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Plants' Bioactive Metabolites and Extraction Methods

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and Mohammed Nedjhioui*

Abstract

Plants are an inexhaustible source of bioactive compounds that have been utilized by Men since antiquity. Plants' bioactive substances are called primary and secondary metabolites. Primary metabolites are dedicated to biological functions and survival. Secondary metabolites are luxury products synthesized mainly to defend against predators and to communicate with other plants. Medicinal plants have ever since been a growing source of interest for scientific research as well as food, chemical, and pharmaceutical for their diverse applications for their anti-oxidant, antibacterial, stimulant, and inhibiting properties. Obtaining secondary metabolite can be challenging as the inadequate choice of extraction methods could alter or destroy bioactive compounds such as thermolabile substances. Hence, a beforehand knowledge of secondary metabolites properties and a mastery of extraction methods are primordial for effective extraction. Extraction methods range from conventional extraction such as solvent extraction to modern techniques like enzymatic assisted extraction EAE and microwave-assisted extraction MAE, which are more efficient methods and more environmentally friendly as they require very little to no use of solvents.

Keywords: plants' metabolites, essential oils, lipids, alkaloids, phenolic compounds, extraction, green extraction

1. Introduction

Plants produce a wide range of bioactive molecules that serve many purposes. Those that are destined for biological functions and surviving are called primary metabolites. On the other hand, those compounds that are luxury products are called secondary metabolites. They serve as extra nutritional and may have adverse effects on other living organisms. Secondary metabolites have been used since antiquity in traditional medicine due to their benefits on human health. They are potent antioxidants, anticancer, antidiabetic, anti-inflammatory, stimulants... Their use has witnessed a dramatic increase in pharmaceutical, chemical, and food industries for the advantages they present. However, obtaining these compounds is a challenging process as they represent a small portion of plants raw material.

Diverse extraction methods, from conventional methods such as solvent extraction to modern techniques like enzymatic assisted extraction EAE, have been used to improve extraction yield in terms of quality and quantity as well as environmental safety. This chapter discusses the main active substances categories, their role for plants and men as well as the different extraction methods used to obtain them.

2. Essential oils

2.1 Definition

Essential oils (EO) are complex mixtures of volatile, lipophilic, and liquid compounds, extracted from different parts of a plant by physical processes. These interesting natural products have the characteristic odor of the plant.

According to ISO norm (ISO/D159235) "An essential oil is a product made by distillation with either water or steam or by mechanical processing of citrus rinds or by dry distillation of natural material. Following the distillation, the essential oil is physically separated from the water phase."

Essential oils are highly concentrated substances and therefore rarely used neat [1], they evaporate slowly when exposed to air at room temperature, and because of this, they are sometimes referred to as volatile oils [1].

EO's quality depends on many factors:

- Origin and parts of the plant used.
- Harvest way and extraction technique.
- Purification process.

2.2 Essential oils composition

The EOs are composed of terpenoids in large proportion, phenylpropanoids, and other compounds of diverse origin such as that resulting from the degradation of fatty acids.

2.2.1 Terpenoids

The Terpenoids represent the major part of essential oil compounds; they are defined as substances composed of isoprene (2-methylbutadiene) units [2]; they are synthesized by acetate via the mevalonic acid pathway.

The Terpenoids are classified according to the number of C₅ isoprene units that they contain; the classes are:

2.2.1.1 Hemiterpenes C₅

Hemiterpenes are the simplest type of terpenoids; they consist of a single unit of isoprene. There is evidence that these compounds may assist in plant defense by repelling herbivores or by attracting predators and parasites of herbivores [3].

2.2.1.2 Monoterpenes C₁₀

Monoterpenes are compounds made up of two units of isoprene, and of chemical formula C₁₀H₁₆, among the classes included in this group of terpenoids: iridoids and pyrethrins; they have anti-inflammatory pharmacological properties.

2.2.1.3 Sesquiterpenes C₁₅

This class of terpenes has a crude formula C₁₅H₂₄ (three units of isoprene), these molecules are found in linear or cyclic form; they have several applications but are rarely found in EOs because of their low volatility.

2.2.1.4 Diterpenes C20

Diterpenes consist of a group of chemically heterogeneous compounds, their crude formula is C₂₀H₃₂ (four units of isoprene). Because of their higher boiling points, they are not considered to be essential oils; instead, they are classically considered to be resins, the material that remains after steam distillation of a plant extract [3].

Diterpenes can be classified as linear, bicyclic, tricyclic, tetracyclic, pentacyclic, or macrocyclic Diterpenes depending on their skeletal core [4].

2.2.1.5 Sesterterpenes C25

Sesterterpenes consist of 5 units of isoprene (25 carbon backbone). They exist in a wide variety of forms, including linear, monocyclic, bicyclic, tricyclic, tetracyclic, and macrocyclic frameworks [4].

2.2.1.6 Triterpenes C30

The triterpenes are made up of six isoprenes, they have relatively complex cyclic structures.

There are also tetraterpenes which consist of 8 units of isoprene (C₄₀H₆₄); and when the number of isoprene units exceeds 8 units, terpenes are called polyterpenes (C > 40).

2.2.2 Phenylpropanoids

The phenylpropanoids are a family of organic compounds with an aromatic ring and a three-carbon propene tail [5]; they are synthesized via shikimic acid pathway (biosynthesis).

2.3 Essential oils properties

The different properties of OEs are related to the species and their chemical composition.

2.3.1 Physicochemical properties

Essential oils are characterized by several physicochemical properties:

- The EOs are known by their volatile, odorous, and flammable character.
- They are colorless or have a yellowish color in the liquid state and at ambient temperature.
- They have a low polarity and a low solubility in water however they are soluble in alcohol and most of the organic solvents.
- They have a lower density than water (0.8 to 1.08).

2.3.2 Biological properties

Essential oils have various biological properties due to the variety of their chemical composition, they are using as:

- Antimicrobial;
- Antioxidant;
- Anti-inflammatory;
- Antiseptic

3. Lipids

3.1 Definition

Unlike the various families of basic molecules in the living world characterized by chemical structures, lipids are defined by their solubility not on the basis of their chemical structure [6].

Lipids are among the primary metabolites, they consist of a heterogeneous group of compounds characterized by their insolubility in water, on the other hand they are soluble in non-polar organic solvents such as: chloroform and alcohols.

Lipids play mainly important roles in plants, as signaling and energy storage compounds [7].

3.2 Classification

Lipids mainly include fatty acids, simple lipids and complex lipids.

3.2.1 Fatty acids

The fatty acids are carboxylic acids $R\text{-COOH}$; radical R is an aliphatic chain of hydrocarbon type of variable length which gives the molecule its hydrophobic character (fatty).

They are generally mono carboxylic, and characterized by a linear chain with an even number of carbons [8].

3.2.1.1 Saturated fatty acids

A fatty acid consists of a hydrocarbon chain, more or less long, strongly apolar, and a polar carboxyl group. The general formula of fatty acids is $\text{CH}_3 - (\text{CH}_2)_n - \text{COOH}$ [8]. The most abundant fatty acids are: palmitic acid and stearic acid (**Figure 1**).

3.2.1.2 Unsaturated fatty acids

The structure of unsaturated fatty acids includes one or more double bonds; the presence of these double bonds gives them specific physicochemical properties (**Figure 2**) [8].

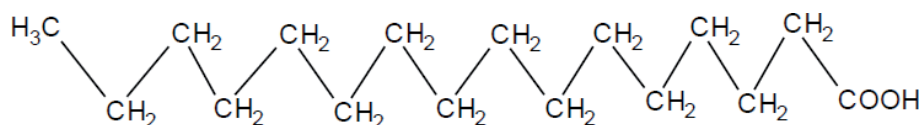


Figure 1.
Palmitic acid.

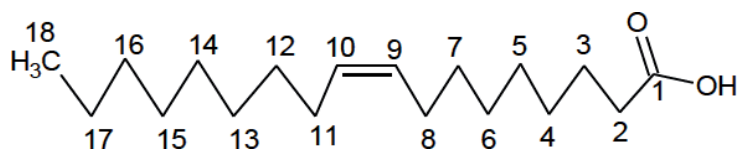


Figure 2.
Oleic acid.

3.2.1.3 Atypical fatty acids

Atypical fatty acids are characterized by an odd number of carbons; they are present in animal fats or in microbial lipids [8].

3.2.2 Simple lipids

Simple lipids or homolipids are ternary bodies (C, H, O), they are fatty acid esters, depending on the alcohol we distinguish the following classes [8]:

- **Acylglycerols:** or glycerides are esters of glycerol,
- **Cerides:** long chain alcohol esters (fatty alcohol),
- **Sterids:** sterol esters (polycyclic alcohol).

3.2.3 Complex lipids

Complex lipids are hetero lipids which contain phosphate, sulfate or carbohydrate groups. They are classified according to the molecule that fixes fatty acids [8].

3.2.3.1 Glycerophospholipids

These are the most numerous and most represented lipids which are constructed from the skeleton of a glycerol mono ester (**Figure 3**) [8].

3.2.3.2 Glyceroglycolipids

The alcohols of the C1 and C2 carbons of glycerol are esterified by fatty acids and the C3 carbon alcohol, unlike the glycerolipids, is not esterified, but it is linked to an ose by a glycosidic bond (**Figure 4**) [8].

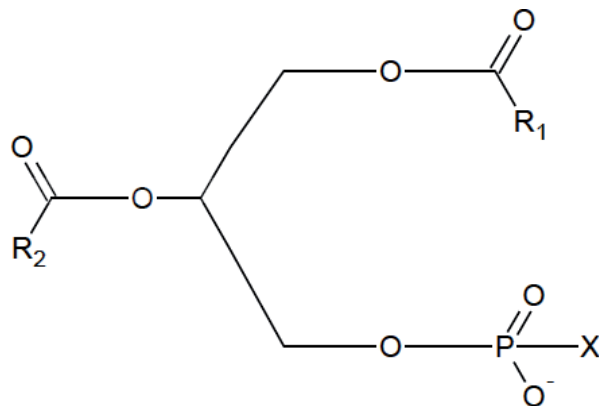


Figure 3.
Glycerophospholipids.

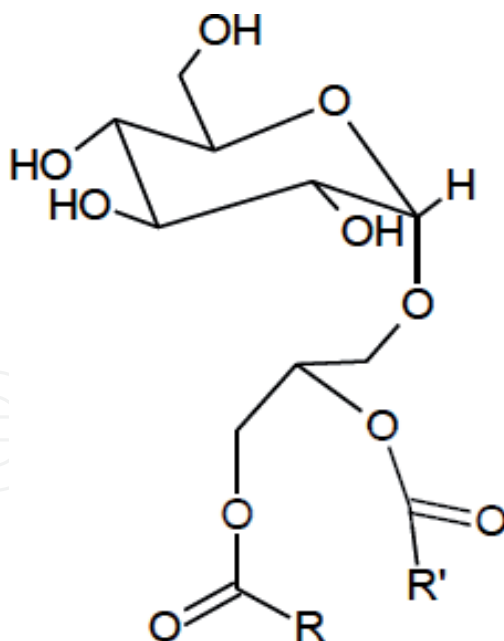


Figure 4.
Glyceroglycolipids.

3.2.3.3 Sphingolipids

The skeleton from which these lipids are made is not glycerol but a long-chain carbon diol amine of the sphingoid type; the binding of a fatty acid to the amine group gives a ceramide which is the lipid precursor molecule of this group [8].

4. Alkaloids

Alkaloids are natural compounds with low molecular weight [9] that are characterized by the presence of nitrogen atoms. They are mostly found in plants as secondary metabolites and some other animals and fungi [10].

They are derived from amino acids containing one or more heterocyclic nitrogen atoms [11]. Alkaloids have played a huge role in traditional medicine in various civilizations since antiquity due to their potential therapeutic properties. Toxic alkaloids were similarly used in poisonous arrows [12]. However, it was until 1804 that they were isolated and characterized by Friedrich Sertürner [13] and Derosne [9].

4.1 Classification

Due to their diverse structures, finding a common classification was complicated [14]. Some studies considered a classification based on the ring systems: indolizidine- and quinolizidine-based systems and quinoline-, quinazoline-, and acridone-based systems (**Figure 5**) [11]. Yet, this classification mode causes confusion as some alkaloids can be categorized in more than one class [15]. Henceforth, a new classification was adopted [9]:

True alkaloids: The nitrogen atom, originating from the precursor amino acid, is part of the alkaloid heterocyclic ring.

Protoalkaloids: The nitrogen atom, originating from the precursor amino acid, is not part of the alkaloid heterocyclic ring.

Pseudoalkaloids: They are not originated from amino acids, including steroids-like, purine-like and terpene-like alkaloids. Each class is divided to subclasses according to the precursor amino acid.

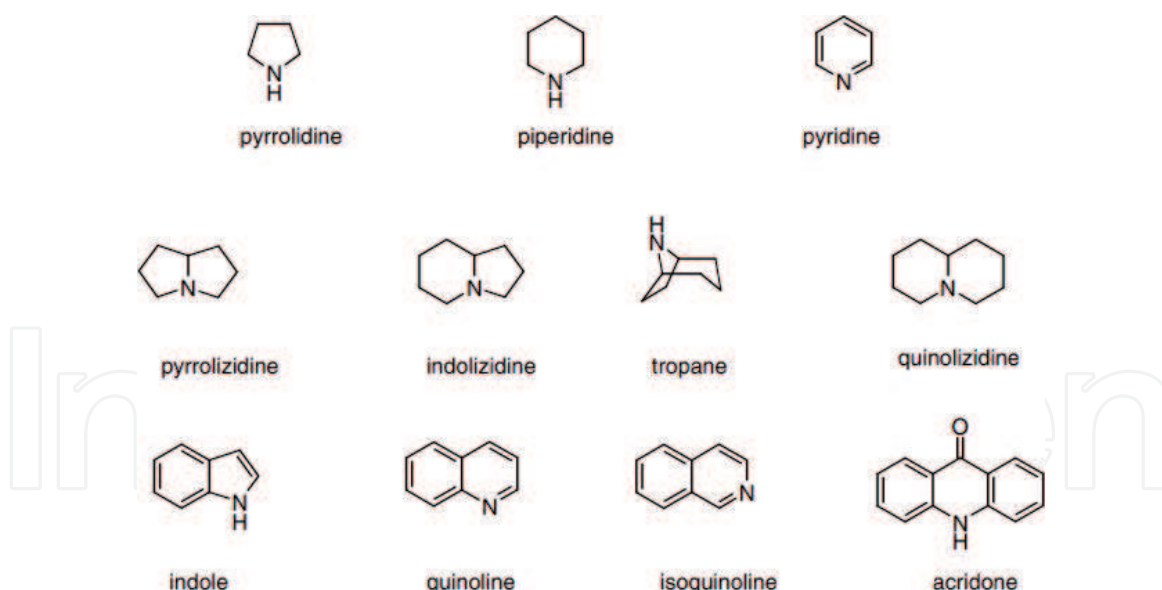


Figure 5.
 Alkaloids classes based on ring systems [11].

4.2 Therapeutic usefulness

Alkaloids have a wide range of potential therapeutic activities. Morphine has narcotic effect, cocaine is a potent central nervous system stimulant, taxol as an effective anticancer chemical, colchicine as an anti-inflammatory [11], tubocurarine is a poisonous alkaloid used to relax muscles prior to surgeries [16], vinblastine, one of the antitumor alkaloids, and quinine being an effective anti-malarial [17].

4.3 Role for plants

Despite the various advantages they present for Man, their role in the plant is not well known. Some studies suggest that they help the process of seed formation [17] and protect the plant against herbivores and unwanted plants [18] whereas some others propose they are waste products [17].

4.4 Extraction methods

There exist as many extraction methods as the diversity of alkaloids structures [15]. They are extracted exploiting their solubility in organic solvents and water for their salts [19]. Base or acidic extractions followed by purification are widely used to obtain alkaloids.

Base extraction consists of the use of alkaline solvents including 1,2-dichloroethane, chloroform, diethyl ether or benzene. Base Alkaloids are then converted to salts by the addition of weak acids and are washed with water to crystallize. The purification method is repeated until the desired purity is reached.

Acidic extraction, on the other hand, processes the finely grounded raw plant material with weak acidic solutions mentioning acetic acid in water, methanol or ethanol... followed by the addition of basic solutions to convert alkaloids to basic forms to allow their extraction with organic solvents. The same purification steps are followed [14].

Different alkaloids are separated based either on the difference of solubility or boiling temperature [19].

5. Phenolic compounds

Phenolics are organic compounds containing one aromatic ring to which is attached one or more hydroxyl groups [11]. Phenolic compounds represent one of the most extensive groups of plants' secondary metabolites [20]. They are contained in a great share in the daily diet due to their countless benefits for human health and well-being. Henceforth, the consumption of phenolic-rich fruits and vegetables, such as berries and spinach, is increasingly recommended [20].

They are synthesized either through the shikimic acid or the acetic acid pathways. Phenylpropanoids are the result compounds of the shikimic acid pathway while simple phenols result from the acetic acid pathway acid. However, both pathways result in the formation of flavonoids, abundant phenolic compounds [21].

In addition to the presence of aromatic rings and hydroxyl substitutions, phenolic compounds are less likely to be found in the free form, rather, they are most frequently bound to other complex compounds as sugars to reduce their toxicity [21].

5.1 Classification

Due to the wide variety of phenolic compounds ranging from simple to polymeric, there exist multiple classifications:

- Based on the carbon chain:

From simple phenols to complicated lignins, 16 different classes can be distinguished. The main classes are shown in **Figure 6**.

- Based on solubility:

Depending on the form phenolics are found in plants (either free from any bound to other compounds or attached to cell wall polysaccharides or proteins), their solubility varies. Therefore, two categories can be defined:

- Soluble phenolics: Free phenolics found in the soluble fraction of the cell such as phenols, flavonoids, and tannins.
- Insoluble phenolics: Bound to other more complicated molecules to form stable insoluble compounds. They majorly include condensed tannins and phenolic acids.

- Based on distribution:

Phenolic compounds are found in nature in different proportions. They thus can be divided to shortly distributed (simple phenols, pyrocatechol, hydroquinone, resorcinol ...), widely distributed (flavonoids and their derivatives, coumarins, and phenolic acids) and polymers (lignins and tannins) [21].

5.2 Main classes

5.2.1 Simple phenols

They are monomers constituted of various substitutions and represent the forming blocs of polymeric phenolic and acid compounds that make up the plant tissue. Some of them (p-hydroxybenzoic acid, protocatechuic acid, vanillic, syringic,


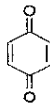


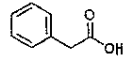
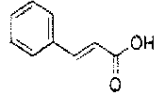

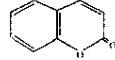

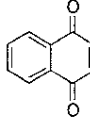
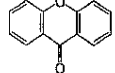
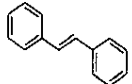
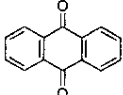
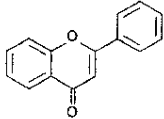
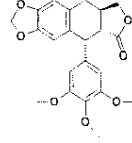
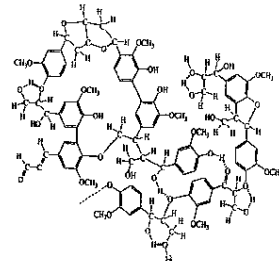
Class	Basic skeleton	Basic structure
Simple phenols	C ₆	
Benzoquinones	C ₆	
Phenolic acids	C ₆ -C ₁	
Acetophenones	C ₆ -C ₂	
Phenylacetic acids	C ₆ -C ₂	
Hydroxycinnamic acids	C ₆ -C ₃	
Phenylpropenes	C ₆ -C ₃	
Coumarins, isocoumarins	C ₆ -C ₃	
Chromones	C ₆ -C ₃	
Naphthoquinones	C ₆ -C ₄	
Xanthenes	C ₆ -C ₁ -C ₆	
Stilbenes	C ₆ -C ₂ -C ₆	
Anthraquinones	C ₆ -C ₂ -C ₆	
Flavonoids	C ₆ -C ₃ -C ₆	
Lignans and neolignans	(C ₆ -C ₃) ₂	
Lignins	(C ₆ -C ₃) _n	

Figure 6.
Classes of phenolic compounds [26].

salicylic, and gallic acids) can be obtained by the hydrolysis of the plant tissue whereas some other free simple phenols do not require the destruction the cell wall polymers (**Figure 7**) [11].

5.2.2 *Flavonoids*

They are compounds containing two aromatic rings attached by a bridge of triple carbon atoms (C₆-C₃-C₆). The bridge often contains double carbon bonds and hetero- elements, which forms another mid-cycle. Therefore, flavonoids can be classified into 13 subgroups among which flavanols, flavones, isoflavones, anthocyanidins or anthocyanins, and flavanones (**Figure 8**) [21].

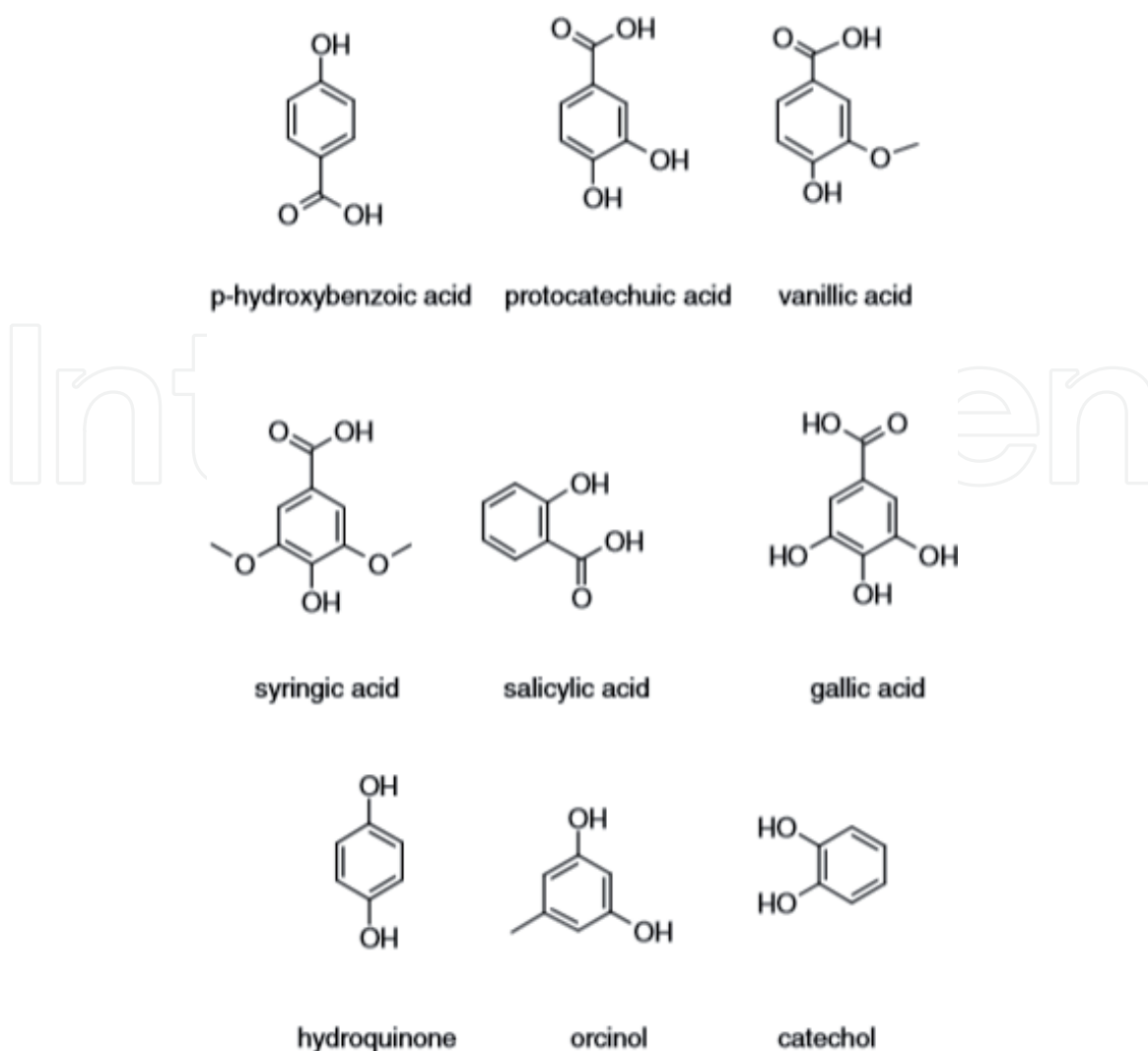


Figure 7.
Simple phenols [11].

5.2.3 Tannins

They are compounds with a medium to high molecular weight that comprise two major classes: hydrolysable and condensed tannins. Hydrolysable tannins are glucose or a polyhydric alcohol centered compounds esterified with gallic acid or hexahydroxydiphenic acid to form gallotannin and ellagitannins, respectively. They are hydrolyzed by enzymatic, acidic or base treatment. On the other hand, condensed tannins, also known as non-hydrolyzable tannins or recently proanthocyanidins, are polymers of catechin and leucoanthocyanidin. Their antioxidant activity is dependent on their degree of polymerization. High molecular weight tannins have been demonstrated to be fifteen to thirty times more active than simple phenols (**Figure 9**) [21].

5.2.4 Phenolic acids

As their name implies, phenolic acids are characterized by the presence of a phenolic ring and a carboxylic group. They comprise two categories:

- Benzoic acids:

They are the simplest phenolic acids in nature. They contain seven carbon atoms C6-C1 with hydroxyl or methoxyl roots.

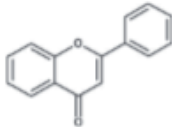
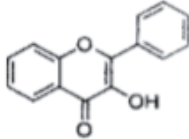
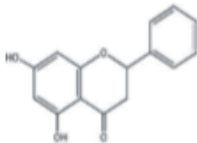
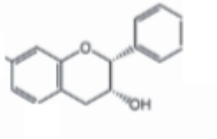
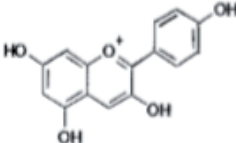
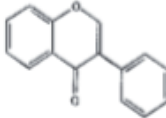
Flavonoid	Basic structure
Flavones	
Flavonols	
Flavanones	
Flavanols	
Anthocyanidins	
Isoflavones	

Figure 8.
Main classes of flavonoids [21].

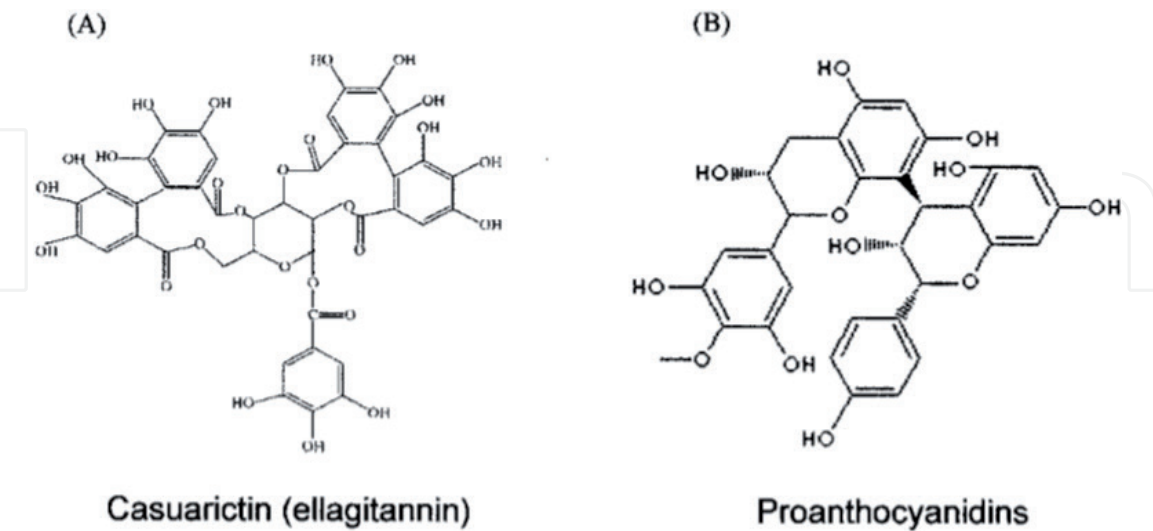


Figure 9.
Chemical structures of (A) hydrolysable tannins and (B) condensed tannins [21].

- Cinnamic acids:

They contain nine carbon atoms C6-C3 with hydroxyl or methoxyl roots.

Their nomenclature varies according to the nature of roots attached to the main ring [21].

5.3 Therapeutic usefulness

Phenolic compounds hold a variety of potential therapeutic properties ranging from antioxidant activity, anticancer [22], bacteriostatic, liver-protecting, anti-infection, cholesterol-lowering, immunity enhancement properties [23], cardio-protective and vasodilatory influences [20]. They have increasingly been part of the human diet for centuries for their benefits through the consumption of fruits and vegetables [21, 24].

5.4 Role for plants

Phenolics stand of great importance to plants. Not only are they responsible for the protection of the plants against exterior hazards [20], but they are also crucial to physiology and cellular metabolism. They play a key role in sensorial traits such as the plants' color and aroma, germination of seeds and reproduction [21].

6. Extraction methods

6.1 Hydrodistillation

6.1.1 Principle of the method

Hydrodistillation is a simple extraction technique which consists in putting in a flask proportional quantity of distilled water and the plant, then heating until boiling; the rising vapor condenses using a refrigerant to recover the distillate.

6.1.2 Mounting

See **Figure 10**.

6.2 Solvent extraction

This method consists in bringing the plant material into contact with the appropriate solvent; this protocol is carried out cold or hot. Among the most used solvents for the extraction of natural products from plant elements: petroleum ether, methanol, ethanol, and hexane. ne of the glassware used for this extraction technique is soxhlet (**Figure 11**).

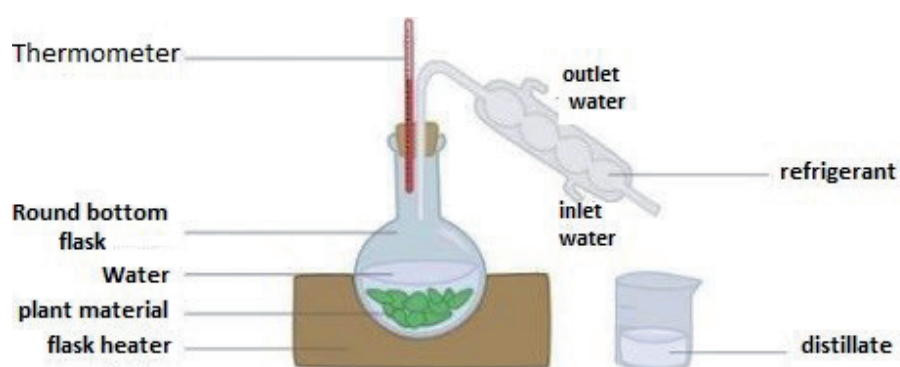


Figure 10.
Installation of Hydrodistillation.

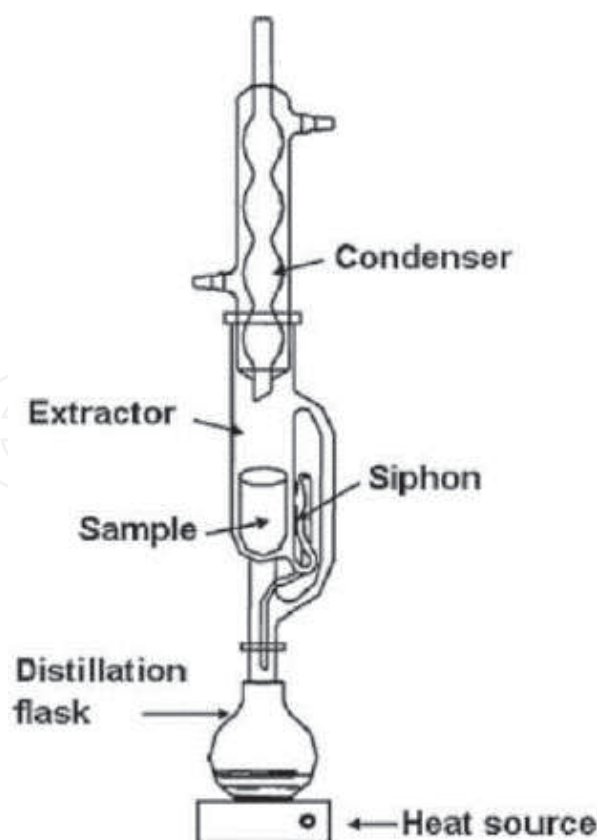


Figure 11.
Soxhlet.

6.3 Enzyme assisted extraction

Enzyme Assisted Extraction is an extraction technique that consists of the destruction of the source material cell wall using specific enzymes to liberate bioactive compounds [25]. Not only does this method augment the extraction yield compared to other conventional techniques, but it also is considered environmentally friendly as it does not require the use of toxic solvents [26, 27].

The most used enzymes are cellulases, hemicellulases, pectinases, and other hydrolytic enzymes [27]. These have the potential of catalyzing the hydrolysis of plant cell wall components such as polysaccharides and proteins. **Table 1** shows some of the useful plants metabolites and enzymes used for their extraction [26].

6.3.1 Plant cell walls

Due to their high insolubility and complex structure, plant cell walls are the major barrier to extracting bioactive compounds. Cellulose, the main component of cell walls, is a carbohydrate polymer that is characterized by low solubility and hydrogen-bonded crystalline fibers, which render its degradation greatly arduous. Components of the cell wall intervene in the process of extraction. Therefore, the nature of the cell wall matrix, the nature of the desired compounds, and their location are key factors controlling the extraction yield [25].

6.3.2 Principle and mechanism of action

Enzymatic assisted extraction is mainly based on the selectivity and ability of enzymes to intrude the matrix of the cell wall through interaction with the cell wall complex components. Thus, the release of bio-actives in the bulk solution is

Product type	Product	Source	Enzyme used	Maximum yield (%)
Oils and carotenoids	Oil	Grape seed	Cellulase, protease, xylase and pectinase	175
	Carotenoids	Marigold flower	Viscozyme, Pectinex, neutrase, corolase and HT-proteolytic	97
	Volatile oil	Mandarin peel	Xylan-degrading enzymes	15
	Carotene	Carrot pomace	Pectinex Ultra SP-L	0.0064
	Lycopene	Tomato	Pancreatin	2.5-fold
Glycosides		Tomato	Cellulase and pectinase	206
	Capsaicin	Chilli	Cellulase, hemicellulase and pectinase	n.d. ^a
	Colourant	Pitaya	Pectinolytic, hemicellulolytic and cellulolytic enzymes	83.5
	Anthocyanin	Grape skin	Pectinex BE3-L	n.d. ^a
	Sugar	Grapefruit peel waste	Cellulase and pectinase	0.6377
	Oligosaccharide	Rice bran	Cellulase	39.9
	Inulin	Jerusalem artichoke	Inulinase	n.d. ^a
	Starch	Cassava	Pectinase enzyme	45.6
	Pectin	Pumpkin	Xylase, cellulose, β -glucosidase, endopolygalacturonase and pectinesterase	14.0
Others	Vanillin	Vanilla green pods	β -glucosidase and pectinase	14–21
	Flavonoid (naringin)	Kinnow peel	Recombinant rhamnosidase	n.d. ^a
	Phenolics	Citrus peel	Celluzyme MX	65.5
	Proteins	Lentils and white beans	Glucoamylases	50.3
	Polyphenols	Grape pomace	Pectinolytic	98.1
	Catechins	Tea beverage	Pepsin	80
	Lignans	Flax	Cellulase and glycosidase	40.75 mg/g
	Soluble fibre	Carrot pomace	Cellulase-rich crude preparation	77.3

^aAbbreviation: n.d., not defined.

Table 1.
List of bioactive compounds of industrial importance obtained by enzyme- assisted extraction from plants [26].

enhanced. Enzymes bind to their specific substrates by conformational complementarity forming the enzyme-substrate complex and therefore, allowing the hydrolysis to occur. This process is a function of various parameters such as temperature,

hydrogen potential, enzyme concentration, the particle size of the substrate, and time of extraction that directly influence the efficiency of EAE. Optimizing these factors implies ensuring a high yield extraction in terms of quality and quantity [25].

6.3.3 Optimum operating conditions

The choice of enzymes is the first parameter to study. It is dependent on the chemical structure of the targeted compounds, the structural complexity of the cell wall, and the nature of the raw material. A combination of different enzymes is possible. Optimum temperature and pH are then selected based on the enzymes chosen (**Table 2**) [27].

Particle size is also a determinant parameter. Small particles were observed to have a better contact between enzymes and substrate and thus lead to a better release of bio-actives.

A prior understanding of the composition of the raw material, the structure of the cell wall, and the nature of the destined compounds are necessary for the determination of the optimum operating conditions as it facilitates the selection of enzymes and optimizing related parameters [27].

6.3.4 Advantages of EAE

Enzyme Aided Extraction is an advantageous technique and has served as an objective for countless recent studies as it remarkably improves the extraction yield, does not alternate the bio-actives properties, and selectively removes the unwanted components of raw material. Moreover, it does not require the use of toxic organic solvents nor does it harm the environment [26–28].

6.4 Steam distillation

Steam distillation is practically the oldest and most famous way of essential oils' extraction [29]. Steam distillation is a separation process for temperature sensitive

Enzyme used	Bioactive extracted	Source material	Conditions used
Cellulase	Polysaccharides	Garlic	Temperature 45 °C, pH 5.0, time 80 min
α-Amylase and glucoamylase	Oleoresin	Turmeric	—
Cellulase, papain, and pectinase	Polysaccharides	Alfalfa	Temperature 52.7 °C, pH 3.87, time 2.73 h
Cellulase, pectinase, and protease	Seed oil	Pumpkin	Temperature 44 °C, time 66 min
Alginate lyase	Fucoxanthin and lipids	<i>Undaria pinnatifida</i>	Temperature 37 °C, pH 6.2
α-Amylase	Polysaccharides	<i>Panax ginseng</i>	—
Pectinase and cellulase	Carotenoids	Tomato waste	—
Lipase and phospholipase	Proteins	Olive pulp and stone	Temperature 30–40 °C, pH 7.0, time 15 min
Papain, protease, and trypsin	Fatty acids	<i>Strongylocentrotus nudus</i>	Temperature 40–55 °C, pH 7.8–8.5, time 180 min

Table 2.
Different enzymes and their optimum operating conditions [27].

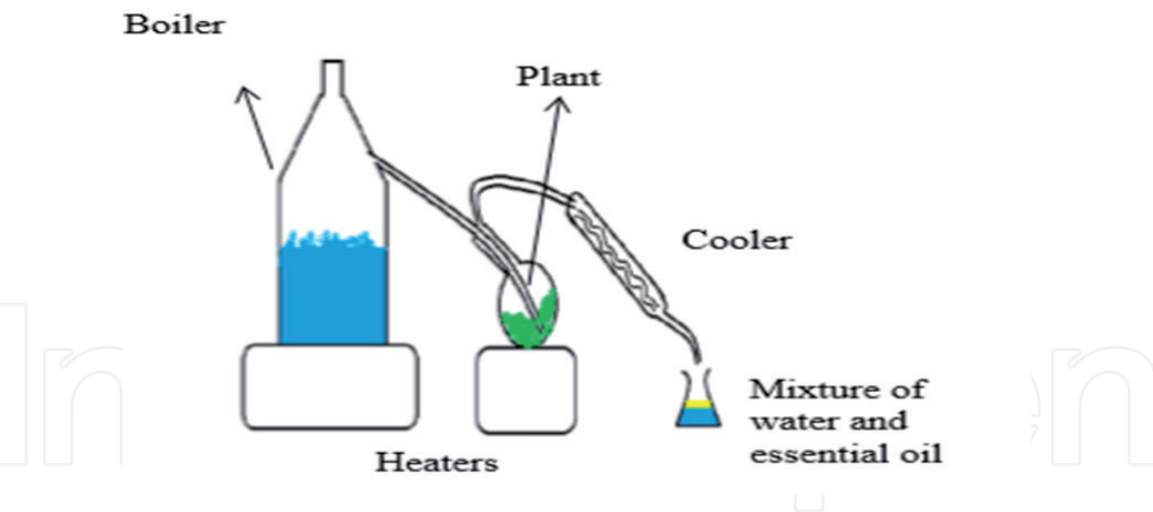


Figure 12.
Steam distillation.

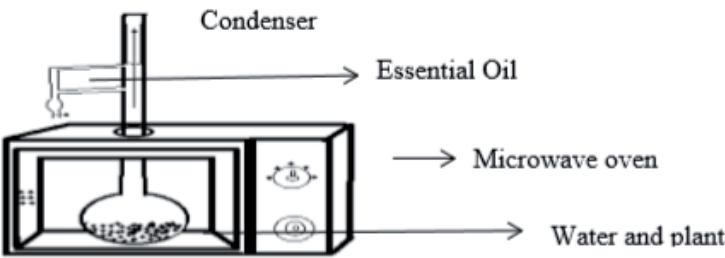


Figure 13.
Microwave assisted extraction.

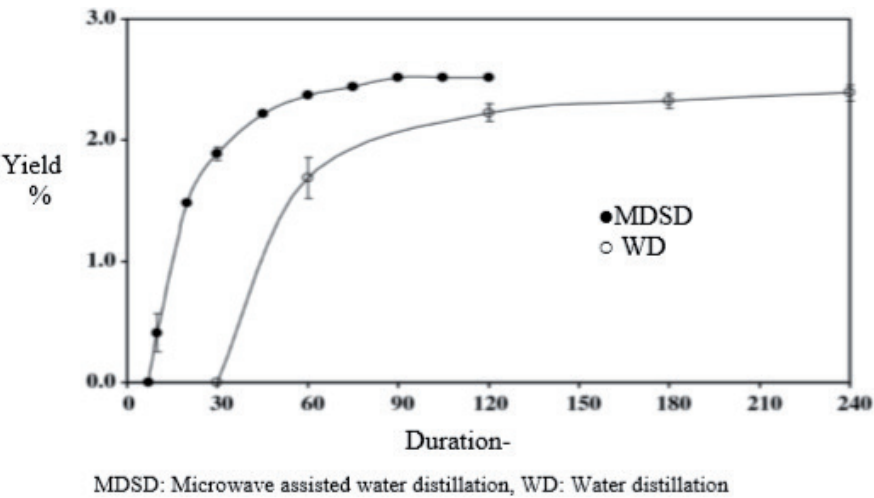


Figure 14.
Comparing water distillation and microwave assisted water distillation of obtaining thyme essential oil.

materials like oils which are insoluble in water and may decompose at their boiling point [30]. It consists of spraying with a certain amount of moisture on the plant material placed on the grid in a similar system to water distillation and allow steam transfer of the essential oils to occur [29]. The plant raw material is placed on a perforated plate and the steam, generated by a separate boiler, volatilizes and carries essential oils as it passes through the plant material [29]. The vapors are then condensed in water cooling system only to be collected at the end of the cooler in a separate appropriate collector. Steam distillation is advantageous as it allows the vaporization of essential oils without reaching their boiling point [30]. Moreover, the boiler being kept in a separate chamber from the plant chamber keeps the ambient temperature at which the material to be

distilled is located is kept below 100° C and hence it diminishes and prevents alterations due to heat effect. However, steam distillation is avoided for the reason that it is time and energy consuming and that, in case of high vapor flow rates, degradation of volatile compounds that can occur (**Figure 12**) [29].

6.5 Microwave assisted extraction

Microwave assisted extraction MAE is modern technique used in extraction. The first patent for MAE was in 1995 for the extraction of a natural product using microwaves by Pare (**Figure 13**) [31].

The target for heating in dried plant material is the minute microscopic traces of moisture that occurs in plant cells. The heating up of this moisture inside the plant cell due to microwave effect, results in evaporation and generates tremendous pressure on the cell wall. The cell wall is pushed from inside due to the pressure and the cell wall ruptures. Thus, the exudation of active constituents from the ruptured cells occurs, hence increasing the yield of phytoconstituents [32]. MAE allows the boiling point to be reached earlier than supported water distillation as shown in **Figure 14**. It has also been determined that the amount and quality of essential oil obtained by microwave-assisted water distillation in 30 minutes is equivalent to the amount and quality of essential oil obtained in 4 hours and thirty minutes by water distillation [29]. Not only does MAE save time and energy, but it also is considered environment friendly as it uses very little or no solvents [31, 32].

7. Conclusion

The rich composition of plants (EOs, primary and secondary metabolites) offers several advantages in the phytotherapy field, therefore the use for different therapeutic purposes.

The extraction method of bioactive molecules depends on several factors and also depends on the plant properties.

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Conflict of interest

The authors declare no conflict of interest.

Notes/thanks/other declarations

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