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A Review on Extraction, Isolation, Characterization and Some Biological Activities of Essential Oils from Various Plants

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Abstract

Essential oils are aromatic and volatile liquids, mixtures of organic compounds extracted from plant materials and characterized by a strong and generally pleasant flavor. The essential oils have been widely used as safe flavoring agents or preservatives in foods, in cosmetic or pharmaceutical products. In recent years, variety of Extraction techniques has been introduced for the recovery of organic compounds. Extraction Methods are widely used in various Industries for Separation of components and has wide range of applications. Essential oils and their volatile constituents have been widely used since the middle Ages, to prevent and treat human disease. They have been widely used for bactericidal, fungicidal, antioxidant, allelochemical, medicinal, cosmetic applications, pharmaceutical, sanitary, cosmetic, agricultural and food industries. They contain some volatile constituents, such as phenol-derived aromatic components, aliphatic components, terpenes and terpenoids. *In vitro* evidence shows that essential oils can act as antibacterial agents against pathogenic fungi and bacterial strains. Today, it is very crucial to develop effective and selective methods for the extraction and isolation of essential oils. The focus of this review paper is to provide a comprehensive view on the analytical methodologies, which include extraction, isolation, characterization and also some biological activities of Essential Oils from various plants.

Key words: Essential oils, extraction methods, isolation, characterization, antibacterial agents; Antifungal agents

1. Introduction

The Egyptians and Asian countries such as China and India have used essential oils and spices for several centuries. Some spices like cloves, cinnamon, mustard, garlic, ginger, and mint were applied as alternative medicine in India. Essential oils have been extracted for 3000 years in Egypt for their importance in various fields. The production of the essential oils dates back to more than 2000 years in the Far East, with early modern technologies taking place in Saudi Arabia in the 9th century [1]. However, during this period, the medical application of essential oils became secondary as they were essentially used as flavors. Extraction methods are not developed because all parts of the aromatic plants and herbs are used.

Over time, several extraction processes emerged. These processes have been developed to optimize the performance of the essential oil in both quantitative and qualitative terms. Alongside the development of the extraction processes, characterization methods and analysis have also experienced a significant progress that has allowed the determination of the chemical composition and physical properties of the various extracts. We can even identify the molecules responsible for each property.

Conventional methods of extraction (Hydro-distillation or steam distillation) have a number of drawbacks. These involve the internal diffusion process that limits the operation. Indeed, the actual structure of the plant cell walls inhibits the transfer of the fluids to the outside. For steam distillation and hydro-distillation, high temperatures can cause chemical changes in the compounds of the essential oils and losses of the volatile compounds [2]. The obtained solvent extract contains a trace amount of solvent. Some volatile compounds can be loosed during distillation of solvent. Thus, the choice of an extraction process depends on the desired objectives, such as cost, energy, compositions, and bioactive molecules.

All the extraction processes are designed to provide more concentrated product form of the desired material. Although the cost should never compromise the quality, it can be a decisive

factor. However, the effectiveness of the extraction and the safety of the process is a priority and, as the limits of solvent residues are increasingly subject to the review, the extracts obtained using supercritical fluids could play a central role to replace toxic solvents. One of the most important aspects of any extraction is probably an intimate substrate; it can be a key element in defining the quality of the extract in order to have a desired product which meets the requirements of consumers.

However, extraction with supercritical fluids allows the extraction of the high quality products which are solvents-free [4]. But, technological conditions for the use of the supercritical fluids expensive, which limits their use [5, 6]. Beside the essential oils, the supercritical CO_2 extracts contains various compounds [7, 8].

The attraction of medicinal and aromatic plants is continuously growing due to increasing consumers demand and interest in these plants for culinary, medicinal, and other anthropogenic applications.

As consumers are becoming more and more informed about issues of food, health, and nutrition, they are also becoming aware of the benefits and potential applications of medicinal and aromatic plants and their metabolites. These plants produce a large variety of secondary metabolites; among them, essential oils.

Despite their rich and complex composition, the use of essential oils remains wide and limited to the cosmetics and perfumery domains. It is worthy to develop a better understanding of their chemistry and the biological properties of these extracts and their individual components for new and valuable applications in human health, agriculture, and the environment. Essential oils could be exploited as effective alternatives or complements to synthetic compounds of the chemical industry, without inducing the same secondary effects.

Essential oils are the complex mixture of several bioactive chemical components, such as terpenes, terpenoids, and phenylpropenes. They can be produced by more than 17,000 aromatic

plant species commonly belonging to angiospermic families, such as Lamiaceae, Zingiberaceae, and Asteraceae [9].

Essential oils, also known as "essences" are volatile and odorous substances found in plants and are extracted by steam distillation, or by co-distillation with solvents [10]. They are concentrated and complex substances which have the form of oily drops present in one or more organs of the aromatic plant: in flowers (Jasmine), leaves (Sage), fruits (Orange), seeds (Fennel), bark (Cinnamon) and in roots (Angelica).

Aromatic plant and their essential oils have been used since antiquity as condiments, spices, antimicrobial, insecticidal and agents to protect stored products [11]. Natural additives from plants can be compounds, groups of com-pounds or essential oils. Essential oils have an antiseptic activity. They exhibit antibacterial, antiviral, antifungal, antioxidant, antiparasitic, and insecticidal effects. They have been shown to exert many biological activities, such as antimicrobial, analgesic, sedative, anti-inflammatory and spasmolytic [12].

The essential oil is a natural secretion of a plant. It is produced by secretory organs that are located in different parts of aromatic plants and trees: seed, root, wood, leaf, flower and fruit. Only an essential oil obtained by distillation of a plant, botanically defined, in an alembic through steam under low pressure corresponds to the french association for standardization (AFNOR standard) [13]. The product, which is obtained by mechanical pressure on citrus essence, is called non-essential oil.

Essential oils are aromatic and volatile liquids, mixtures of organic compounds extracted from plant materials and characterized by a strong and generally pleasant flavor. The essential oils have been widely used as safe flavoring agents or preservatives in foods, in cosmetic or pharmaceutical products [14]. Essence is a natural substance secreted by the aromatic plant. For citrus, essences are extracted by expression of the zest, also known as lemon oil and lemon essential oil note. At its transformation by distillation, gas undergoes biochemical modifications and becomes essential oil. The essential oil is the essence of the distilled plant. It is made up of

volatile molecules and a pure essential oil contains no natural fats. Essential oils are used in foods, medicines and cosmetics [15].

2. Extraction Methods For Essential Oils

Several parts of various aromatic plants can be extracted and form essential oils which subsequently have many applications in cosmetics, pharmaceutical and food safety fields. The manufacturing method and technique used to extract essential oils are dependent on the characteristics and components required in the botanical extract. The main factor to ensure the quality of essential oils is the extraction method used, since inappropriate extraction procedures may cause the destruction and vary the action of phytochemicals present in aromatic oils. The resulting effects can be, for example, the loss of pharmacological constituents, stain effect, off flavor/ odour, and physical change of essential oils [16].

Such extraction techniques can be categorized into two categories: classical methods and innovative methods. The application of innovative techniques, such as ultrasonic and microwave enhanced processes, has improved the efficiency of extraction process in terms time required for isolation of the essential oil and energy dissipation, as well as improvement in production yield, and high quality of essential oils [17].

2.1. Conventional Extraction Methods

Conventional techniques applied to extract essential plant oils are based on water distillation by the heating process.

2.1.1. Hydrodistillation

Hydrodistillation is the oldest and simplest oils extraction method which was discovered by Avicenna and the first to develop extraction through the alembic. Rose was the first plant extract used and purified by this method. The procedures start with immersing the plant materials directly into water inside the alembic (vessel), and whole mixture was boiled. The devices include a heating source, vessel (Alembic), a condenser to convert vapor from vessel onto liquid, and a decanter to collect the condensate and to separate essential oils with water (Figure 1)).

This extraction technique is considered as a unique method to extract plant materials like wood or flower and is frequently used for extractions involving hydrophobic natural plant material with a high boiling point. As the oils are surrounded by water, this method is able to protect

essential oils to be extracted at a certain degree without being overheated. The main advantage of this extraction technique is its ability to isolate plant materials below 100°C [17].

Few studies have been conducted on the extraction of essential plant oils by using hydrodistillation. Okoh et al. investigated the comparison between extraction process, Hydrodistillation (HD) and Solvent-free Microwave Extraction (SFME) on the properties and yield of essential oil from rosemary (*Rosmarinus officinalis* L.). Through hydrodistillation, a total yield of the volatile fraction was 0.31%, while 0.39% was obtained for the SFME method [18].

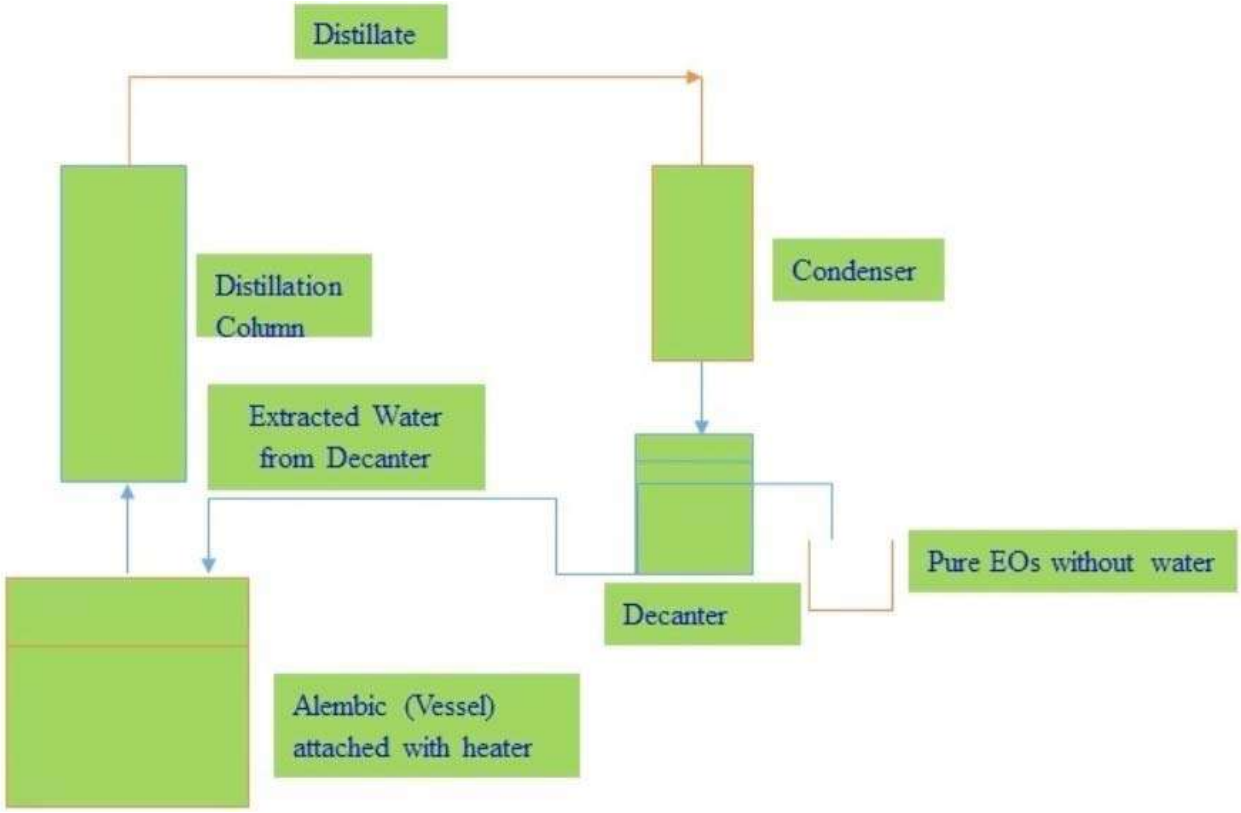

Figure 1: Flow diagram of hydrodistillation extraction process [17].

The general hydrodistillation process has been modified by using new technologies as reported by a few researchers. Golmakani and Rezaei developed advanced HD extraction process technique named Microwave-assisted HD (MAHD) which showed superiority in energy

dissipation and isolation period (75 min compared to 4 h in HD) [19]. Additionally, Ohmic assisted HD (OAHD) is the other advanced HD extraction technique, discovered by Gavahian and co-workers. Through OAHD, thyme essential oil can be extracted in a period of only 25 min compared to HD method. No change was observed in characteristics of components in thyme obtained by OAHD and HD [20].

2.1.2. Steam Distillation

In essential plant oil extraction, steam distillation method is the broadest technique applied. The percentage of essential oils being extracted by this technique is 93% and the remaining 7% can be further extracted by other methods [21]. Basically, the process started by heating of plant material using steam which is supplied from steam generator (Fig. **5**). Heat is the main factor determining how effectively the plant material structures break down and burst and release the aromatic components or essential oils [22].

Masango developed an innovative steam distillation extraction technique to increase the isolated essential oil yields and reduce the amount of wastewater produced during the extraction process. The system uses a packed bed of the plant samples, placed above the steam source. Only steam is allowed to pass through the plants and boiling water does not mix with the botanical materials. Therefore, the process requires less steam and the amount of water in the distillate can be reduced [21].

In another study, Yildirim *et al.* reported a component 2,2- diphenyl-1-picryl hydrazyl (DPPH) used to evaluate the antioxidant properties of essential oils by using steam distillation extraction process. It was reported to have a higher yield of antioxidant components than the oils extracted by hydrodistillation (HD) [23].

2.1.3. Hydrodiffusion

Hydrodiffusion extraction method is an extraction process in which steam is supplied to a container which holds plant materials.This technique is only applied on dried plant samples that can be damaged at boiling temperature. In the steam distillation process, steam is applied from the bottom of the steam generator, whereas in the hydrodiffusion method, steam is supplied from

the top of the generator. This process was carried out at low pressure or vacuum and steam temperature can be reduced below 100°C [24].

This steam diffusion method was further enhanced by adding microwave technology. Bousbia and research team have investigated the difference in performance between innovative Microwave Hydrodiffusion and Gravity (MHG) and a traditional method like hydrodistillation [25]. In another study, the isolation of essential oil from orange peel was studied using an innovative steam diffusion technique (SDf) called microwave steam diffusion (MSDf). The extraction performance results showed that the isolation period of the essential oils by MSDf technique is within 12 minutes and had similar yield and aromatic profile to those obtained by SDf for 40 minutes [25].

2.1.4. Solvent Extraction

Ordinary solvents like acetone, petroleum ether, hexane, methanol, or ethanol have been implemented by this technique to extract fragile or delicate flower materials which cannot be extracted using heat or steam supplied [16]. Generally, the plant samples are mixed with solvents to be extracted by mildly heating the mixture, and the process is followed by filtration and evaporation of the solvents. The filtrate contains a resin (resinoid), or the mixture of wax, fragrance, and essential oil. Alcohol is combined with the filtrate mixture in order to dissolve the essential oil into it and thereafter distilled at low temperature. During the distillation process, the alcohol absorbs fragrance and is evaporated while the aromatic absolute oil remains in the pot residue. Compared to other methods, this method is more complicated for essential oils extraction, and as a result, time-consuming and more expensive [26].

In another study, authors investigated the antioxidant activity of Ptychotisverticillata by solvent extraction technique for essential oils extraction. It was found that 48% of phenolic compounds are present and contain 44.6% and 3.4% of carvacol and thymol, respectively, as the main compounds [27]. In other studies, the essential oils were separated from *Thymus praecox* subsp. *Skorpilii* var. *Skorpilii* (TPS), and its chemical constituents and antioxidant activity were investigated by mixing the plant extractant with different solvents like ethanol, methanol, and water. These extracts showed significant free-radical scavenging activity with 40.31% of thymol

and 13.66% of o-cymene. The results also showed that the extraction process using water as the solvent gives highest phenolics and flavonoids compared to other types of solvents [28].

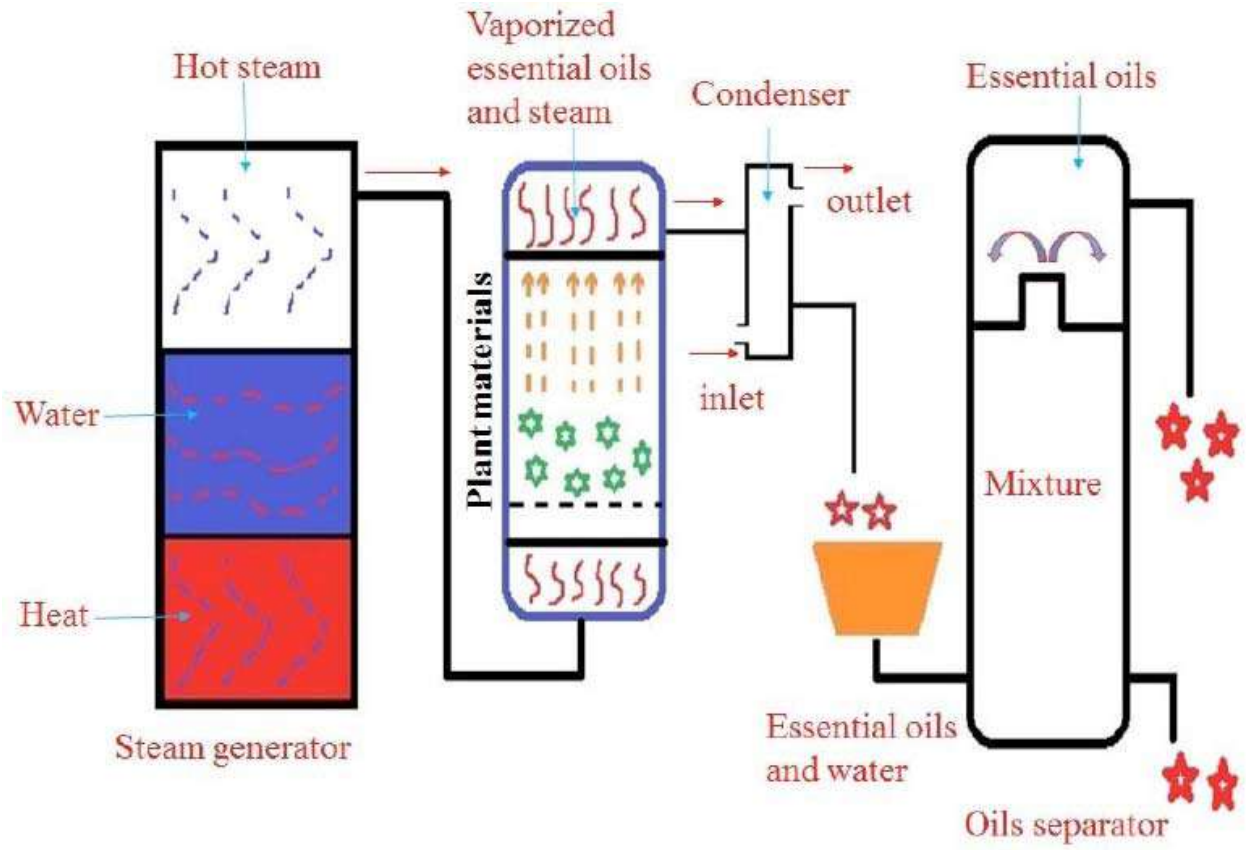

Figure 5: Diagrammatic illustration of steam distillation method [29].

2.2. Innovation of Extraction Methods

The further modification of extraction techniques is due to various disadvantages of conventional methods which encourage essential oils to undergo chemical alteration like hydrolysis, isomerisation, and oxidation. These processes involve high temperature and affect the quality of essential oils, at the same time prolonging the extraction period. In the field of essential oils extraction process, it is very important to maintain the oils chemical constituents and natural proportion at its original state. The parameters that need to be considered in new extraction techniques are reduction of extraction period, energy consumption, solvent used and carbon dioxide emission [17].

2.2.1. Supercritical Fluid Extraction

Conventional extraction techniques such as solvent extraction and steam distillation need more time to undergo the extraction process and a large amount of organic solvents are required [30]. Additionally, the disadvantages of these techniques like various volatile components losses, poor efficiency of oils extraction, degradation of unsaturated compounds, and toxic residues from extraction process need to be encountered [31, 32].

The supercritical fluid state is mainly depending on two factors which are the fluids critical pressure, Pc and critical temperature, Tc. Fluids with these critical parameters exhibit very interesting properties such as low viscosity, high diffusivity, and density closer to liquids [17]. Carbon dioxide is used as a supercritical solvent for the extraction of essential oils due to its numerous attractive properties: (i) easily reach critical point (low critical pressure, Pc : 72.9 atm, and temperature, Tc : 31.2°C); (ii) unaggressive for thermo labile molecules of the plant essence; (iii) chemically inert and toxic; (iv) nonflammable; (v) available in high purity at relatively low cost; (vi) easily eliminated; (vii) its polarity similar to pentane which makes it suitable for extraction of lipophilic compounds [33, 34].

Generally, the principle of supercritical fluid extraction process involves the use and recycling fluid in repeated steps of compression/ decompression. The supercritical state of CO_2 can be achieved by highly compressing and heating this fluid. Then, it passes through the raw plant material to load volatile matter and plant extracts. The process is followed by decompression steps, where the mixture of CO_2 and plant extracts are routed to two separators where the fluid is gradually decompressed to separate the obtained extracts from the CO_2. The CO_2 is released from second separator and recycled into storage tank, and no solvent residue remains in the final product since CO_2 easily reverts to a gas under normal atmospheric pressure and temperature [35].

Several plant materials have been extracted by using supercritical carbon dioxide extraction method such as rose geranium, Eugenia caryophyllata, clove buds, and marchantia convolute, and their chemical constituents are revealed by some researches [36, 37, 38, 39]. In a study about the comparison between supercritical fluid extractions with hydrodistillation method, by using the supercritical fluid technique, an essential oil was successfully isolated and revealed as

advance aromatic oil, with superior performance and pharmacological activities [25]. Other than that, a carrot essential oil obtained by supercritical fluid extraction method was found to give better antibacterial and antifungal properties against Bacillus cereus as compared to the oil obtained by hydrodistillation [40].

2.2.2. Subcritical Extraction Liquid

The use of water at subcritical state has been reported by many researchers and found that this is a better and powerful alternative of essential oils extraction technique [39]. The definition of subcritical stage of liquid is the time when liquid reaches pressure higher than the critical pressure, Pc and lower than the critical temperature, Tc or *vice-versa*. The fluids that are used to extract essential oils using this method are water and CO_2. The subcritical state of fluid offers several superior characteristics such as lower viscosity, lower density, and enhanced diffusivity between gas and liquids. This extraction technique is considered the best alternative approach as it enables a fast essential oil isolation process, conducted at a low working temperature. Moreover, it is a costefficient extraction, simple and environmental friendly process [39].

In this process, the required duration of extraction is only 15min compared to 3h required to extract essential oils by using conventional methods. Essential oils with more valuable properties which are a higher amount of oxygenated components with no significant presence of terpenes can be obtained and allow substantial cost saving in terms of both energy and plant materials [2].

Kubatova and co-workers investigated the lactones extraction from a Piper methysticum root by using subcritical water extraction, and this method was compared with Soxhlet extraction with water. The working temperature for subcritical water extraction was at 100°C and 175°C, and the extraction time required to extract the lactones was 20 min and 2h, respectively. Soxhlet extraction method showed a large difference in extraction time compared to subcritical water method, and required 6 hours to extract the oils and produced lower yields by 40% to 60% [40].

2.2.3. Solvent Free Microwave Extraction

The impediments of ordinary extraction techniques, such as solvent and hydrodiffusion, are the losses of several evaporative constituents, poor isolation coherence, and toxic solvent residues at the final product stage. These challenges prompted the consideration of Solvent-Free Microwave

Extraction (SFME) for various applications [25]. This technique is an expeditious isolation of essential oils from spices, aromatic herbs, and dry seeds. Several advantages of SFME have been reported by researchers, which can be summarized: to obtain essential oils with high yield and selectivity, shorter extraction time and environmentally friendly process [25].

SFME involves a combination of two techniques which are heating plant samples using microwave technology followed by dry distillation which operates at an atmospheric pressure in the absence of any solvent. Bayramoglu et al. applied SFME method to extract oregano at different microwave power; 622W, 498W, 373W, and 249W, while the essential oil yields were determined depending on each different microwave power used. The results showed maximum yields achieved at 0.054, 0.053, 0.052 and 0.049 mL/g of oregano essential oil at 622W, 498W, 373W, and 249W power levels, respectively. Exception with working at lowest microwave power (249W), all other yields were found to be higher ($p \square 0.05$) [41]. Compared to hydrodistillation, the yield extracted oregano essential oil was only 0.048 mL/g which about 6% slightly lower than SFME oregano oil highest yield. Later, Ferhat et al. presented the comparison of SFME method with traditional methods in terms of extraction periods, yields, impact of the technique used towards the environment, solvent residues content, and antimicrobial activities. It was demonstrated that microwave extraction offers a shorter isolation period of essential oil (30 min compared to 3 h for hydrodiffusion and 1 h for cold pressing); 0.24% of yields from SFME which is much better than hydrodiffusion and cold pressing with 0.21% and 0.05%, respectively; high energy consumption for performing hydrodiffusion and cold pressing (using mechanical motors) compared to rapid microwave extraction; no water and solvent used in SFME make the extraction process as cleaner features, and high antimicrobial activities of essential oils obtained by SFME technique [42].

3. Isolation and Purification

The components in the extracts from the above methods are complex mixture and contains various type of natural products with different polarities. To obtain pure bioactive compound involves further separation and purification. Their separation remains a big challenge for the process of identification and characterization of pure bioactive natural product. Purification and isolation of natural products has undergone new development in recent years [43]. Many bioactive natural products have been isolated and purified by using different separation

techniques such as TLC, HPTLC, Paper chromatography, Column chromatography, Gas chromatography, OPLC and HPLC. Column chromatography and thin-layer chromatography (TLC) are still mostly used due to their convenience, economy, and availability in various stationary phases [44].

Besides that, non-chromatographic techniques uses such as immunoassay, which use monoclonal antibodies (MAbs), phytochemical screening assay. The pure compounds are then used for the determination of structure and biological activity [45]. Several of the commonly used separation techniques of the natural products are discussed below:

a) Thin Layer Chromatography (TLC)

TLC is the most commonly used planar chromatographic method in natural product research. This is the easiest and cheapest technique and can be applied in the analysis, isolation and setting the parameters for column chromatography [46]. Usually, silica or alumina (more polar) is used as the stationary phase and organic solvents (less polar) are used as the mobile phase. This situation is categorized as normal phase chromatography. In contrast to this, reverse phase TLC is available, in which stationary phase is alkyl bonded silica or alumina (less polar) and mobile phase is polar solvent like water, alcohol etc.

b) Column Chromatography (CC)

Column chromatography is the most effective technique used in separation of crude plant extracts into its components in pure form. This is a preparative chromatographic method and the stationary phase (silica gel) is packed in a column and then the mobile phase (eluent) is passed through the column after loading the extracts on the top of the stationary phase. The mobile phase carries the natural products present in the mixture at different rate based on their affinities to the stationary and mobile phase. Separated compounds can be collected along with the mobile phase [46].

c) Gas Chromatography (GC)

It is an analytical technique for separating compounds based primarily on their volatilities. GC provides both qualitative and quantitative information for individual compounds present in a sample. The gas phase is flowing and the liquid phase is stationary. The rate of migration for the chemical species is determined through its distribution in the gas phase. For example, a species that distributes itself 100% into gas phase will migrate at the same rate as the flowing gas, whereas, a species that distributes itself 100% into stationary phase will not migrate at all. Species that distribute themselves partly in both phases will migrate at an intermediate rate [47]. Gas chromatography involves a sample being vaporized and injected onto the head of the chromatographic column. The sample is then transported through the column by the flow of inert, gaseous mobile phase. The column itself contains a liquid stationary phase, which is adsorbed onto the surface of an inert solid.

d) High Performance Liquid Chromatography (HPLC)

It is a versatile, robust, and widely used technique for the isolation of natural products. HPLC is an analytical technique for the separation and determination of organic and inorganic solutes in any samples especially biological, pharmaceutical, food, environmental, industrial etc. Currently, this technique is gaining popularity among various analytical techniques as the main choice for fingerprinting study for the quality control of medicinal plants. In order to identify any compound by HPLC, a detector must first be selected, The extent or degree of separation is mostly determined by the choice of stationary phase and mobile phase. Modern HPLC uses a non-polar solid phase, like C18 and a polar liquid phase, generally a mixture of water and another solvent. High pressure up to 400 bars is required to elute the analyte through column before they pass through a diode array detector (DAD). A DAD measures the absorption spectra of the analytes to aid in their identification. HPLC is useful for a compound that cannot be vaporized or that decompose under high temperature and it provides a good complement to gas chromatography for detection of compounds [48].

e) High Performance thin Layer Chromatography (HPTLC)

It is a planar chromatography where separation of natural compounds is achieved on high performance layers with detection and data acquisition. These high performance layers are pre-coated plates coated with a sorbent of particle size 5-7 microns and a layer thickness of 150-200 microns. The reduction in thickness of layer and particle size results in increasing the plate efficiency as well as nature of separation [49]. HPTLC plates are substantially more expensive (4- to 6-times more) than normal plates but are an efficient alternative when high sensitivity, accuracy and precision are required in situations demanding high performance [50].

f) Optimum performance laminar chromatography (OPLC)

It is a new concept in parallel chromatography; OPLC combines the advantages of both TLC and HPTLC. OPLC is both an analytical and preparative tool, suitable for research and quality control laboratories. It is a powerful liquid chromatography separation technique that combines the user- friendly interface and resolution of HPLC with the capacity of flash chromatography and multi dimensionality of TLC. The basis of OPLC is similar to that of other chromatographic techniques in that a pump is used to force a liquid mobile phase through a stationary phase, such as silica. The OPLC columnhousing structure allows flat planar columns to be used in the same way as cylindrical glass or stainless steel ones. The flat column is pressurized up to 50 bars and mobile phase is forced through it at constant linear velocity via a solvent delivery pump [51].

4. Structure Determination

Determination of the structure of natural products uses data from a wide range of spectroscopic techniques such as UV-Visible, Infrared (IR), Nuclear Magnetic Resonance (NMR) and Mass spectroscopy. The basic principle of spectroscopy is passing electromagnetic radiation through an organic compound that absorbs some of the radiation, but not all. By measuring the amount of absorption of electromagnetic radiation, a spectrum can be produced. The spectra are specific to certain bonds in a compound. Depending on these spectra, The structure of the natural compound can be identified. Scientists mainly use spectra produced from either three or four regions—Ultraviolet (UV), Visible, Infrared (IR), Radio frequency (FTIR), and electron beam for structural clarification [52].

a) UV-Visible Spectroscopy

UV-visible spectroscopy can be performed for qualitative analysis and for identification of certain classes of compounds in both pure and biological mixtures. Preferentially, UV-visible spectroscopy can be used for quantitative analysis because aromatic molecules are powerful chromophores in the UV range. Natural compounds can be determined by using UV-visible spectroscopy [49]. Moreover, spectro-scopic UV-Vis techniques were found to be less selective and give information on the composition of the total polyphenol content. This technique is not time-consuming, and presents reduced cost compared to other techniques [53].

b) Fourier Transform Infrared Spectroscopy (FTIR)

Fourier- transform infrared spectroscopy is a valuable tool for the identification of functional groups present in the plant extract. It helps for identification and structure determination of the molecule [53].It is a high-resolution analytical tool to identify the chemical constituents and elucidate the structural compounds. FTIR offers a rapid and nondestructive investigation to fingerprint herbal extracts or powders.

c) Nuclear Magnetic Resonance Spectroscopy (NMR)

Nuclear Magnetic Resonance Spectroscopy gives physical, chemical and biological properties of matter. One dimensional technique is routinely used but the complicated structure of the molecules could be achieved through two dimensional NMR techniques. Solid state NMR spectroscopy is used for the determination of molecular structure of solids. Radiolabeled 13C NMR is used to identify the types of carbon are present in the compound. 1 H-NMR is used to find out types of hydrogen are present in the compound and to find out how the hydrogen atoms are connected [51].

d) Mass Spectroscopy

Mass spectrometry is a powerful analytical technique for the identification of unknown compounds, quantification of known compounds andto elucidates the structure and chemical properties of molecules. Through MS spectrum, the molecular weight of sample can be determined. This method mostly employed for the structural elucidation of organic compounds,

for peptide or oligonucleotide sequencing and for monitoring the existence of previously characterizes compounds in complex mixtures with a high specificity by defining both the molecular weight and a diagnostic fragment of the molecule simultaneously [47].

5. Biological Activities of Essential Oils

a) Essential oils as antibacterial agents

The Ancient Egyptians used aromatic plants (and the essential oils content in them) in embalming, in that manner, bacteria stop to growth and decay was prevented. This was confirmed from strong in vitro evidence. In fact, essential oils can act as antibacterial agents against a wide spectrum of pathogenic bacterial strains, including: Listeria monocytogenes, L. innocua, Salmonella typhimurium, Escherichia coli O157:H7, Shigella dysenteria, Bacillus cereus, Staphylococcus aureus and Salmonella typhimurium [54-58], and many more [59]. Also, Commiphora africana (A.Rich.) Endl. essential oil can inhibit some pathogenic bacterial strains, such as Staphylococcus aureus, Escherichia coli, Candida albicans [60] and Helicobacter pylori [61]. Helicobacter pylorus is a Gram-negative microaerophilic bacterium. It is a highly motile and thought to be an infective agent widely spread on the world population (more than 50%), this makes it the most common chronic infection for humans. H. pylori is widely recongnized as a gastrointestinal pathogen. It is the causative of chronic superficial gastritis, and is major factor contributing to the pathogenesis of duodenal ulcer disease.The medical treatment for H. pylori include a combinations of different active substances: antibiotics, H_2-blockers, bismuth subsalicylate, proton pump inhibitors, is well known that multidrug therapy is associated with considerable side effects, but there is an alternative. Few studies have been shown that some traditional herbal medicines can act against H. pylori; one of this (C. africana) was tested by Epifano et al. [61]. Antibacterical activity against H. pylori, Grampositive (S. aureus, S. epidermis, E. faecalis) and Gram-negative (E. coli, P. aeruginosa) bacteria was tested in vitro by Epifano et al. [61]. In this study in vitro agar dilution method was employed for the assessment, as reccomended by the National Committee for Clinical Laboratory Standard (2002/2003). The results pointed out that C. africana essential oil has shown a potent anti-H. pylori activity with MIC values of 1 μl/ ml (much lower than those of the reference compound metronidazole), while little or no activity against different species of Gram-positive and Gram-negative bacteria has been showed. The results show a selective antibacterical activity of C. africana essential oil against H. pylori. The activity of C. africana essential oil against H. Pylori, is comparable to the

one of known antimicrobial agents, but the latter may favour the emergence of resistant colonies and also present a potential for the disruption of intestinal microbial flora, which is responsible for side effects [61].

b) Essential oils as antifungal agents

Despite of modern knowledge on slaughter hygiene and food, production techniques show an increasing during the last years, food safety remaining an increasingly important public health issue [62]. It has been estimated that as many as 30% of people in industrialised countries suffer from a food borne disease each year, and in 2000, at least two million people died from diarrhoeal disease worldwide [63]. There is, therefore, still a need for new methods of reducing or eliminating foodborne pathogens, possibly in combination with existing methods [58]. At the same time, Western society appears to be experiencing a trend of 'green' consumerism [64,65], desiring fewer synthetic food additives and products with a smaller impact on the environment. Moreover, the World Health Organization has recently asked for a worldwide reduction in the consumption of salt that is correlated to the incidence of cardio-vascular disease [63]. If the level of salt in processed foods is reduced how reccomend WHO, it is necessary that other additives will be develop to maintain the safety of foods. There is, hence, scope for new methods of making food safe, which have a natural or 'green' image. One such possibility is the use of essential oils as food additives that can act as antibacterial and antifungal additives.

Angelini et al. [66] pointed out the use of essential oils in the food industry, as natural sanitizing agents; in this study, Angelini et al. [66] evaluate some antimicrobial activity parameters as mycelial growth inhibition, minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of six essential oils against Aspergillus niger, Aspergillus terreus, Chaetomium globosum, Penicillium chrysogenum, Penicillium pinophilum, Trichoderma harzianum and Trichoderma viride. The antimicrobial activity of essential oils was monitored by the macrodiluition technique. The mycelial growth inhibition, fungistatic and fungicidal concentrations were recorded for each strain that showed sensitivity to the essential oils. The essential oils of catnip, cinnamon, tea tree and thyme essential oils exhibited large spectrum antimicrobial activities; those of clary sage and laurel inhibited the mycelial growth in a few

fungal strains. The essential oils of cinnamon and thyme had the lowest MIC and MFC values against all the fungi assayed, followed by catnip, tea tree, clary sage and laurel [66].
In the last two decades, there has been a considerable increase in the incidence of life-threatening systemic fungal infections. The challenge has been to develop strong strategies for treating fungal diseases, to treat opportunistic fungal infections in human immunodeficiency virus-positive patients, and others who are immunocompromised due to cancer chemotherapy or the indiscriminate use of antibiotics [67, 68].

Most clinically-used antifungal drugs have various drawbacks. They are pretty toxic, they have a low efficacy and high cost, furthermore, their frequent use has produced resistant strains [69]; therefore, there is a great need for new antifungals that concern to a wide range of structural classes, that can selectively work on new targets with fewer side effects [70,71].

Strong in vitro evidence indicates that some essential oils like Thymus schimperi Ronniger essential oil, can act as antibacterial agents against a wide spectrumof pathogenic fungal isolates including (Penicillium chrysogenum, Verticillium sp., Aspergillus tubingensis, Aspergillus minutus, Beauveria bassiana and Microsporum gypseum) [72]. In vitro susceptibility testing of the isolates to conventional antifungal agents and to two chemically well-defined chemotypes of T. schimperi essential oil was performed. Most of the isolated fungi were resistant to amphotericin B (except A. minutus), and itraconazole, while terbinafine was quite active on these fungi. T. schimperi essential oil showed antifungal activity against all of the tested fungal isolates. The minimal inhibitory concentration values was similar or lower than those of terbinafine. Considerable morphological and cytological changes revealed by transmission electron microscopy analyses, occur when essential oil inhibit fungal growth [72].

Also, Tirillini et al. [73] focused our investigation on the antifungal activities of Laserpitium garganicum subsp. Garganicum (Ten.) Bertol essential oil. L. garganicum subsp. garganicum (Ten.) Bertol. (=Laserpitium siler L. subsp. garganicum (Ten.) Arcangeli) is a perennial herb belonging to the Apiaceae family. The distribution is limited to the southern area of the Balkan peninsula and Italy. In Italy, this plant is found in the central Apennines, Sicily and Sardinia. This plant is described as a subspecies of L. siler or a species of Laserpitium in the Flora

Europaea and the Flora d' Italia, respectively. Tirillini et al. [73] tested L. garganicum subsp. garganicum essential oil against some phytopathogens and opportunistic human fungi. A few studies have reported the biologically active components isolated from L. siler, mainly sesquiterpene lactones, and one refers to sesquiterpene lactones from the roots of L. garganicum. Tirillini et al. [73] identified fifty-six compounds in L. garganicum essential oil, representing 92.3% of the total oil.

Table 1 shows the anfungal activity of the essential oil of L. garganicum [73].

Microorganism		% Inhibition*		
	0.125 µL/mL**	0.250 µL/mL**	0.5 µL/mL**	1 µL/mL**
A. niger	21 ± 7	31 ± 6	32 ± 4	28 ± 4
A. terreus	n.i.	14 ± 5	17 ± 5	22 ± 6
C. globosum	n.i	22 ± 3	22 ± 4	20 ± 4
P. chrisogenum	n.i.	10 ± 4	15 ± 5	47 ± 5
P. pinophilum	23 ± 6	28 ± 5	34 ± 5	54 ± 4
T. viride	13 ± 4	33 ± 3	42 ± 4	67 ± 2

*The data are the mean of triplicate values ± SD.

**Essential oil content (µL/mL cultured medium)

n.i.: no inhibition.

Table 1: Antimicrobic activity of the essential oil of L. garganicum

c) Essential oils as antioxidant agents

Free radicals and other reactive oxygen species produce oxidation of proteins, amino acids, unsaturated lipids and DNA. Reactive oxygen species produce molecular alterations related to aging, arteriosclerosis and cancer [74], Alzheimer's disease [75], Parkinson's disease, diabetes and asthma [76]. The human body has defense mechanisms against free radicals present in almost all cells [77]. Is possible that occur an imbalance between free radical production and their removal by the body's antioxidant system; this imbalance bring to a phenomena known as 'oxidative stress' [78, 79]. Balance between free radicals and antioxidants can be recovered from an external supply of antioxidants.

Essential oils are rich in phenolic compounds, and for this reason, attract investigators to evaluate their activity as antioxidants or free radical scavengers. The essential oils of basil, cinnamon, clove, nutmeg, oregano and thyme have proven radical-scavenging and antioxidant properties in the DPPH radical assay at room temperature [80]. The order of effectiveness was found to be: clove>>cinnamon>nutmeg>basil ≥ oregano>>thyme. The essential oil of Thymus serpyllum L. showed a free radical scavenging activity close to that of the synthetic butylated hydroxytoluene (BHT) in a β-carotene/linoleic acid system [81]. The antioxidant activity was attributed to the high content of the phenolics thymol and carvacrol (20.5% and 58.1%, respectively).

Bertuzzi et al. [82] investigates the action of Citrus×limonum Risso essential oil to control free radical-induced lipid peroxidation and preventing tissue damage in skin. In this study, the essential oil was analized by GC-MS technics. The superoxide anion scavenging activity of C. limonum essential oil was evaluated by the enzymatic hypoxanthine/xanthine oxidase system. The antiradical activity was tested on human volunteers after UV ray ex position. The essential oil was diluted in DMSO or grape-seed oil, then it was spread on the face of human volunteers. The presence of peroxyl radicals was detected on a sample skin lipids that has been previously collected. The detection of peroxyl radicals based on the measurement of light emitted (chemiluminescence), when the excited carbonyl and singlet oxygen decay to ground state. Bertuzzi et al. [82] demonstrate that the lemon essential oil is more active than a-tocopherol against O2- and peroxide free radical inhibition at 1: 100 dilution, therefore, protocol for controlling free radical-induced lipid peroxidation in human skin was thus proposed. The results of the study by Bertuzzi et al. [82] suggest that lemon essential oil has properties that could benefit human skin, as it undergoes environmental and chronological ageing, therefore, the scavenging action of lemon essential oil could have a practical application for treating human skin against oxidative damage [82]. The scavenging action of lemon essential oil solubilized in grapeseed oil could have a practical application in aesthetic medicine for treating human skin against oxidative damage. Therefore, continuous application of lemon essential oil solubilized in grape-seed oil might contribute to the prevention of lifestyle-related skin diseases by regulating the balance of oxidative stress [82].

d) Essential oil as allelochemical agents

Although oleogumresins/essential oils are well known antimicrobial agents, they stimulates some microorganisms and use them as carbon energy sources [83,84]. Angelini et al. [85] suggest that the weak parasitism of P. eryngii spp.-complex on roots and stems of umbellifers (family Apiaceae, genera Eryngium, Ferula, Ferulago, Cachrys, Laserpitium, Diplotaenia and Elaeoselinum) is mediated by allelopathic interactions. The oleogum-resin/essential oils (or their components) shifts the microrganism balance in favour of those microrganisms (e.g. Pleurotus spp.) that can tolerate them. Some even use them as a carbon and energy source [85,86].

The term "Allelopathy" has undergone several changes over time [87,88]. The definition adopted by the International Allelopathy Society (IAS) in 1996 is "The science that studies any process involving secondary metabolites produced by plants, algae, bacteria and fungi that influences the growth and development of agricultural and biological systems". Allelopathic interactions derive from the production of secondary metabolites. The secondary metabolites are synthesized for a wide range defense by plant and microorganisms. The secondary metabolites involved are called allelochemicals [89].

Trichoderma harzianum is a fungal contaminant that causes extensive losses in the cultivation of Pleurotus species. Melaleuca alternifolia (Maiden and Betche) Cheel (tea tree) essential oil was investigated by Angelini et al. [85]. This essential oil have "in vitro" allelopathic ability to control Trichoderma harzianum. The antifungal activity of M. alternifolia essential oil and antagonist activities between Pleurotus species against three T. harzianum strains were studied in dual-culture experiments. The dual-culture was realized on an agarbased medium, in which different concentrations of essential oil were incorporated. M. alternifolia essential oil at a concentration of 0.625 l L/mL, inhibited T. harzianum mycelial growth by 5.9-9.0%, depending on the strain. At the same concentrations P. ferulae and P. nebrodensis stimulated mycelial growth by 5.2-8.1%. All strains of T. harzianum were antagonistic to the Pleurotus species in the control. When essential oil was added to the substrate cultural, the antagonistic activity of T. harzianum against the Pleurotus species was weak (0.0625 l L of essential oil) or non-existent (0.125 l L of essential oil). Currently, synthetic chemicals are currently used to prevent and

control T. harzianum in mushroom cultivation; M. alternifolia essential oil could be an alternative to the synthetic [90].

Essential oils, aromatherapy: From at least 4000 years, essential oils are used by man to for prevention and treatment of many disorders. Due to the balancing properties of essential oils, a type of "alternative medicine" called aromatherapy has been developed. Aromatherapy is defined as the treatment or disorders prevention by the use of essential oils. Aromatherapy is a complementary medicine that can be considered a branch of phytotherapy; it combines two words: aroma (a fragrance) and therapy (a treatment). Our sense of smell access to the brain's limbic system, which is an anatomical structure that is our emotional "part", to spread the 'essential oil in the environment is used burners, nebulizers and diffusers. A source of heat to evaporate the essential oil previously diluted in water. The heat is used to dissolve the oil in the water, which otherwise would not be water-soluble, only aroma delivery through inhalation, to induce psychological or physical effects, can be defined as aromatherapy [91]. Nevertheless, the clinical use of essential oils and their volatile constituents via inhalation or massage has expanded worldwide.

Conclusion

The various essential oil extraction processes were reviewed. The conventional methods were compared with the improved process. These improvements focus on optimizing yields of essential oil as well as the operating parameters of the processes. The results showed the benefits of the new processes over the older one in terms of the environmental preservation and energy consumption. However, the costs of the new technologies are still high and are therefore not available to all manufacturers. Toxicological studies should be conducted to determine the effect of the obtained essential oils on human health. Further, in this article are intended for retrieving the attention of scientific community on the wide range of application of essential oils. They can provide to develop new drugs from natural products. Thus, essential oils and their constituents can hopefully be considered in the future for more clinical evaluations and possible applications,

and as adjuvants to current medications. The data presented provide a basis for reviving investigation on the pharmaceutical diversity of essential oils.

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