants [92].

9.2 Extraction of fats and oils

Lipids contain a broad category of non-polar molecules that are barely soluble or completely insoluble in water, but soluble in an organic solvent such

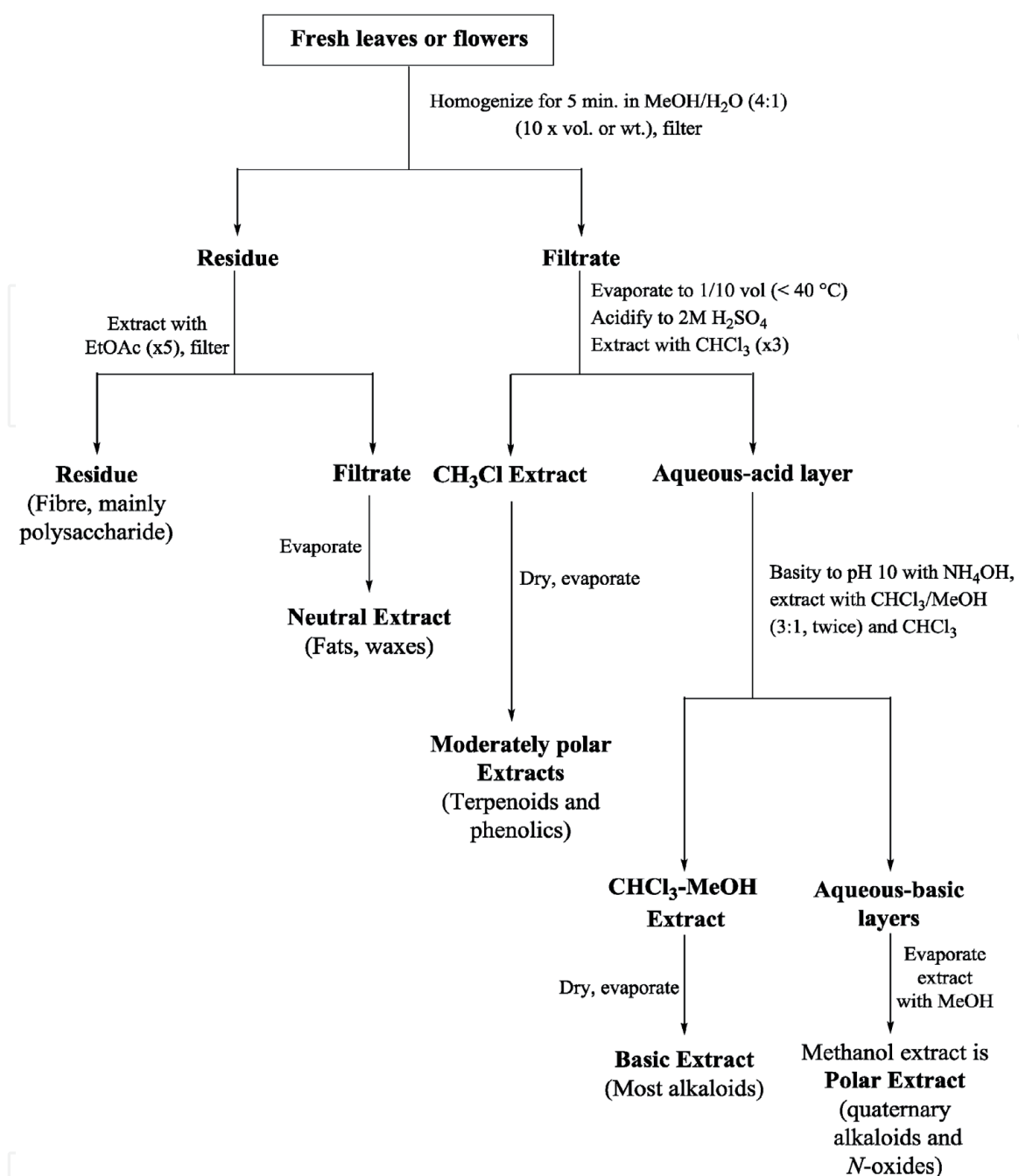


Figure 10.
A general procedure for extracting fresh plant tissues and fractionating into different classes according to polarity.

as *n*-hexane, diethyl ether, chloroform, and alcohol [98]. Fats are triglycerides that are solid or semi-solid at room temperature, while oil is also triglycerides that are liquid or clear liquid at room temperature, however, their chemistry is determined by the degree of solubility. Fats and oil may be of vegetable, animal, and marine origin [99]. Oilseeds and fats production requires several units-operations, starting with a pre-treatment stage. It is often necessary to dry the sample before oil extraction using solvents because many organic solvents are not miscible with water and cannot easily penetrate the matrix and extraction would be inefficient [100]. The processing methods used are usually neither specific to lipids, nor insure 100% recovery of the lipid material because of the nature of the matrix. Diethyl ether and petroleum ether stands as favorite solvents in the case of crude fat because they are relatively non polar, hence extract most non-polar components [98].

Extraction process of edible oils may have negative effects on taste, stability, appearance or nutritional value, preserve tocopherols, and prevent chemical changes in the triacylglycerol. Fats and oil can be extracted from plants using conventional and advanced techniques that include hot water extraction, cold pressing, solvent extraction, high-pressure solvent extraction, microwave –assisted extraction, and supercritical fluid extraction [99]. Extraction of oil involves several mechanisms for removing a liquid from a solid such as leaching, washing, diffusion and dialysis [98]. In the case of palm oil (seeds of *Elaeis oleifera*), crude oil is obtained after a digestion step followed by a pressing stage. Digestion helps the rupture or breaking down the oil-bearing cells, thus releasing the palm oil in the fruit [101–103]. Enzyme-assisted extraction (EAE) is an efficient method to improve lipid extraction from several different biomasses such as soybean, sunflower, and microalgae [104, 105].

The main side reactions reported during oil processing are (i) *trans* fatty acid formation, (ii) *cis-trans* isomerization, (iii) and physical loss [99]. Before oilseeds processing, moisture must not exceed a certain limit to prevent growth of fungi and the occurring lipase formation, resulting in a free fatty acid increase [98].

9.3 Volatile organic compounds

Volatile organic compounds (VOCs) are odorant compounds emitted from plant tissues. Plants can produce a high diversity of VOCs. They are responsible for the distinct aroma of certain dried plants, including the tea, *Camellia sinensis*. VOCs can therefore be used as an indicator of tea quality [106, 107]. Several VOCs are emitted as a natural defense mechanism against arthropods and pathogen attacks [108, 109].

Hydro-distillation (HD), steam distillation (SD), simultaneous distillation solvent extraction (SDE), microwave-assisted hydro-distillation (MWHd), supercritical fluid extraction (SFE), purge and trap, and solid phase microextraction (SPME), are used to extract VOCs [110].

Verde and collaborators conducted a work to optimize the MAE of the volatile oil terpenes from *Pterodon emarginatus* fruits and characterize the volatile compounds. According to their study, MAE proved to be feasible with a particular interest in avoiding the need of organic solvents in volatile oil extraction from plants. They proved that a minimum amount of water could be enough to bring result in extraction. That green methodology appears to be an excellent alternative to extract terpenes from aromatic plants [111].

9.4 Alkaloids

The alkaloids are low molecular weight nitrogen-containing compounds found mainly in plants and a lesser extent in microorganisms and animals. They contain one or more nitrogen atoms, typically as primary, secondary, or tertiary amines, which usually confers basicity on the alkaloids. If the free electron pair on the nitrogen atom is not involve in mesomerism, the salt formation can occur mineral acids. This fundamental property of alkaloids is used in their extraction and further clan-up. According to the nature of the nitrogen-containing structure, alkaloids are classified as pyrrolidine, piperidine, quinoline, isoquinoline, indole, etc. [27].

Two methods may be used for alkaloids extraction. One is to basify the plant material using diethylamine or ammonia and extract with an organic solvent [112, 113]. Alkaloids are substances with a basic character and their solubility is a function of pH. They are soluble in low polar organic solvents in basic medium, while in acidic medium, they are soluble in water.

Alkaloids containing basic amines can be selectively extracted using a modified version of the classical “acid–base shake-out” method (**Figure 11**).

As recommendations, mineral acids and strong bases should be avoided in extracting alkaloids (and plant material in general) because of the risk of artifact formation [3, 114, 115].

9.4.1 Extraction of caffeine

Caffeine is a natural product found in Coffee, cocoa beans, kola nuts, and tea leaves in a substantial amount. Its efficient extraction from Coffee relies heavily on the properties of caffeine and other components present in Coffee. One of the most popular species of the genus whose seeds contains caffeine is *Coffea arabica* (**Figure 12**). Several methods can be used to extract caffeine, including Ultrasonic extraction, Heat Reflux extraction, and Soxhlet extraction. Heat Reflux extraction is commonly used methods to extract caffeine from Coffee [116]. The initial solvent used in the extraction of caffeine is water. Caffeine is sparingly soluble in water at ambient temperature (2 g/100 mL) but increasing when mixed in boiled water (100°C) with a yield of 66 g/ 100 mL. Meanwhile, the solubility of caffeine in chloroform, toluene, acetone and ethyl acetate is relatively high at ambient temperature [116, 117]. Caffeine is a weakly basic, white colorless powder in its anhydrous state.

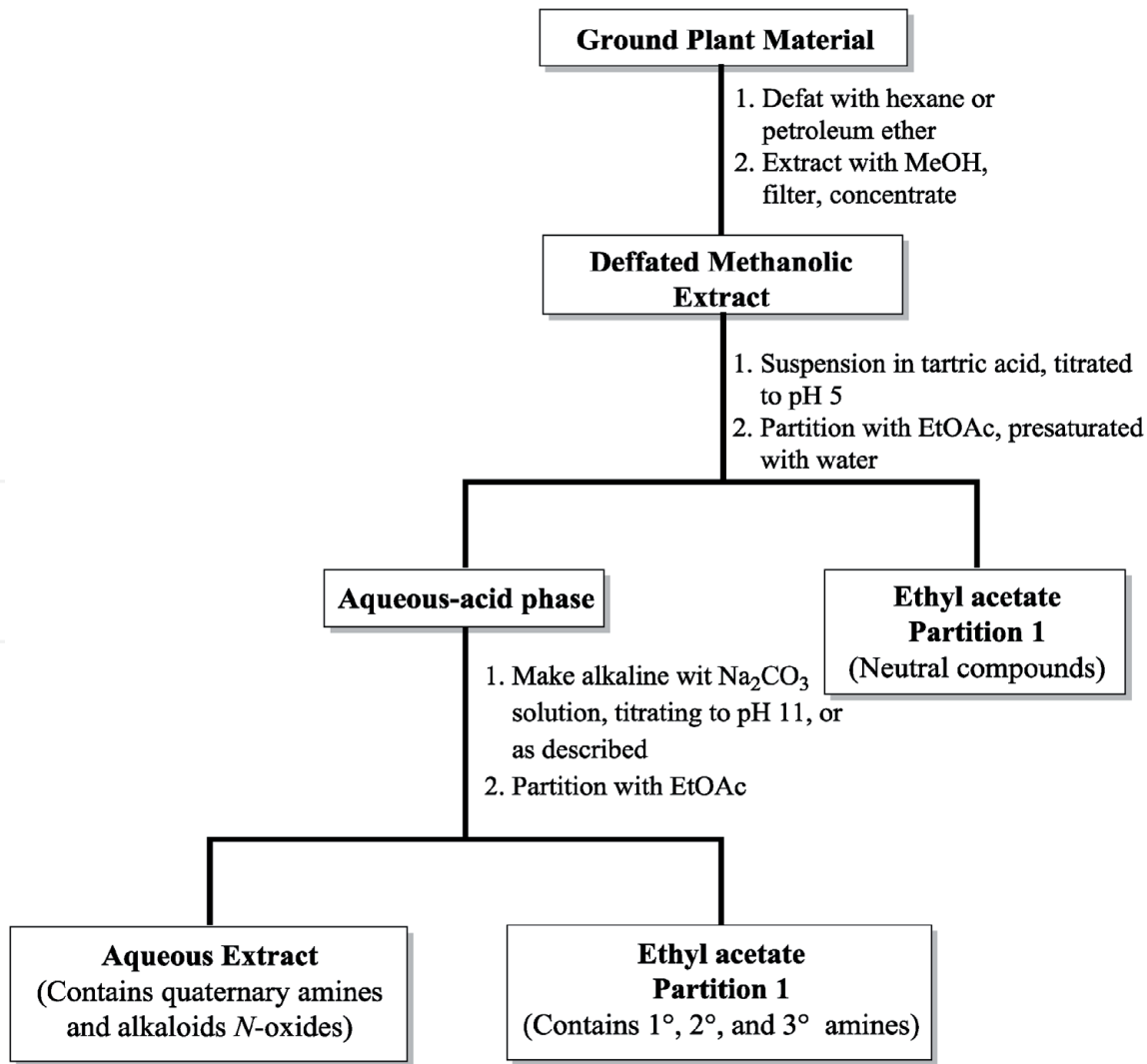
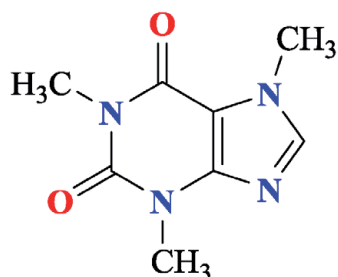


Figure 11.
General procedure to obtain alkaloidal extracts from crude plant material [114].



Chemical Formula: $C_8H_{10}N_4O_2$

Molecular Weight: 194.1940

Figure 12.
Chemical structure and a few data of caffeine.

There are several ways to remove caffeine from coffee. Here are few reported procedures:

9.4.1.1 Extraction procedure I: solvent extraction using dichloromethane (DCM)

Coffee seeds are firstly grounded and refluxed in an aqueous sodium carbonate solution for about 20 minutes under constant stirring. After filtration of the resulted mixture to filtrate is allowed for cooling at room temperature. The DCM is use to perform the partition of the aqueous filtrate. The process is repeated several times to extract more caffeine. The DCM fractions are then mixed with anhydrous sodium sulfate to remove water traces, the DCM-caffeine solution is filtered through reverse-phase filter paper, which will trap any water and residual matter. The DCM solution is allowed to evaporate and the white amorphous powder of caffeine is obtained [118].

The addition of sodium carbonate converts the protonated form of caffeine, which is naturally present in coffee, to its free caffeine form. During the extraction of caffeine, tannins being soluble in water and organic solvents can interfere with extraction. A weak base such as calcium carbonate or sodium sulphate can be added to break down tannins esters bonds into glucose and calcium or sodium salts of gallic acid, both of which will not be extracted into the organic solvent.

9.4.1.2 Extraction procedure II: supercritical carbon dioxide extraction

Some benefits are reported when using this method: caffeine is easily extracted from the final product after avoiding the use of flammable and toxic solvents. In this process, caffeine diffuses into supercritical CO_2 with water. Coffee beans are introduced at the top while fresh CO_2 is introducing at the bottom of an extractor vessel in a continuous extraction to remove caffeine. The recovery is accomplished in a separate absorption chamber containing water. Higher temperature and pressure are mandatory to obtain great yields. A pretreatment step is needed in this process. The addition of polar cosolvents affects cosolvent solute specific chemical or physical interactions. The extraction rate is accelerated by the solvent–cosolvent interaction and makes the extraction easier. The material is humidified with ultrapure water for prewetting, this will destroy the hydrogen bonds that link the caffeine to its natural matrix. Cell membrane swelling enhances solute diffusion. Subsequently, the quality of caffeine extracted can reach a purity >94%, which is generally the standard criteria for use in the soft drink and drug companies [119].

9.4.1.3 Extraction procedure III: activated charcoal

There are some benefits to use charcoal: it is cheaper, “green,” and ease to regenerate by heat and steam. The choice of active charcoal with the appropriate

number of micropores and a specific area up to 1000 m²/gram is mandatory for good absorption performance.

Cleaned green coffee beans are firstly soak in water, and the caffeine and other soluble content transferred to the aqueous phase. During the filtration through the activated charcoal, solely caffeine will continue to migrate in water. The recovered and dried coffee beans are now decaffeinated [30].

9.4.2 Extraction of morphine

The poppy straw (*Papaver somniferum* capsules) produces a white sticky latex known as opium. Usually, two weeks after the petals fall from the bud farmers harvest and collect opium. To allow the viscous latex to ooze out slowly farmers generally use sharp blade to do two to five incisions into the pod's skin. 24-hours after incisions of the pod, opium is then collected. This gummy latex, or opium (poppy tears), is a complex mixture containing at least 50 different alkaloids (**Figure 11**). Morphine is the major alkaloid, making up to 8–17% of the dry weight of opium. The chemical structure of morphine was established in 1925 despite de fact it has been used for centuries. Even if the immense majority of morphine continues to be harvested from the opium poppy, there are at least three classical processes (all old) for the extraction of morphine from simple starting material [120].

9.4.2.1 Extraction by Merck process

Cold water is used to treat the opium and the obtained aqueous solution concentrated until syrupy consistence. Powered sodium carbonate is added to precipitate hot and heated as long as ammonia given off; it is recommended that the solution remain alkaline to phenolphthalein and left aside four 24 hours at room temperature. After standing, the precipitate is filtered and cold water is use to wash several times until the wash-water become colorless. The precipitate is dissolved in alcohol at 85°C and the alcoholic solution is allowed for evaporation until dryness, and the residue is exhausted after neutralization with little amount of acetic acid. Decolorizing charcoal is used to treat the acidic solution and afterward precipitated with ammonia, avoiding excess is important. After filtration, the precipitate is washed and purified by crystallization in alcohol; concentration of the alcoholic mother-liquor yields a further quantity of morphine. This procedure was reported to be impossible to be consider for industrial scale because of the slight solubility of morphine in alcohol [120].

9.4.2.2 The Thiboumery and Mohr process

The gummy opium is divided into thin slices and treated with hot water thrice of its weight until obtain a homogeneous paste. After filtration the residue is pressed and treated again with thrice its weight in water. The resulted solutions are combined and allowed to evaporate until half their volume and poured into boiling milk of lime. One part of lime in ten parts of water should be used for four parts of opium; it is then filtered off again. The lime solutions are united and concentrated to a quantity twice the weight of the opium used. The solution is filtered, heated to boiling, and morphine is precipitated by adding ammonium chloride. The solution is filtrated after cooling at room temperature, and the precipitate is washed, then purified by solution in hydrochloric acid and crystallization of the morphine hydrochloride. It is an attractive process since there are no technical difficulties and the morphine is well separated from the secondary alkaloids. The morphine solutions are relatively clean; however, the yield might be bad. The contributory factors may be the oxidation of morphine in alkaline solution, and the fact that the lime always retains morphine [120].

9.4.2.3 The Roberson-Gregory process

Five to ten times its weight of cold distilled water is used to completely exhaust the opium. The resultant solution is evaporated to the consistency of a soft extract. The process is repeated with cold distilled water. This aqueous re-extraction causes impurities to precipitate, they are filtered off and the solution obtained is evaporated until its density is 10° Baumé. For each kilogram of opium, one hundred and twenty grams of calcium chloride are added to the boiling liquor, which is further diluted with an amount of cold water equal to its volume. A mixture of a precipitate of meconate and sulfate of calcium is thus formed and is filtered off. After filtration, the filtrate is once more concentrated to produce a new deposit which consist almost entirely of calcium meconate. After removal of the residue by filtration, the filtrate is left to stand for few days until it becomes a crystalline mass called “Gregory’s salt”. It is a mixture of hydrochloride and codeine hydrochloride. The crystals obtained are drained and then placed in a cloth and squeezed out in the presser. Successive crystallization is employed and each time animal charcoal is used to decolorize the solutions. To separate morphine to codeine, sufficiently pure crystals are dissolve in water and ammonia is therefore added to precipitate morphine while codeine remains in aqueous solution.

The first disadvantage of this procedure is that 20 to 25% of the morphine is left with the secondary alkaloids in the brown and viscous mother-liquids after filtration of the Gregory’s salt. The second drawback is that the hydrochloride of morphine and codeine crystallize in furry needles retains the mother-liquids in which the crystallization occurred. Several successive crystallization and subsequent recoveries are required for purification, which is a time-consuming process [120].

Later in 1957, an efficient method of extraction of morphine from poppy straw was developed by Mehlretter and Weakley. Water-saturated isobutanol containing 0.23% ammonia was used to extract morphine. Almost all the alkaloid was absorbed by passing off the raw opium through a cation exchange ions resin bed. Quantitative elution of morphine from the bed was achieved with dilute aqueous alkali. After neutralization and concentration, the crude morphine is obtained, and the eluate can be converted to hydrochloride pharmaceutical grade without difficulty. The general recovery of morphine was 90% [121].

Cooper and Nicola have reported recently a straightforward process for extraction of morphine with a good overall yield (**Figures 11 and 13**). Morphine and related alkaloids can be purified from opium resin and crude extracts by extraction in the following manner: first, soaking the resin with diluted sulfuric acid, which releases the alkaloids into solution. Either ammonium hydroxide or sodium carbonate then precipitates the alkaloids. The last step separates morphine from other opium alkaloids. Today, morphine is isolated from opium in relatively large quantities: over 1000 tons per year (**Figure 14**) [30].

Till date, morphine is used as a powerful painkiller to alleviate severe pain by acting straightaway on the brain. It also possesses euphoric and hallucinatory

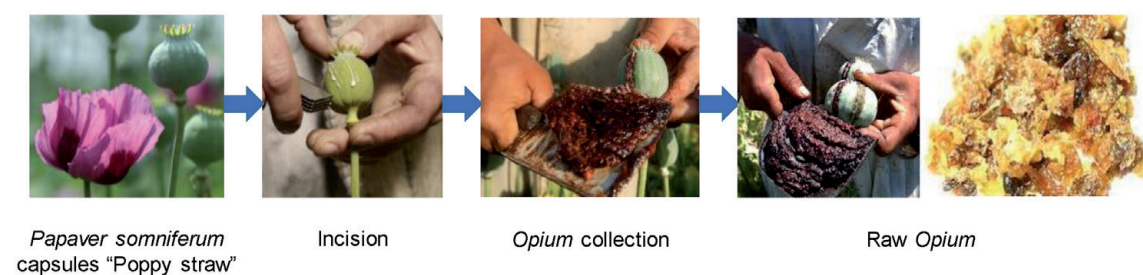


Figure 13.
Extraction of raw opium from poppy straw.

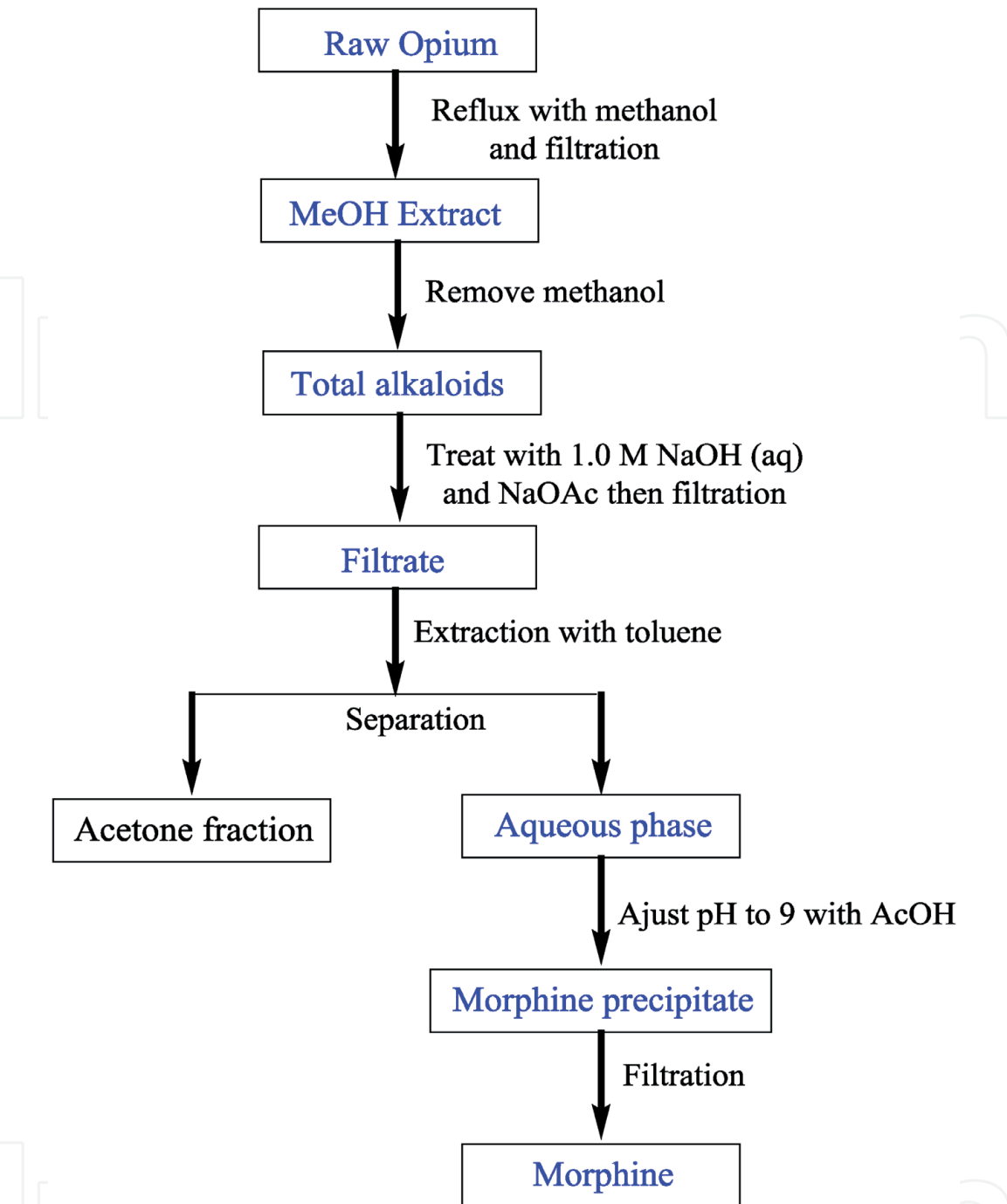


Figure 14.
Extraction protocol of morphine from raw opium by Cooper and Nicola [30].

effects. Morphine can also be chemically converted by an acetylation reaction using acetic anhydride and pyridine to create a much more potent form of the narcotic drug known as heroin [30].

9.5 Glycosides

Glycosides are relatively polar, and their polarity depends on both the number and type of sugar moieties attached to the aglycone. Cardiac glycosides have bulky steroidal aglycone, which are soluble in chloroform. However, most glycosides are extracted using polar solvents like acetone, methanol, ethanol, water or mixtures of these solvents. When extraction is done using water as solvent, enzymatic breakdown can happen. This will be avoided by using boiling water or add important proportions of alcohol or ammonium sulfate to the extract. In some cases, it may be the hydrolytic separation of the aglycone and sugar before or after extraction [122, 123].

9.6 Total phenolic and total flavonoids content

Phenolic compounds are well-known phytochemicals found in almost all plants. They can be simple phenols, benzoic and cinnamic acid derivatives, coumarins, tannins, lignins, lignans, and flavonoids [124]. Flavonoids are a group of plant constituents, the most common phenolic compound produced by plants as secondary metabolites in response to diverse biotic and abiotic factors [63, 82, 124]. They are responsible for the characteristics of flavor, color and pharmacological activities [67, 80, 125]. Because of their positive effects on human and animal health, and medical application for disease therapy and chemoprevention, interest in flavonoids increases [126, 127]. Complete extraction of phenolics is the next critical step after the sample preparation. The most common procedures of extraction of phenolics employ solvents, either organic or inorganic. Different parameters may influence the extraction yield, that includes temperature, the solvent used, time, solvent-to-sample ratio, as well as the number of repeated extractions of the plant material [124].

There is no universal extraction method and each optimized procedure is unique [82]. Due to the complex nature of the sample matrix and diverse chemical characteristics of flavonoids, it is consensual among scholars that there is no single or/and standard method to be used for every material or flavonoids to be extracted at present [67]. Maceration, water infusion, and Soxhlet extractions are generally used in research laboratories and/or in small manufacturing companies. The choice of solvent for extraction such as water, acetone, ethyl acetate, alcohols (methanol, ethanol, and propanol), and their mixtures will influence phenolics' extraction [124, 128]. The extraction of flavonoids-containing sample material are still performed by simple direct solvent extraction. It can also be extracted in a Soxhlet apparatus, first with *n*-hexane or diethyl ether to remove fats, and then with ethyl acetate or ethanol to obtain total phenols. This procedure is unsuitable for thermolabile components. A commodious and frequently used technique is sequential solvent extraction. Dichloromethane is used in the first step to remove flavonoid aglycones and non-polar components. A subsequent step using alcohol or alcohol-water mixtures will therefore extract flavonoid glycosides and other polar constituents. Cowan indicated that acetone was the most selective solvent for extracting flavonoids [129]. Anokwuru and collaborators discovered that acetone and *N,N*-dimethylformamide (DMF) were highly influential for removing antioxidants [130]. In most cases, flavonoids and polyphenols are coextracted [82]. Furthermore, several promising methods (Microwave-assisted extraction (MAE), Enzyme-assisted extraction (EAE), Pressurized liquid extraction (PLE), Ultrasound-assisted extraction (UAE), Matrix solid-phase dispersion (MSPD), and Supercritical fluid extraction (SFE) are nowadays used with increased yields and lower cost as main advantages [8, 82].

Due to the multiplicity of hydroxyl functions, phenols tend to be relatively polar and dissolve in aqueous alcohols. They may also be extracted or partitioned into aqueous alkali as phenolate salts as they are weak acids. A problem encountered with phenolic compounds is that they can undergo extensive polymerization reaction by polyphenol oxidation. This reaction is responsible for developing brown coloration in damaged plant material when exposed to the air and in certain extracts. The polymerization reaction is catalyzed by acid [131].

9.7 Total mixture of crude saponins

The procedure for isolating mixtures of crude saponins (i.e., steroidal or triterpene glycosides) is shown in **Figure 15**. Fats are removed from the plant material by treating with *n*-hexane and after extraction with methanol. The resultant methanol extract

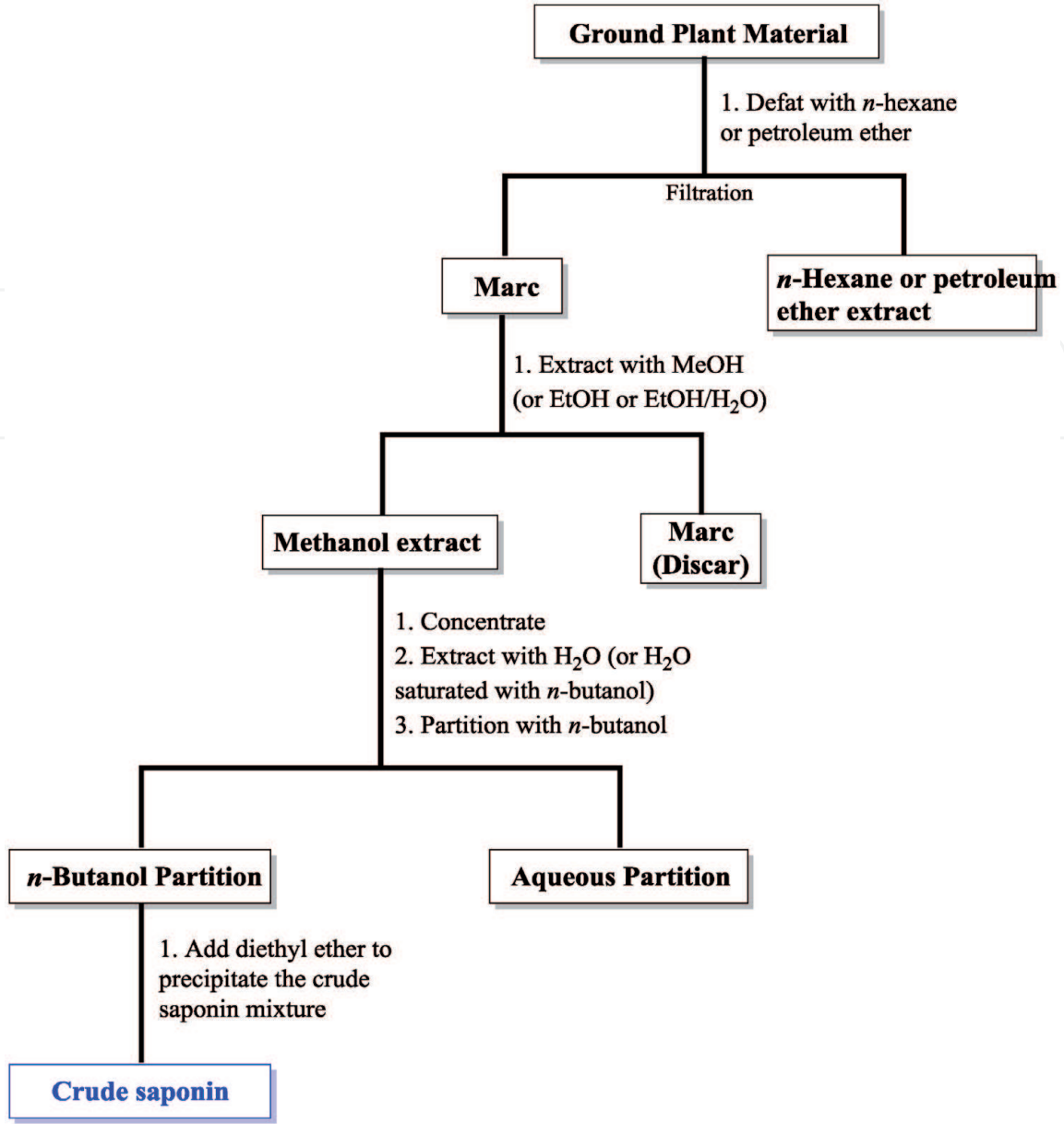


Figure 15.
General fractionation procedure to obtain a precipitate of crude saponin from plants, adapted from the literature [132].

is evaporated under vacuum and suspended in deionized water (presaturated with *n*-butanol), and partitioned with *n*-butanol. Diethyl ether is added to the butanol solution to precipitate the saponins [3, 132]. Selective extraction and fractionation of plant sterols (including sapogenins, bufadienolides, and cardiac glycosides) using manipulations and liquid/liquid partitioning have been described [3, 133]. Partitioning between the aqueous phthalic anhydride and organic solvent can be used to separate alcohols from non-alcohols. The alcohols partition into the aqueous layer as half-phthalates and can be regenerated by treatment with sodium methoxide in methanol. Sterols with ketone functional groups can be set-apart from non-ketones by liquid/liquid partition between organic and aqueous layers using Girard's hydrazide reagents ($\text{H}_2\text{N.NH.CO.CH}_2.\text{NR}_3^+\text{Cl}^-$), and generate ketones by acid hydrolysis [3, 133].

10. Conclusion

There is a clear and growing interest in the extraction procedure of natural products and their isolation, identification, and applications. Research innovation and

safe extraction processes are of primary importance in modern analytical processes, which are economically viable and environmental friendly. In the process of plant extracting plant material, it is peremptory to reduce interference of components that may be co-extracted with the target compounds, and to bypass contamination of the extract, moreover to prevent degradation of necessary metabolites or the formation of artifact as a result of extraction conditions or solvent impurities. Regardless of the extraction procedure, the resulting solution should be filtered to remove any particulate matter. Plant extracts should be stored for short time at room temperature or in sunlight to avoid increasing risks associated with the production of artifact making and additionally degradation or isomerization of extract components. The most suitable extraction procedure depends on the matrix of the plants and the type of compost, and should follow clear selection criteria.

Conflict of interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript or in the decision to publish the results.

Author details

Fongang Fotsing Yannick Stéphane^{1*}, Bankeu Kezetas Jean Jules²,
Gaber El-Saber Batiha³, Iftikhar Ali⁴ and Lenta Ndjakou Bruno⁵

1 Department of Chemistry, Higher Teachers Training College, University of Maroua, Maroua, Cameroon

2 Department of Chemistry, Faculty of Science, University of Bamenda, Bambili, Cameroon

3 Department of Pharmacology and Therapeutics, Faculty of Veterinary Medicine, Damanhour University, Damanhour, AlBeheira, Egypt

4 Department of Chemistry, Karakoram International University, Gilgit, Gilgit-Baltistan, Pakistan

5 Department of Chemistry, Higher Teacher Training College, University of Yaoundé I, Yaoundé, Cameroon

*Address all correspondence to: fongangfys@yahoo.fr

IntechOpen

© 2021 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Aman D. Introductory chapter: Plant extracts. 2019. DOI:<http://dx.doi.org/10.5772/intechopen.85493>
- [2] Swamy MK and Akhtar MS. Natural Bio-active Compounds. Volume 2: Chemistry, Pharmacology and Health Care Practices, 2019. Springer Nature Singapore Pte Ltd. (eBook) <https://doi.org/10.1007/978-981-13-7205-6>
- [3] Jones WP and Kinghorn AD. Extraction of Plant Secondary Metabolites. In, Natural Products Isolation, 2nd Ed. Humana Press Inc. 999 Riverview Drive, Suite 208 Totowa, New Jersey 07512, 2006
- [4] Cosa P, Vlietinck AJ, Berghe DV, Maes L. Anti-infective potential of natural products: How to develop a stronger *in vitro* 'proof-of-concept'. Journal of Ethnopharmacology 2006; 106: 290-302.
- [5] Daily G. Nature's Services: Societal Dependence on Natural Ecosystems. Covelo, CA: Island Press. 1997; 392 pp.
- [6] Botanic Gardens Conservation International, 1996. "CITES and Medicinal Plants Study: A Summary of Findings." Web resource: www.bgci.org/wellbeing/CITES_and_Med_Plants_Summary/.
- [7] Wang J, Soisson S, Young C, Shoop W, Kodali S, Galgoci A, Painter R, Parthasarathy G, Tang YS, Cummings R, Ha S, Dorso K, Motyl M, Jayasuriya H, Ondeyka J, Herath K, Zhang C, Hernandez L, Alloco J, Basilio A, Tormo JR, Genilloud O, Vicente F, Palaez F, Colwell L, Lee SH, Michael B, Felcetto T, Gill C, Silver LL, Hermes JD, Bartizal K, Barrett J, Schmatz D, Becker JW, Cully D, Singh SB. Platensimycin is a selective FabF inhibitor with potent antibiotic properties. Nature 2006; 441: 358-361. Doi: 10.1038/nature04784
- [8] Azwanida NN. A review on the extraction methods use in medicinal plants, principle, strength and limitation. Medicinal & Aromatic Plants 2015; 4:3. Doi: 10.4172/2167-0412.1000196
- [9] Santos GS, Sinoti SBP, Cunha de Almeida FT, Silveira D, Simeoni LA, Gomes-Copeland KKP. Use of galantamine in the treatment of Alzheimer's disease and the strategies to optimize its bisynthesis unsing *in vitro* culture technique. Plant Cell Tiss Organ Cult 2020; 143: 13-29. Doi.org/10.1007/s11240-020-01911-5
- [10] Scott LJ and Goa KL. Galantamine: a review of its use in Alzheimer's disease. Adis Drugs Evaluation 2000; 60(5): 1095-1122.
- [11] Tajuddeen N and Van Heerden FR. Antiplasmodial natural products: an update. Malaria Journal 2019, 18; 404: 1-62. Doi.org/10.1186/s12936-019-3026-1
- [12] Armstrong MJ and Okun MS. Diagnosis and treatment of Parkinson Disease: A review. F1000Research 2020; 9: 862
- [13] Ho JFV, Yaakup H, Low GSH, Wong SL, Tho LM, Tan SB. Morphine use for cancer pain: a strong analgesic used only at the end of life? A qualitative study on attitudes and perceptions of morphine in patients with and advanced cancer and their caregivers. Palliative medicine 2020; 34(5): 619-629.
- [14] Rathmes G, Rumisha SF, Lucas TCD, Twohig KA, Python A, Nguyen M, Nandi AK, Keddie SH, Collins EL, Rozier JA, Gibson HS, Chestnut EG, Battle KE, Humpheys GS, Amratia P, Arambepola R, Bertozzi-Villa A, Hancock P, Millar JJ, Symons TL, Bhatt S, Cameron E,

- Guerin PJ, Gething PW, Weiss DJ. Global estimation of antimalarial drug effectiveness for the treatment of uncomplicated *Plasmodium falciparum* malaria 1991-2019. *Malaria Journal* 2020; 19: 374. Doi.org/10.1186/s12936-020-03446-8
- [15] Pandey A and Tripathi S. Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. *Journal of Pharmacognosy and Phytochemistry* 2014; 2(5): 115-119.
- [16] Fellows LE. Pharmaceuticals from traditional medicinal plants and others: Future prospects. A paper presented at the symposium “New drugs from natural sources” sponsored by I.B.C. Technical services Ltd, London, 1991. Royal Botanic Gardens, Kew.
- [17] Farnsworth NR and Soejarto DD. Potential consequence of plant extinction in the United States on the current and future availability of prescription drugs. *Economic Botany* 1985; 39: 231-240.
- [18] Doughari JH. Phytochemicals: Extraction methods, basic structures, and mode of action as potential chemotherapeutic agents, phytochemicals—a global perspective of their role in nutrition and health. In: A Global Perspective of Their Role in Nutrition and Health. Venketeshwer R. Editor. InTech; 2012. Available from: www.intechopen.com.
- [19] Rungsung W, Ratha KK, Dutta S, Dixit AK, Hazra J. Secondary metabolites of plants in drugs discovery. *World J Pharm Res* 2015; 4:604-613
- [20] Sofowora A, Ogunbodede E, Onayade A. The role and place of medicinal plants in the strategies for disease prevention. *Afr J Tradit Complement Altern Med.*, 2013; 10(5); 210-229.
- [21] Evans WC. Trease and Evans’ Pharmacognosy. 16th Edition. WB Saunders Company Ltd., London, 2008.
- [22] Sofowora A. The present status of knowledge of the plants used in traditional medicine in western Africa: A medical approach and a chemical evaluation. *J Ethnopharmacol* 1980; 2:109-118.
- [23] Astutik S, Pretzsche J, Kimengsi JN. Asian medicinal plants’ production and utilization potentials: A review. *Sustainability*, 2019; 11: 5483. Doi:10.3390/su11195483
- [24] Smith-Hall, C.; Larsen, H.O.; Pouliot, M. People, plants and health: A conceptual framework for assessing changes in medicinal plant consumption. *J. Ethnobiol. Ethnomed.* 2012; 8, 43.
- [25] Schippmann, U.; Leaman, D.; Cunningham, A.B. A comparison of cultivation and wild collection of medicinal and aromatic plants under sustainability aspects. In Bogers J, Craker LE, Lange D. *Medicinal and Aromatic Plants: Agricultural, Commercial, Ecological, Legal, Pharmacological and Social Aspects*; Eds.; Springer: Dordrecht, The Netherlands, 2006; pp. 75-95. ISBN 978-1-4020-5447-1.
- [26] Slikkerveer, L.J. The challenge of non-experimental validation of MAC plants. In *Medicinal and Aromatic Plants: Agricultural, Commercial, Ecological, Legal, Pharmacological and Social Aspects*; Bogers, R.J., Craker, L.E., Lange, D., Eds.; Springer: Dordrecht, The Netherlands, 2006; pp. 1-28.
- [27] Peter KV. *Handbook of herbs and spices*. Woodhead Publishing Limited Abington Hall, Abington Cambridge CB1 6AH England, 2001.
- [28] Akhtar I, Javad S, Yousaf Z, Iqbal S, Jabeen K. Microwave assisted extraction

of phytochemicals an efficient and modern approach for botanicals and pharmaceuticals. Pak. J. Pharm. Sci. 2019; 32(1):223-230.

[29] Omeroglu PY, Acoglu B, Özdal T, Tamer CE, Çopur OU. Extraction techniques for plant-based bio-active compounds. In Mallappa KS and Mohd SA. Natural Bio-active Compounds. Volume 2: Chemistry, Pharmacology and Health Care Practices. Springer, Springer Nature Singapore, 2019. (eBook) <https://doi.org/10.1007/978-981-13-7205-6>.

[30] Cooper R and Nicola G. Natural Products Chemistry: Sources, Separations, and Structures. CRC Press Taylor & Francis Group 6000 Broken Sound Parkway NW, Suite 300 Boca Raton, 2015.

[31] Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga Latha L. Extraction, isolation and characterization of bioactive compounds from plants' extracts. Afr J Tradit Complement Altern Med 2011; 8:1-10.

[32] Bendary E, Francis RR, Ali HMG, Sarwat MI, El Hady S. Antioxidant and structure-activity relationships (SARs) of some phenolic and anilines compounds. Annals of Agricultural Science 2013, 58(2): 173-181. <http://dx.doi.org/10.1016/j.aoas.2013.07.002>

[33] Nath R, Roy S, De B, Choudhury MD. Anticancer and antioxidant activity of *Croton*: a review. International Journal of Pharmacy and Pharmaceutical Sciences 2013; 2(2): 1-8.

[34] Huang W, Zhang X, Wang Y, Ooi V, Chung HY, Li Y. Chinese Medicine 2010; 5(23): 1-6.

[35] Chouna JR, Tamokou JDD, Nkeng-Efouet-Elango P, Lenta BN, Sewald N. Antimicrobial triterpenes from the stem bark of *Crossopteryx febrifuga*. Z. Naturforsch. 2015; 70(7-8c): 169-173.

[36] Sidjui LS, Nganso YOD, Toghueo RMK, WAKEU BNK, Dameue JT, Mkoumga P, Adhikari A, Lateef M, Folefoc G, Ali MS. Kostchyienones A and B, new antiplasmodial and cytotoxicity of limonoids from the roots of *Pseudocedrela kotschy* (Schweinf.) Harms. Z. Naturforsch. 2015: 1-8. <https://doi.org/10.1515/znc-2017-0102>

[37] Online Etymology Dictionary (website): <http://www.etymonline.com>; 2013.

[38] Bernard G and Dromard A. Book of etymology and medical terminology: Lexicon etymology (in French). Livret d'étymologie et de terminologie médicale: Lexique d'étymologie, 2011: 1-4.

[39] Alain R. Le Robert micro-poche (2nd Ed) (in french). 1994: 14 and 126.

[40] Cammack R, Atwoot TK, Campbell PN, Parish JH, Smith AD, Stirling JL, Vella F. Oxford Dictionary of Biochemistry and Molecular Biology (2nd Ed). Oxford University Press, Oxford New York 2006: 74-75.

[41] Guaadaoui A, Benaicha S, Elmajdoub N, Bellaoui M, Hamal A. What is a bioactive compound? A combined definition for a preliminary consensus. International Journal of Nutrition and Food Sciences 2014; 3(3): 174-179. Doi: 10.11648/j.ijnfs.20140303.16

[42] Dictionary of Food Science and Technology (2nd Ed). International Food Information Service (IFIS Editor), 2009: 47-48.

[43] Neeraj Varma. Phytoconstituents and Their Mode of Extractions: An Overview. Res. J. Chem. Env. Sci., 2016; 4(2): 08-15.

[44] Biesalski H-K, Dragsted LO, Elmadfa I, Grossklaus R, Muller M,

- Schrenk D, Walter P, Weber P. Bioactive compounds: Definition and assessment of activity. *Nutrition*. 2009; 25: 1202-1205.
- [45] Patel K, Panchal N, Ingle P. Techniques Adopted for Extraction of Natural Products Extraction Methods: Maceration, Percolation, Soxhlet Extraction, Turbo distillation, Supercritical Fluid Extraction. *International Journal of Advanced Research in Chemical Science* 2019; 6(4): 1-12. DOI: <http://dx.doi.org/10.20431/2349-0403.0604001>.
- [46] Zhang Z and Li G. A review of advances and new developments in the analysis of biological volatile organic compounds. *Microchemical Journal* 2010; 95(2):127-139.
- [47] Harborne JB. *Phytochemical methods*. Chapman and Hall 1998, 3rd edition, London. 302 pages.
- [48] Sing CC, Hasnida MN, Siti H M-S, Sarajul FM, Akil A, Waseem AW, Mohd M, Abdullah A. A Glimpse into the Extraction Methods of Active Compounds from Plants. *Critical Reviews in Analytical Chemistry*. 2020; DOI: 10.1080/10408347.2020.1820851
- [49] Hussain MK, Saquib M, Khan MF. Techniques for Extraction, Isolation, and Standardization of Bio-active Compounds from Medicinal Plants. In Swamy MK and Akhtar MS. *Natural Bio-active Compounds. Volume 2: Chemistry, Pharmacology and Health Care Practices*, 2019. Springer Nature Singapore Pte Ltd. (eBook) <https://doi.org/10.1007/978-981-13-7205-6>.
- [50] Handa SS. An Overview of extraction techniques for medicinal and aromatic plants. In Handa SS, Khanuja SPS, Longo G, Rakesh DD. *Extraction technologies for medicinal and aromatic plants*. ICS-UNIDO, Trieste, Italy. 21-52. 2006.
- [51] Chuo S C, Ahmad A, Mohd-Setapar SH, Ripin A. Reverse micelle extraction - an alternative for recovering antibiotics. *Der Pharma Chemica* 2014 ; 6 : 37.
- [52] Jurinjak TA, Benkovic M, Valinger D, Jurina T, Belcak-Cvitanovic A, Gajdos KJ. Optimizing Bioactive Compounds Extraction from Different Medicinal Plants and Prediction through Nonlinear and Linear Models. *Ind. Crops Prod.* 2018; 126: 449-458. DOI: 10.1016/j.indcrop. 2018.10.040
- [53] Visht S and Chaturvedi S. Isolation of natural products. *Current Pharma Research*, 2012, 2(3), 584-599.
- [54] Handa SS, Khanuja SPS, Longo G, Rakesh DD. *Extraction Technologies for Medicinal and Aromatic Plants*, (1st edn), no. 66. Italy: United Nations Industrial Development Organization and the International Centre for Science and High Technology. 2008.
- [55] Rasul MG. Extraction, Isolation and Characterization of Natural Products from Medicinal Plants. *International Journal of Basic Sciences and Applied Computing* 2018; 2(6): 1-6.
- [56] Rassem HA, Nour AH, Yunus MR. Techniques for extraction of essential oils from plants: a review. *Aust J Basic Appl Sci.*, 2016; 10: 117-127.
- [57] Wang L and Weller CL. Recent advances in extraction of nutraceuticals from plants. *Trends in Food Science & Technology* 2006, 17; 300-312.
- [58] Soxhlet F. Die gewichtsanalytische Bestimmung des MilCHFettes. *Dingler's Polytechnisches Journal* (in German) 1879; 232: 461-465.
- [59] Luque de Castro MD and Garcia-Ayuso LE. Soxhlet extraction of solid materials: An outdated technique with a promising innovative future. *Analytica Chimica Acta* 1998; 369: 1-10.

- [60] Mandal V, Mohan Y and Hemalath S. Microwave assisted extraction-an innovative and promising extraction tool for medicinal plant research. *Pharmacognostic Reviews* 2007; 1(1): 7-18.
- [61] Alupului A, Călinescu I, Lavric V. Microwave extraction of active principles from medicinal plants. *U.P.B. Sci. Bull., Series B*, 2012; 74(2): 129-142.
- [62] Castro-López C, Rojas R, Sánchez-Alejo EJ, Niño-Medina G, Martínez-Ávila GCG. Phenolic compounds recovery from grapefruit and by products: an overview of extraction methods. In Morata A and Loira I. *Grape and Wine Biotechnology*. IntechOpen: London, UK, 2016. DOI: 10.5772/64821. Available from: <https://www.intechopen.com/books/grape-and-wine-biotechnology/phenolic-compounds-recovery-from-grape-fruit-and-by-products-an-overview-of-extraction-methods>.
- [63] Chavez-Gonzales ML, Sepulveda L, Verma DK, Luna-Garcia HA, Rodriguez-Duran LV, Ilin A, Aguilar CN. Conventional and emerging extraction processes of flavonoids. *Processes* 2020; 8, 434. 1-30. Doi:10.3390/pr8040434
- [64] Jaitak V, Bikram SB, Kaul VK. An efficient microwave-assisted extraction process of stevioside and rebaudioside-A from *Stevia rebaudiana* (Bertoni), *Phytochem Anal.*, 2009; 20(3): 240-245.
- [65] Bandar H, Hijazi A, Rammal H, Hachem A, Saad Z, Badran B. Techniques for the extraction of bioactive compounds from Lebanese *Urtica dioica*. *American Journal of Phytomedicine and Clinical Therapeutics* 2013; 1(6): 507-513.
- [66] Rodsamran, P.; Sothornvit, R. Extraction of Phenolic Compounds from Lime Peel Waste Using Ultrasonic-Assisted and Microwave-Assisted Extractions. *Food Biosci.* 2019; 28 : 66-73. DOI : 10.1016/j.fbio.2019.01.017
- [67] Chaves JO, De Souza MC, Da Silva LC, Lachos-Perez D, Torres-Mayanga PC, Machado APF, Forster-Carneiro T, Vasquez-Espinoza M, Gonzalez-de-Peredo AV, Barbero GF, Rostagno MA. Extraction of flavonoids from natural sources using modern techniques. *Frontiers in Chemistry* 2020; 8(507887): 1-20. Doi: 10.3389/fchem.2020.507887.
- [68] Richter BE, Jones BA, Ezzell JL, Porter NL, Avdalovic N, Pohl C. Accelerated solvent extraction: a technique for sample preparation. *Analytical Chemistry* 1996; 68(6): 1033-1039
- [69] Wu K, Ju T, Deng Y, Xi J. Mechanochemical assisted extraction: a novel, efficient, eco-friendly technology. *Trends in Food Science & Technology* 2017, 66: 166-175.
- [70] Suan L. A review on plant-based rutin extraction methods and its pharmacological activities. *Journal of Ethnopharmacology* 2013; 150(3): 805-817.
- [71] Wijngaard, H., Hossain, M. B., Rai, D. K., and Brunton, N. (2012). Techniques to extract bioactive compounds from food by-products of plant origin. *Food Res. Int.* 46, 505-513. doi: 10.1016/j.foodres.2011. 09.027
- [72] Evstafev SN and Chechikova EV. Transformation of wheat straw polysaccharides under dynamic conditions of subcritical autohydrolysis. *Russ. J. Bioorg. Chem.* 2016; 42: 700-706. Doi: 10.1134/S1068162016070050
- [73] Lachos-Perez D, Brown AB, Mudhoo A, Martinez J, Timko MT, Rostagno MA, Forster-Carneiro T. Applications of subcritical and supercritical water conditions for

extraction, hydrolysis, gasification, and carbonization of biomass: a critical review. *Biofuel Research Journal* 2017; 4:611-626. Doi: 10.18331/BRJ2017.4.2.6

[74] Plaza, M., and Turner, C. Trends in analytical chemistry pressurized hot water extraction of bioactives. *Trends Anal. Chem.*, 2015; 71, 39-54. Doi: 10.1016/j.trac.2015.02.022

[75] Lv GP, Huang WH, Yang FQ, Li J, Li SP. Pressurized liquid extraction and GC–MS analysis for simultaneous determination of seven components in *Cinnamomum cassia* and the effect of sample preparation. *Journal of Separation Science* 2010; 33(15): 2341-2348. <https://doi.org/10.1002/jssc.201000208>

[76] Verma, D.K.; Dhakane, J.P.; Mahato, D.K.; Billoria, S.; Bhattacharjee, P.; Srivastav, P.P. Supercritical Fluid Extraction (SCFE) for Rice Aroma Chemicals: Recent and Advance Extraction Method. In *Science and Technology of Aroma, Flavour and Fragrance in Rice*; Verma, D.K., Srivastav, P.P., Eds.; Apple Academic Press: Waretown, NJ, USA, 2018; pp. 179-198.

[77] Bubalo MC, Vidovic S, Redovnikovic IR, Jokic S. New perspective in extraction of plant biologically active compounds by green solvents. *Food and Bioproducts Processing* 2018. DOI: <https://doi.org/10.1016/j.fbp.2018.03.001>

[78] Conde-Hernández LA, Espinosa-Victoria JR, Trejo A, Guerrero-Beltrán JÁ. CO₂-supercritical extraction, hydrodistillation and steam distillation of essential oil of rosemary (*Rosmarinus officinalis*). *J Food Eng.* 2017; 200: 81-86.

[79] Khaw KY, Parat MO, Shaw PN, Falconer JR. Solvent supercritical fluid technologies to extract bioactive compounds from Natural sources: A

review. *Molecules* 2017; 22: 1186: 1-22. Doi: 10.3390/molecules22071186

[80] De Luna SL, Ramirez-Garza RE, Salvidar SOS. Environmentally freiendly methods for flavonoids extraction from plant material: Impact of their operating conditions on yield and antioxidant properties. *The Scientific World Journal* 2020, 2020(6792069): 1-38. Doi. [org/10.1155/2020/6792069](https://doi.org/10.1155/2020/6792069)

[81] Bonizou E, Karageorgou I, Batra G, Dourtoglou VG, Lalas SI. Pulse electric field extraction of antioxidant activity determination of *Moringa oleifera* dry leaves: a comparative study with other extraction technique. *Beverages*. 2019; 5(8): 1-13. Doi:10.3390/beverages5010008

[82] Tzanova M, Atanasov V, Yaneva Z, Ivanova D, Dinev T. Selectivity of current extraction techniques for flavonoids from plant materials. *Processes* 2020, 8, 1222:1-30. Doi:10.3390/pr8101222.

[83] Martel JP. Device for High Speed Production of Aromatic Essential Oils from Perfume-Generating Plants or Parts Thereof. U.S. Patent 4,406,745, 27 September 1983.

[84] Périno S, Chemat-Djenni Z, Petitcolas Emmanuel, Giniès C, Chemat F. Downscaling of Industrial Turbo-Distillation to Laboratory Turbo-Clevenger for Extraction of Essential Oils. Application of Concepts of Green Analytical Chemistry. *Molecules* 2019; 24: 2734. Doi:10.3390/molecules24152734w.

[85] Sonaglio D, Ortega GG, Petrovick PR, Bassani VL. Desenvolvimento tecnológico de produção de fitoterápicos. In C.M.O. Simões, *et al.* (Eds.), *Farmacognosia: Da planta ao medicamento*, UFRGS/UFSC, Porto Alegre/Florianópolis (2003), pp. 289-326.

- [86] Martins PM, Lanchote AD, Thorat BN, Freitas LAP. Turbo-extraction of glycosides *Stevia rebaudiana* using a fractional factorial design. *Revista Brasileira de Farmacognosia* 2017; 27(4): 510-518. Doi: 10.1016/J.Bjp.2017.02.007
- [87] Arsenault JC. Beginner's guide to SPE Solid-Phase Extraction. Waters Corporation. Water, Sep-Pak, Oasis, UPLC, Nova-Pak, 34 Maple street, Milford, MA 01757. 2012.
- [88] Sridhar A, Ponnuchamy M, Kumar PS, Kapoor A, Vo D-VN. Techniques and modeling of polyphenol extraction from food: a review. *Environmental Chemistry Letters* 2021; 1-35. [Hhttps://doi.org/10.1007/s10311-021-01217-8](https://doi.org/10.1007/s10311-021-01217-8)
- [89] Martinez-Correa HA, Bitencourt RG, Kayano AC, Magalhaes PM, Costa FTM, Cabral FA. Integrated extraction process to obtain bioactive extracts of *Artemisia Annua* L. Leaves using Supercritical CO₂, Ethanol and Water. *Ind. Crops Prod.* 2017; 95: 535-542. DOI: 10.1016/j.indcrop.2016.11.007
- [90] Tamborrino, A.; Romaniello, R.; Caponio, F.; Squeo, G.; Leone, A. Combined Industrial Olive Oil Extraction Plant Using Ultrasounds, Microwave, and Heat Exchange: Impact on Olive Oil Quality and Yield. *J. Food Eng.* 2019, 245, 124-130. DOI: 10.1016/j.jfoodeng.2018.10.019
- [91] Fongang FYS and Bankeu KJJ. Terpenoids as Important Bioactive Constituents of Essential Oils. [Online First], IntechOpen 2020, DOI: 10.5772/intechopen.91426. Available from: <https://www.intechopen.com/online-first/terpenoids-as-important-bioactive-constituents-of-essential-oils>.
- [92] Benmoussa H, Elfalleh W, He S, Romdhane M, Benhamou A, Chawech R. Microwave hydrodiffusion and gravity for rapid extraction of essential oil from Tunisian cumin (*Cuminum cyminum* L.) seeds: optimization by response surface methodology. *Industrial Crops & Products* 2018; 124: 633-642. Doi: 10.1016/j.indcrop.2018.08.036
- [93] Dawidowicz AL, Rado E, Wianowska D, Mardarowicz M, Gawdzik J. Application of PLE for the determination of essential oil components from *Thymus Vulgaris* L. *Talanta* 2008; 76: 878-884.
- [94] Fahlbusch KG, Hammerschmidt FJ, Panten J, Pickenhagen W, Schatkowski D, Bauer K, Garbe D, Surburg H. *Flavors and Fragrances. Ullmann's Encyclopedia of Industrial Chemistry* 2003. Doi:10.1002/14356007.a11_141
- [95] Meyer-Warnod B. Natural essential oils: extraction processes and application to some major oils. *Perfume. Flavorist* 1984, 9: 93-104.
- [96] Arnould TWE. *Aromatherapy for the Whole Person*. UK: Stanley Thornes 1981, pp: 22-26.
- [97] Yousefi M, Rahimi-Nasrabadi M, Pourmortazavi SM, Wysokowski M, Wysokowski T, Ehrlich H, Mirsadeghi S. Supercritical fluid extraction of essential oils. *Trends in Analytical Chemistry* 2019; 118: 182-193. Doi: 10.1016/j.trac.2019.05.038
- [98] Ghouila Z, Sehailia M, Chemat F. Vegetable oils and fats: extraction, composition and applications. In Li Y and Chemat F. plant based "Green chemistry 2.0". moving from evolutionary to revolutionary. Springer Nature Singapore Ltd. 2019. Ebook ISBN 978-981-13-3809-0. Doi: 10.1007/978-981-13-3810-6.
- [99] Ogori AF. Source, extraction and constituents of fats and oils. *Journal of*

Food Science and Nutrition 2020, 6;
060: 1-8. Doi: 10.24966/
FSN-1076/100060

[100] Chemat F, Fabiano-Tixier AS,
Vian MA, Allaf T, Vorobiev E. Solvent-
free extraction of food and natural
products. TrAC Trends Anal. Chem
2015; 71: 157-168.

[101] Cakaloglu B, Ozyurt VH, Otles S.
Cold press in oil extraction. A review.
The Ukrainian Food Journal 2018; 7(4):
640-654.

[102] Virgilio P, Tuomas H, Marjukka K,
Risto S, Jussi H, Linnanen L.
Sustainability of palm oil production
and opportunities for finnish
technology and know-how transfer.
Lappeenranta University of Technology
Press 2009.

[103] Gunstone F. Palm oil supplying
much of world demand for fats and oils.
INFORM 2001, 12:141.

[104] Gerken HG, Donohoe B,
Knoshaug EP. Enzymatic cell wall
degradation of *Chlorella vulgaris* and
other microalgae for biofuels
production. Planta 2013, 237:239-253.
[https://doi.org/10.1007/
s00425-012-1765-0](https://doi.org/10.1007/s00425-012-1765-0)

[105] Shankar D, Agrawal YC, Sarkar BC,
Singh BPN. Enzymatic hydrolysis in
conjunction with conventional
pretreatments to soybean for enhanced
oil availability and recovery. J Am Oil
Chem Soc 1997; 74:1543-1547.

[106] Zheng X-Q, Li Q.-S, Xiang L-P,
Liang Y-R. Recent advances in volatiles
of teas. Molecules 2016, 21, 338.

[107] Yang Z, Baldermann S,
Watanabe N. Recent studies of the
volatile compounds in tea. Food Res. Int.
2013, 53, 585–599.

[108] Villamar-Torres R, Jazayeri SM,
Luiba-Delfini G, Cruzaty LCG, Viot

C-R. Volatile organic compounds: plant
natural defense mechanisms against
herbivorous arthropods and an
opportunity for plant breeding of
cotton. Scientia Agropecuaria 2018,
9(2): 287-297. Doi: 10.17268/sci.
agropecu.2018.01.14.

[109] War AR, Paulraj MG, Ahmad T,
Buhroo AA, Hussain B, Ignacimuthu S,
Sharma HC. Mechanisms of plant
defense against insect herbivores. Plant
Signaling and Behavior 2012, 7(10):
1306-1320.

[110] Adamova T, Hradecky J, Panek M.
Volatile organic compounds (VOCs)
from wood and wood-based panels:
methods for evaluation, potential health
risks, and mitigation. Polymers 2020;
12(2289): 1-21. Doi: 10.3390/
polym12102289

[111] Verde GMV, Barros DA,
Oliviera MS, Aquino GLB, Santos DM,
De Paula JR, Dias LD, Pinero M,
Pereira MM. A green protocol for
microwave-assisted extraction of
volatile oil terpenes from *Pterodon*
emarginatus Vogel. (Fabaceae).
Molecules 2018, 23,651: 1-12. Doi:
10.3390/molecules23030651.

[112] Mukherjee PK. Quality control of
herbal drugs: an approach to evaluation
of botanicals, 1st edition. Business
Horizons Pharmaceutical Publishers;
India: 2002.

[113] Dermarderosian A. The Review of
Natural Product, 1st edition. Fact and
comparisons; St. Louis, Missouri: 2001.

[114] Cordell, G. A. Introduction to the
Alkaloids: A Biogenetic Approach.
Wiley-Interscience, New York, 1981.

[115] Hesse, M. Alkaloids: Nature's Curse
or Blessing? Wiley-VCH, Weinheim,
Germany, 2002.

[116] Shinde RR and Shinde NH.
Extraction of caffeine from Coffee and

preparation of anacin drug.
International Journal of Engineering
research and Technology 2017; 10(1):
236-239.

[117] Subila S and Sirley N.
Determination of Caffeine in different
tea samples. IOSR, Journal of Applied
Chemistry 2016; 9(1): 75-78.

[118] Chaugule A, Patil H, Pagariya S,
Ingle P. Extraction of Caffeine.
International Journal of Advanced
Research in Chemical Sciences 2019;
6(9): 11-19. DOI : <http://dx.doi.org/10.20431/2349-0403.0609002>

[119] Tello J, Viguera M, Calvo L.
Extraction of caffeine from Robusta
coffee (*Coffea canephora* var. Robusta)
husks using supercritical carbon
dioxide. Journal of Supercritical Fluids
2011; 59: 53-60.

[120] Barbier A. The extraction of
Opium alkaloids. United Nations Office
on Drugs and Crime 1950; 3: 22-29.

[121] Mehtretter CL and Weakley FB.
Extraction of morphine from Poppy
capsules and its recovery by ion
exchange. Journal of the American
Pharmaceutical Association (Scientific
ed.) 1957; 46(3):193-196.

[122] Kalia AN. Textbook of industrial
pharmacognosy, 1st edition. CBS
Publishers and Distributors Pvt. Ltd;
Noida, India: 2005.

[123] Mohammed A. Pharmacognosy
and Phytochemistry, 1st edition. CBS
Publishers and Distributors Pvt. Ltd;
Noida, India: 2008.

[124] Khoddami A, Wilkes MA,
Roberts TH. Techniques for analysis of
plant phenolic compounds. Molecules
2013; 18: 2328-2375. Doi:10.3390/
molecules18022328

[125] Qing-Wen Z, Li-Gen L, Wen-Cai Y.
Techniques for extraction and isolation
of natural products: a comprehensive

review. Chinese Medecine 2018; 13(20):
1-26. <https://doi.org/10.1186/s13020-018-0177-x>

[126] Panche AN, Diwan AD,
Chandra SR. Flavonoids: An overview. J.
Nutr. Sci. 2016; 5: e47.

[127] Rana AC and Gulliya B. Chemistry
and Pharmacology of Flavonoids—A
Review. Indian J. Pharm. Educ. Res.
2019; 53: 8-20.

[128] Garcia-Salas, P.; Morales-Soto, A.;
Segura-Carretero, A.; Fernández-
Gutiérrez, A. Phenolic compound-
extraction systems for fruit and
vegetable samples. Molecules 2010; 15:
8813-8826.

[129] Cowan MM. Plant products as
antimicrobial agents. Clin. Microbiol.
Rev. 1999; 12: 564-582.

[130] Anokwuru CP, Anyasor GN,
Ajibaye O, Fakoya O, Okebugwu P.
Effect of extraction solvents on
phenolic, flavonoid and antioxidant
activities of three nigerian medicinal
plants. Nat. Sci. 2011; 9: 53-61.

[131] Bohlin L. Natural Products
Isolation, Drug Discovery Today. 1998;
3(12): 536-537.

[132] Hostettmann K, Hostettmann M,
Marston A. Saponins: in Terpenoids
(Charlwood B. V., Banthorpe D. V.,
eds.), Methods in Plant Biochemistry
1991. Dey PM and Harborne JB eds., 7,
Academic Press, San Diego, CA, pp.
435-471.

[133] Klyne W. The Chemistry of the
Steroids. Wiley, New York, 1957.