International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Advancements in bioactive components isolation from essential oils: A review

Ghulam Meheuddin[*1], *Elham Ahmed Kazerooni*[2]

[1]*Department of Chemistry, University of Agriculture, Faisalabad-38040-Pakistan*

[2]*Department of Plant Sciences, College of Agriculture and Marine Sciences, Sultan Qaboos University, Al-Khoudh 123, Muscat, Oman*

Abstract

Bioactive components found in essential oils have a wide range of advantageous qualities, including antimicrobial, antifungal, antiviral, anti-inflammatory, anticancer, and antioxidant actions. Conventional techniques for the isolation of essential oils have disadvantages like loss or degradation of certain components, prolonged extraction time, and using substantial amounts of solvent. The use of advanced techniques offers higher yields, better quality, and faster separation. Modern techniques including pressurized liquid extraction (PLE), microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), and sub- and supercritical fluid extraction (SFE) improve yield and quality while classic methods like solvent extraction, distillation, and pressing have also been used. Differences in the partition coefficient of components are used in isolation techniques such as high-speed countercurrent chromatography to get around problems with traditional techniques (solid support, solute adsorption, contaminants, pH limitations). The separation, purification, and concentration of bioactive compounds are further refined by advanced technologies like Membrane Filtration Gel Filtration Chromatography, Microfiltration, Ultrafiltration, Nanofiltration, Molecular Distillation, Supercritical Fluid Chromatography, Simulated Moving Bed Chromatography, Prep-GC, Multi-dimensional Chromatographic Separation. These techniques take advantage of larger molecules, continuous functioning, economical solvent use, and enhanced component separation.

Keywords: essential oils, bioactive components, advanced techniques, isolation, extraction, bioactivity.

Full length review article **Corresponding Author*, e-mail: *ghulammeheuddin@gmail.com*

1. Introduction

Essential oils referred to as volatile oils or etheric oils are complex mixtures of volatile components obtained and biosynthesized by various aromatic plants. Various parts of plants such as flowers, bark, buds and leaves, seeds and fruits, rhizomes, and roots contain and are employed for essential oil extraction [1-3]. Essential oils are globally recognized due to their possession of applications in cosmetics, industries, food systems, aromatherapy, and pharmaceuticals [4-8]. Essential oils (EOs) have been used for centuries in traditional medicine, numerous studies have been conducted on their possible uses in food, cosmetics, and pharmaceuticals. Researchers are trying to characterize the biological properties of these volatile oils which include antibacterial, antioxidant, antifungal, antimicrobial, antiviral, and synergistic activities as well as antimutagenic, anticancer, anti-inflammatory, and immunomodulatory [9-10]. Essential oils show solubility in alcohols, ethers, and specific oils but are insoluble in water. Essential oils exist as colorless liquids at normal temperatures and have a density of less than one. Their optical activity is quite high and they have a high refractive index. Essential oils have distinct smells except cinnamon, sassafras, and vetiver. Plants release smell because they contain essential oils or volatile oils in them, this is the reason essential oils have extensive applications in cosmetics, aromatherapy, and perfumery. These volatile oils are also used in massage, inhalations, and baths as a therapeutic approach. Additionally, EOs have deterring and antifungal properties similar to those of insecticides. Chemical classification of essential oils suggests that they fall under alcohols, phenols, ethers, esters, aldehydes and ketones, amines, amides, and primarily terpenes. Numerous fragrant notes, including fruity ((E)-nerolidol), floral (Linalool), herbal (γ-selinene), citrusy (limonene), and citrusy (limonene), are present in alcohols, ketones, and aldehydes [11]. Modern non-conventional extraction techniques are developed to increase yield and reduce mass transfer limits quickly while using the least amount of extraction solvents possible. Ultrasound-assisted extraction, microwave-assisted extraction, pressurized liquid extraction, supercritical and

subcritical fluid extraction, and pulsed electric fields are some of these advanced techniques. Compared to traditional methods, several strategies have shown to be beneficial in producing plant extracts of superior quality faster, at lower temperatures, and with less energy usage [12]. In the future, combining non-thermal processing technologies will be essential to provide safe outputs and sustainable processing, even if there are still many obstacles to overcome. This might lead to the development of novel methods for producing plant extracts of high quality and essential oils. More research work is required to produce therapeutic bioactive chemicals and essential oils in an ecologically friendly, efficient, and "green" manner [13].

Essential oils or plant extracts are complex mixtures containing a large number of natural products. To separate or purify active fractions different isolation and purification techniques are applied. Difference in the chemical and physical properties of natural products leads to their isolation from the mixture. Chromatographic techniques (e.g. column chromatography) are the major tools to isolate pure natural products and essential oils from complex mixtures [14].

2. Sources of essential oils

Different parts of various aromatic plants are used for extraction of essential oils including flowers of chamomile, rose, and lavender and leaves of peppermint, basil, and rosemary. Seeds of some plants like cumin, cardamom, and fennel are commonly employed for essential oil extraction. The bark and wood of some plants like cinnamon, cassia, sassafras, camphor, and sandalwood as well as the roots and rhizomes of some plants such as ginger, vetiver, and turmeric are sources of essential oil. Along with other parts of plant resins some plants like myrrh and frankincense are also good sources of essential oils. A wide variety of botanical families synthesize and serve as sources of essential oils. Some of the most important and common families are Rosaceae (e.g., rose), Lamiaceae (e.g., mint), Myrtaceae (e.g., allspice, myrtle, clove), and Oleaceae (e.g., jasmine) [15-16].

Out of 3000 recognized essential oils, 300 are significant from a commercial standpoint [17]. Around 60 plant families that belong to different genera are known for producing essential oils valuable in pharmaceutical, therapeutic, fragrance, flavors, and agriculture industries. Certain plant families such as Alliaceae, Apiaceae, Asteraceae, Lamiaceae, Myrtaceae, Poaceae commonly known as grass family and Rutaceae (citrus family) are famous for their ability to provide essential oils of medicinal as well as industrial importance [9]. Many species found worldwide belong to the genera Hyssopus, Origanum, Leonurus, Salvia, Mentha, Nepeta, Perovskia, Scutellaria, and Ziziphora. These species are sources of essential oils that are commonly employed to treat inflammation, dermatitis, gastritis, wounds, and infections. Numerous chemicals, as well as essential oils with therapeutic and other commercial benefits, have been identified as a result of extensive research on the chemical components of these plants [18].

3. Extraction of Essential oil from plant material

Extraction is the first step in isolating the desired natural compounds from the basic materials. Solvent extraction, distillation, pressing, and sublimation are examples of extraction methods that adhere to the extraction principle. Solvent extraction is a popular technique. Extraction process of essential oil from a plant material consist of four stages, firstly the solute (plant extract) diffuses out of the solid matrix, this solute gets dissolved in added solvent, solid matrix absorbs the solvent and finally the extracted solutes or essential oil is collected. Any element that increases diffusivity and solubility in the earlier phases will speed up the extraction process. Within a specific range of time, the extraction efficiency rises as the extraction duration does. Once the solute is at equilibrium within and outside the solid substance, more time does not affect the extraction process. An extremely high ratio of solvent will result in an excess of solvent and require a long time to concentrate, but it will also improve the extraction yield. The use of conventional techniques like maceration, percolation, and reflux extraction offers some drawbacks such as using a substantial amount of solvent and delayed extraction time. Some of the more recent and ecologically friendly extraction methods that have also been used in the extraction of natural products include microwave-assisted extraction (MAE), supercritical fluid extraction (SFC), and pressurized liquid extraction (PLE). The advantages of these methods include less usage of organic solvent, faster product extraction, and increased selectivity [14].

4. Isolation of bioactive components of essential oils based on partition coefficient

Partition chromatography adheres to the liquid–liquid extraction concept, which relies upon the difference in solubility of two distinct immiscible liquids. Initially, a single liquid phase was affixed onto a solid substrate such as silica gel, or paper (cellulose). Solid substrate acts as stationary phase and another liquid as the mobile phase. Challenges emerged due to the potential detachment of the stationary phase and inconsistent outcomes, resulting in limited contemporary use of this approach in partition chromatography (PC). Additionally, use of a solid support such as silica gel in liquid chromatography gives rise to disadvantages like irreversible absorption of solutes, impurities, size exclusion, persisting silanol residues, and constraints related to pH, all impacting the overall yield and purity of the process [19]. Counter-current chromatography (CCC), being a support-free liquid–liquid partition method prevents the irreversible adsorption of the sample onto solid surfaces [20]. Recent advancements in CCC equipment such as high-speed countercurrent chromatography (HSCCC) and centrifugal partition chromatography (CPC) provide an additional advantage in performing preparative-scale separations [21].

4.1 High-speed counter current chromatography (HSCCC)

HSCCC employs a liquid mobile phase, a centrifugal force field, and a support-free liquid stationary phase. The use of a multi-layer spiral tube with synchronous planetary centrifugal motion in HSCCC swiftly captures the

sample, enabling rapid and efficient separation. Separation column in HSCCC is either a transparent spiral tube or a straight PTFE tube, uses a two-phase solvent system. Due to centrifugal force, one immiscible solvent phase stays stationary inside the column, while the other phase, carrying the sample, moves through the column as a moving phase propelled by a continuous pump. In the separation tube, two-phase solvents are consistently blended and layered to swiftly distribute the sample across both phases. Selection of a mobile phase and stationary phase liquid is an important step to achieve better peak resolution and retention time. The solvent system is selected based on stability and solubility, setting time, and partition coefficient of sample in the solvent system [22].

Head-to-tail or tail-to-head elution techniques are used to pump the mobile phase. Through the injection valve, the sample is injected once the hydrodynamic equilibrium has been reached throughout the column. Variations in components and their solvent distribution capacity influence their speed within the pipeline. Consequently, the sample components separate based on their distribution coefficients [23]. A scholarly publication reported isolation threenmajor of bioactive components i.e. zingerone, 6-gingerol, and sesquiterpenes from essential oil of ginger with biphasic solvent system utilizing HSCCC [24].

4.2 High-performance counter-current chromatography (HPCCC)

HPCCC represents a recent advancement in chromatographic techniques, enabling rapid separations within minutes, a stark contrast to the lengthier process encountered in HSCCC. HPCCC is a variant of Counter-Current Chromatography (CCC) that employs a support-free liquid stationary phase secured by centrifugal force, facilitating the isolation of chemical constituents from mixtures. Functioning akin to HSCCC but at significantly higher gravitational force (g-level), HPCCC employs centrifugal force fields reaching 240 times gravity (240 × g), enhancing retention of the liquid stationary phase. This feature allows for escalated flow rates, resulting in abbreviated run times and elevated throughput compared to traditional CCC methods [25]. Early HSCCC equipment was operated at g-levels of less than 80g and took several hours for separation. In addition to providing greater g-level, Use of HPCCC reduce the analysis time to less them an hour [26]. A study conducted by Skalicka and colleagues elucidates use of n-heptane, methanol, ethyl acetate, and water solvent system (5:2:5:2, v/v/v/v) in HPCCC to isolate linalool, terpinene-4-ol, α-terpineol, and p-anisaldehyde from *Pimpinella anisum* essential oil through a stepwise gradient elution process [27]. Patuletin-3-O-glucoside, kaempferol, quercetin, astragalin, and isorhamnetin were effectively extracted from *Flaveria bidentis (L.) Kuntze* via elution pump out HPCCC [28].

4.3 Centrifugal partition chromatography (CPC)

Hydrostatic CCC, such as centrifugal partition chromatography (CPC), operates with a single rotating axis along with a set of interconnected chambers to retain the stationary phase. In contrast to HSCCC a CPC system has a greater stationary phase retention and higher system pressure. Elevated pressure in the CPC system restricts resolution enhancement by extending the column length. Using centrifugal partition chromatography, volatile compounds from essential oil of *Curcuma wenyujin*, curdione, curcumol, germacrone, curzerene, 1,8-cineole, and β-elemen were successfully extracted. In this procedure, a nonaqueous two-phase solvent solution in a ratio of 4:3:1 v/v/v was used to combine petroleum ether, acetonitrile, and acetone [29].

5. Isolation based on difference in molecular size

Isolation of bioactive components of essential oil through membrane filtration and gel filtration chromatography relies on their respective molecular dimensions [14].

5.1 Membrane filtration (MF)

In membrane filtration uses a semipermeable membrane that permits smaller molecules to pass while trapping larger ones. The use of membrane technology for the concentration, purification, and separation of bioactive substances from organic and aqueous solutions has gained popularity in recent days. Natural product MF is categorized into microfiltration, ultrafiltration, and nanofiltration, determined by the membrane's pore size. The utilization of coupled membrane filtration emerges when a singular membrane filtration step falls short in efficacy. Extraction of bioactive constituents from leaf extract of olive using a consecutive series involving micro, ultra, and nanofiltration has been reported in a study. In the MF process (0.2 μm), it filters out large particles. Ultrafiltration eliminates molecules larger than 5 kDa, while nanofiltration concentrates substances up to 300Da in size [30]. A research describes the concentration of antioxidant components in rosemary (*Rosmarinus officiallis L.*) essential oil, such as flavones, rosmarinic acid, carnosic acid, and carnosol, using nanofiltration [31].

5.2 Gel filtration chromatography (GFC)

Gel filtration chromatography is also known as gel permeation or molecular sieve chromatography, separates molecules based on their size. It operates on the principle of partitioning molecules between a moving phase over a stationary phase, where a porous matrix separates them by size. In this technique, a column filled with this matrix, often in bead form, contains two liquid volumes one is the external liquid between the beads and the other is internal liquid within the beads. This process effectively isolates molecules by their relative sizes. Upon sample application, the void volume (V_0) denotes the external volume, while the total volume (V_t) represents the combined external and internal volumes. Larger molecules are quickly removed from the inner bead volume and travel quickly through the column to surface at V0. The migration and emergence of smaller and intermediate-sized molecules, on the other hand, occur more slowly and occur at an elution volume (V_e) that is greater than V_0 as a result of their equilibration with both internal and exterior liquids. Hence, molecules are sequentially eluted based on decreasing molecular size. Gel filtration matrices are crafted from various materials like dextrans (Sephadex), agarose (Sepharose), polyacrylamide (bio-Gel), cellulose, silica-based elements, or blends like dextran-polyacrylamide

(Sephacryl) or dextran–agarose (Superdex). Each material presents distinct advantages and drawbacks [32].

Sephadex, a product of dextran cross-linking, specifically the G-types, effectively separates hydrophilic compounds like peptides and carbohydrates (oligosaccharides, polysaccharides) [33]. The hydroxypropylated form of Sephadex G25, known as Sephadex LH-20, uses the adsorption process and demonstrates both hydrophobic and hydrophilic qualities. It effectively uses aqueous or non-aqueous solvents to separate a wide variety of natural materials. For example, feruloylated arabinoxylan oligosaccharides from intermediate wheat, a perennial cereal grain, were effectively separated utilizing 100% water-mobile phase of Sephadex LH-20 [34].

6. Alternative contemporary methods of separation

6.1 Molecular distillation (MD)

Molecular distillation represents a specialized iteration of vacuum distillation. Operating within an ultra-high vacuum environment (< 10 mbar), this method enables the handling of compounds at notably reduced temperatures, significantly beneath their boiling points. This aspect safeguards the retention of crucial attributes inherent in thermosensitive products, thereby averting the potential loss of desirable characteristics during the separation process [35]. Borgarello and colleagues utilized molecular distillation, guided by artificial neural networks, to extract a concentrated thymol fraction from oregano essential oil. This resultant fraction exhibited antioxidant qualities and demonstrated the capacity to stabilize sunflower oil [36]. D-limonene, a primary component of essential oil of orange (*Citrus sinensis*), is vulnerable to heat, light, and water, which compromises the oil's overall quality. Utilizing molecular distillation enables the isolation of a D-Limonene-enriched fraction, mitigating these stability issues. Molecular distillation stands as a "non-thermal" method, showcasing efficacy in concentration of oxygenated compounds (like linalool, decanal, and valencene) within the heavy fraction at low temperatures (around 30–35 °C) [37].

6.2 Supercritical fluid chromatography (SFC)

SFC involves the use of supercritical fluid as moving or mobile phase which combines the merits of gas chromatography and high-performance liquid chromatography (HPLC), fostering SFC advancement. While various supercritical gases have found utility, supercritical CO_2 has asserted its dominance in contemporary SFC. Unlike GC, packed columns are predominantly used in SFC applications. While most stationary-phase materials align with SFC, manufacturers are now tailoring columns explicitly for SFC purposes. 2-Ethylpyridine-bonded silica is still a frequently mentioned stationary phase in achiral separations, but materials based on polysaccharides are predominant in chiral separations. Mass spectrometry (MS) prevails as the primary detection mode in SFC, followed by UV/Visible absorption, typically recorded through photodiode arrays (PDA). For analytes challenging to ionize or lacking chromophores, universal detectors like Evaporative Light Scattering Detector (incompatible with GC) or flame ionization detector FID (unsuitable for UHPLC) are viable options [38]. Polymethoxylated flavones such as tangerine, heptamethoxyflavone, tetra-O-methylscutellarein, hexamethoxyflavone, and sinensetin have undergone isolation and quantification within the EOs of sweet orange (*Citrus sinensis)* and mandarin (*Citrus reticulate*) [39]. The active elements (Verbenone, Trans-caryophyllene, Terpinen-4-ol, Borneol, Camphor, 1,8-cineole, and Linanool) found in *Rosmarinus officinalis L.* essential oil, known for their antioxidant and antimicrobial qualities, are effectively obtained and separated using a preparative-SFC method [40].

6.3 Simulated moving bed chromatography (SMBC)

SMBC employs several columns holding stationary phases. Movement of bed that resembles the counter-current flow, is orchestrated by rotary valves. These valves intermittently redirect the feed and eluent through the inlet as well as extract and raffinate through the outlet. SMBC stands out as a continuous separation technique, ideal for efficiently isolating natural products on a large scale while consuming less solvent in a shorter duration. SMB chromatography has demonstrated efficacy in segregating multi-component blends. In contrast to the conventional single-column method, SMB has excellent separation effectiveness and purity and performs well when it comes to large-scale separation of various combinations. A sequential simulated moving bed (SSMB) technique was developed by Cheng et al. to continue isolating the high-value tri-alpha-linolenic monomer from silkworm pupae oil [41].

6.4 Isolation using preparative Gas chromatography (Prep-GC)

Prep-GC is an important technique to recover bulk materials in pure form for isolation and purification of specific constituents in a mixture for later purposes like structural elucidation. This technique is also used to isolate EO components for use in commercial applications. After GC separation, prep-GC adds mass to a single component or isolated zones of compounds from a sample. Prep-GC can be used using packed-column GC, which permits adding a larger sample mass to the column, multidimensional approaches that combine two capillary columns of different phases, or capillary column GC for improved component separation [42]. An autosampler periodically injects the oil-bearing desired components into a GC fitted with a preparative capillary column and a cooled injection system. A zero dead volume effluent splitter at end of the column transfer some of the effluent (1.0%) to the detection system and transported the bulk (99.0%) to a preparative fraction collector (PFC) unit, which trapped certain fractions. The isolated chemicals have been created in sufficient amounts to enable their subsequent identification using various spectroscopic techniques by trapping them in collector vials chilled with liquid nitrogen over numerous injections [43].

Preparative multidimensional gas chromatography (prep-MDGC) has gotten easier because of enhanced pressure/flow control, better column connections, and the availability of computer software to help with dimensions and conditions of column for experiment computation. The prep-MDGC approach was used to a composition of peppermint, spearmint, and lavender essential oils that had been spiked

with geraniol (1.1 mg/mL). The results showed that linalyl acetate, carvone, and menthol could be resolved and separated [44]. The oil of *Crinitaria tatarica* was split into many components using preparative capillary gas chromatography (PCGC). 87 components, or 98.5% of the total, were identified from the oil study. These included two unknown compounds, I (21.4%) and II (3.4%), as well as β-pinene (8.8%) and sabinene (32.1%). The primary groups found in the oil were isocoumarins, oxygenated monoterpenes, and hydrocarbons of sesquiterpenes, oxygenated sesquiterpenes, and hydroterpene hydrocarbons. Each analysis in an essay requires good PCGC analytical circumstances. Thus, a variety of parameters are tested and optimized, including injection volumes, cooling temperatures, PFC transfer and distribution temperatures, and column and flow rates. The ideal separation and successful collection conditions are attained and implemented [43].

6.5 Multidimensional chromatographic separation

GC using a single column remains the preferred technique for routine essential oils investigation. One-dimensional GC can sometimes be not able to isolate every compound of the mixture. MDGS system introduces another column with a different stationary phase, this increases the peak capacity, resolution, and selectivity. The second column is connected with the first vis transferring device. This technique is known as multidimensional gas chromatography (MDGC). MDGC relies heavily on the efficient functioning of the transferring mechanism in which the fractions eluting from the first column are transferred to the second-dimension column. Fractions from the first column can be concentrated into a narrow band during this process and then transferred to a second dimension (2-D) column, this improves the sensitivity and aid in identifying components at the trace level [45].

"Two-dimensional gas chromatography" (GCXGC) is the multi-dimensional Gas chromatographic approach. The advantages of quicker analysis times and more separation power are combined by using the valve less on-column interface among the two gas chromatographic columns and high-speed second dimension. The first dimension in the GC x GC system yields separation similar to 1D GC. The effluent from the primary column is injected into the secondary column via a modulator located at the intersection of the two columns. The second-dimension column produce a secondary chromatogram which separates all the concentration pulses into their constituent parts. The modulator periodically generates the concentration pulses and a secondary chromatogram is developed for each pulse. Every time a secondary chromatogram develops, a modulator collects samples for the next concentration pulse. Hence, both separation dimensions are applied to every part of the sample. A "snapshot" of the second separation dimension is produced by each high-speed chromatogram that makes up the second-dimension chromatogram. To concurrently see the peak profiles in two dimensions or one dimension the retention time of both dimensions and signal intensity is recorded along with the snapshots. Snapshots take place at a specific frequency which permits continuous inspection of separation in both dimensions. Four large peaks in the chromatogram of peppermint essential oil represent menthone, menthol, menthyl acetate, and eucalyptol while menthofuran, isomenthone, neomenthol, limonene, and pulegone have smaller abundances. Carvone, menthol, limonene, and menthone are the four main peaks on the spearmint chromatogram; iso-menthone, neomenthol, and cis-dihydrocarvo are present in lesser amounts [46].

Essential oil of *Artemisia argyi* was investigated by comprehensive 2D (CCC×GC) chromatographic separation. First-dimensional chromatography was chosen to be CCC, and GC examination was performed on every fraction that was collected from the first dimension. The orthogonality of this extensive 2D chromatography method for the essential oil separation of *A. argyi* has been investigated. The EO of *A. argyi* was thoroughly characterized using 2D CCC × GC, a parallelism metric for 2D CCC × GC, and a 2D contour plot map for chemical component categorization utilized in a study. The linear correlation coefficient and geographical coverage demonstrated the entire obliqueness of the 2D CCC × GC. Research on this 2-D separation technique might significantly lower peak overlaps in 1D chromatography and enhance resolution for complex natural crude extracts. The comprehensive 2D CCC × GC may offer a productive way for analyzing EOs chromatographically. A 2D contour plot facilitates the visualization of various chemical substance classifications [47].

7. Bioactivity of essential oils

7.1 Antioxidant activity

When free radicals oxidize biomolecules like proteins, lipids, or DNA, they can have hazardous consequences. This process often results in damage to the cell and its ultimate demise. Foods lose their sensory and nutritional value during the oxidation procedure. Antioxidants, or substances that prevent oxidation in living things and fat-based meals, especially in meat and dairy products or fried foods, remove and minimize the harmful effects of free radicals. Antioxidants are utilized extensively in the medical field as well as other sectors. They are classified as, synthetic and natural. The second category is frequently utilized as meal additives to shield meals from oxidative deterioration [66]. The biological activities of several EOs are significantly influenced by their antioxidant capabilities. These characteristics result from the naturally occurring capacity of its components to inhibit or postpone the oxidation of organic matter, especially phenols. However, because many of these compounds are non-volatile, the distillation process, which extracts the oil from the raw material, restricts the amount of phenolics in the final matrix. Many EOs without phenol possess anti-oxidant qualities [67]. It is being proposed that terpenes and terpenoids, two major fragrance elements of essential oils, contribute to their antioxidant capacity. The aforementioned components are found in *Mentha aquatica L.*, *Mentha longifolia L.*, and *Mentha alternifolia L.; in Melaleuca alternifolia* tea trees, they are menthone and iso-menthone; in Black Cumin, Cinnamon Bark, and Ginger; and in thyme and clove leaves [68].

Table 1. Bioactive components of essential oils, isolated by advanced isolation techniques.

Plant	Main constituents	Isolation technique	Bioactivity	References
Flaveria bidentis.	caryophyllene (2.85%), caryophyllene oxide (1.6%),	HSCCC	Antibacterial, antiinflammatory	[48]
ginger	zingiberene (16.72%), 6-gingerol (11.7%), 8-gingerol (11.37%)	HSCCC	anti-proliferative	[24]
Pimpinella anisum L.	Anethole (7.14%), foeniculin (1.0%)	HPCCC	Antimicrobial, Antifungal	[27]
Nigella damascena L.	β-elemene (47%)	HPCCC	Antimicrobial	[49]
Curcuma wenyujin	Curzerene (6%), β-elemene (5.6%)	CPC	anti-tumor, anti-viral	[29]
Acorus calamus	β-asarone	CPC	anti-inflammatory, neuroprotective	[50]
Rosmarinus officiallis L.	rosmarinic acid, carnosic acid, flavones	Membrane filtration	Antioxidant	[31]
Agelas axifera	*pyrimidine diterpenes, axistatins*	GFC	Anticancer	[51]

Table 2. Major bioactive components of some common essential oils and their bioactivity.

Essential oils	Major Components	Bioactivity	Reference
E. tereticornis	Myrcene, α-thujene, α-pinene, camphene, β-pinene, α-phellandrene, limonene, α-fencho, trans-pinocarveo, borneo, α-terpineo, aromadendrene	antibacterial	[52-53]
E. citriodora	*Trans*-caryophyllene, γ-elemene, *Trans*-caryophyllene, γ-elemene, Geranyl acetate, methyleugenol, Citronellyl acetate	Antibacterial, antifungal	[53-54]
Coriander (*Coriandrum sativum L.)*	Camphene, myrcene, limonene, a-pinene, geraniol, terpinen-4-ol, α-terpineol, γ-terpinene, r-cymene, limonene, a-pinene, lauricene, Phenol, camphor, and linalyl acetate	antibacterial, antifungal and anti-oxidative	[55-56]
Thymbra spicata L	α-Dihydroxyphene, α-Pinene, Camphene, Sabinen, α-Terpinene, p-Dihydroxyphene, o-Dihydroxyphene, γ-Terpinene, 3-caren-2-ol, Oxide of caryophyllene	antioxidant, antimicrobial, antibiofilm, cytotoxic	[57]
Kaffir Lime *(Citrus hystrix DC)*	Myrcene, β-Phellandrene, 2-Carene, β-Pinene, γ-Terpinene, 3,8, p-Menthadiene, Terpinolene, Citronellal, Geraniol, Terpin, Hydroxycitronellal	antioxidant and antibacterial	[58]
Flaveria bidentis	Patuletin-3-O-glucoside, kaempferol, quercetin, astragalin, and isorhamnetin	antioxidant	[28]
Rosmarinus officiallis L.	rosmarinic acid, carnosic acid, carnosol and flavones	Antioxidant	[31]
Citrus oil *(Citrus sinensis L., Citrus paradise, Citrus deliciosa, Citrus limon)*	1-Decanol, α-terpineol, geraniol, and linalool	antimicrobial	[59]
Rosmarinus officinalis L.	Verbenone, Trans-caryophyllene, Borneol, Terpinen-4-ol, 1,8-cineole, Linalool, and Camphor	Antioxidant,antimicrobial	[40]
Crinitaria tatarica	β-pinene, sabinene	antifungal	[43]

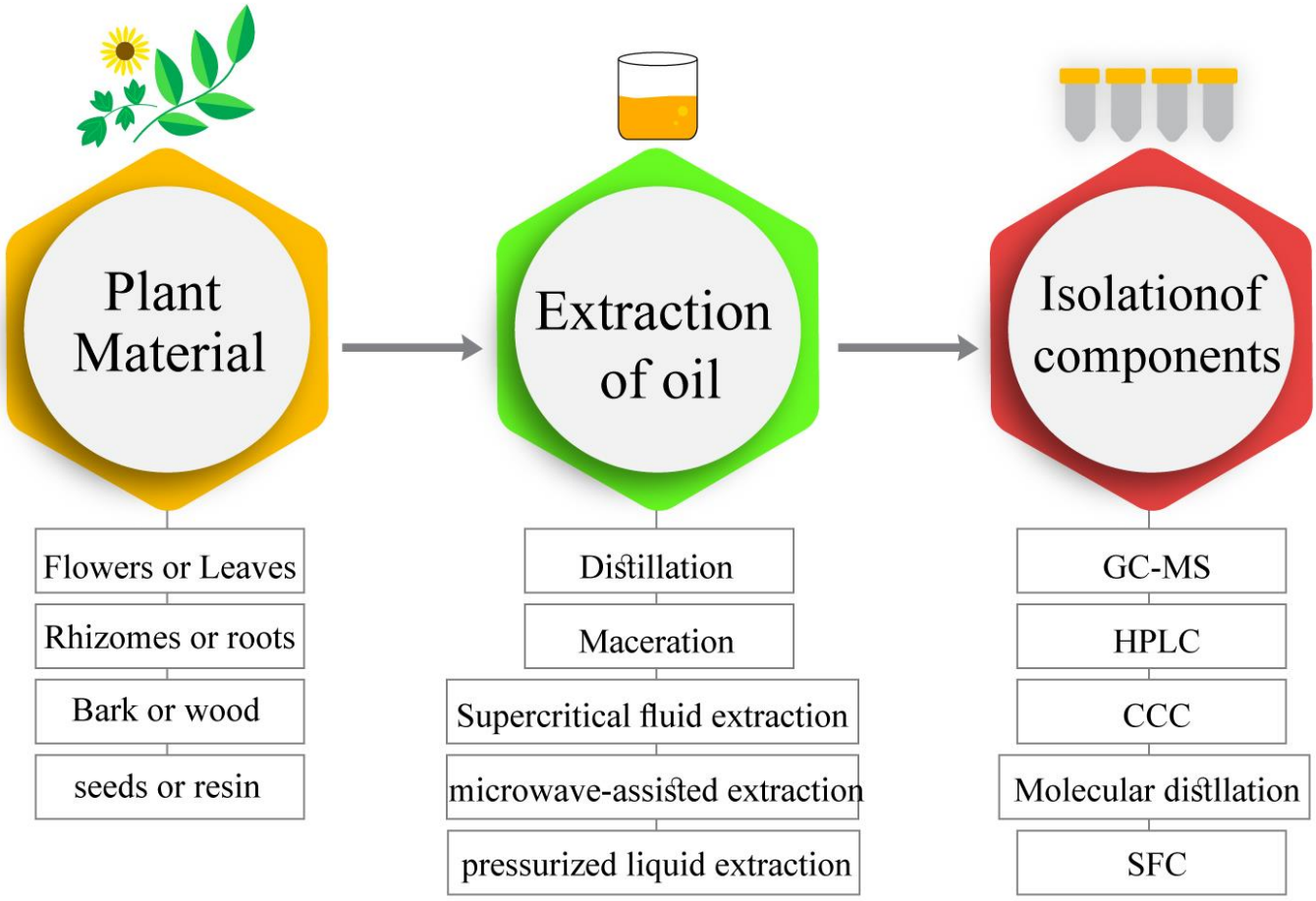

Figure 1. Stages involved in obtaining bioactive components of essential oil.

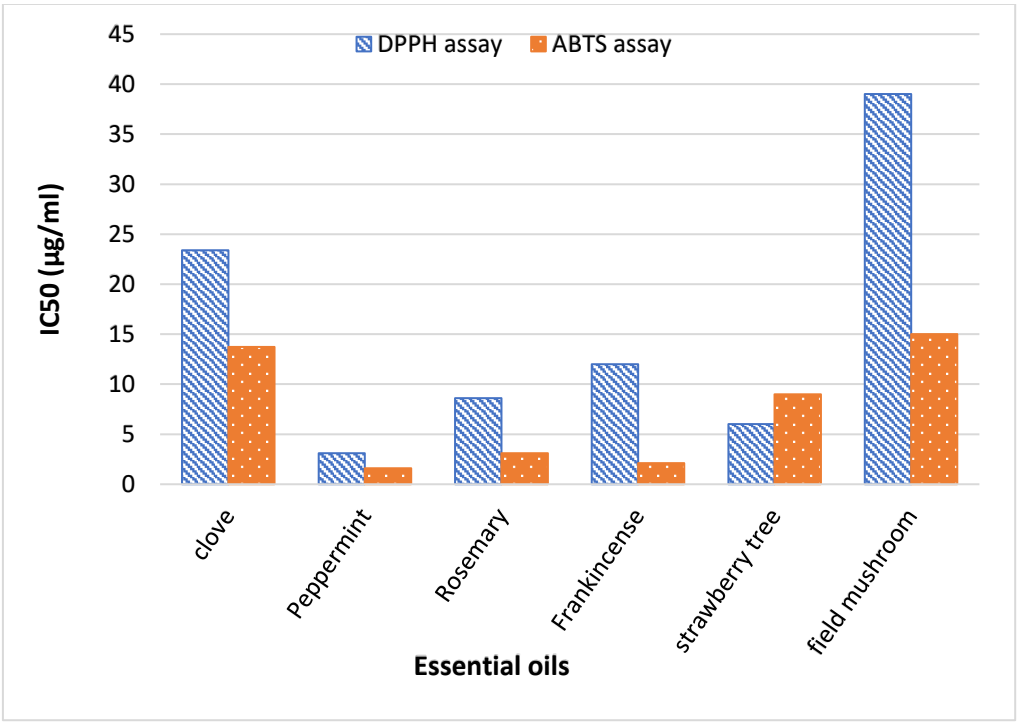

Figure 2(a). Comparison of IC50 of essential oils determined by DPPH and ABTS assay [60-62].

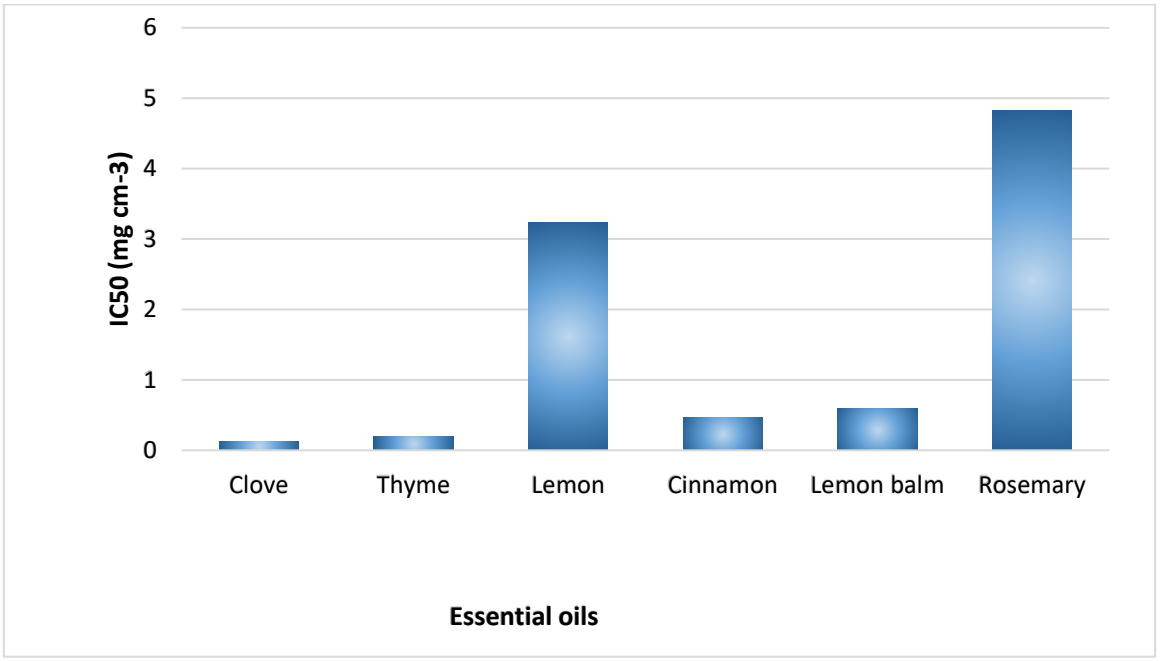

Figure 2(b). Comparison of IC50 of essential oils determined by β-carotene bleaching assay [63].

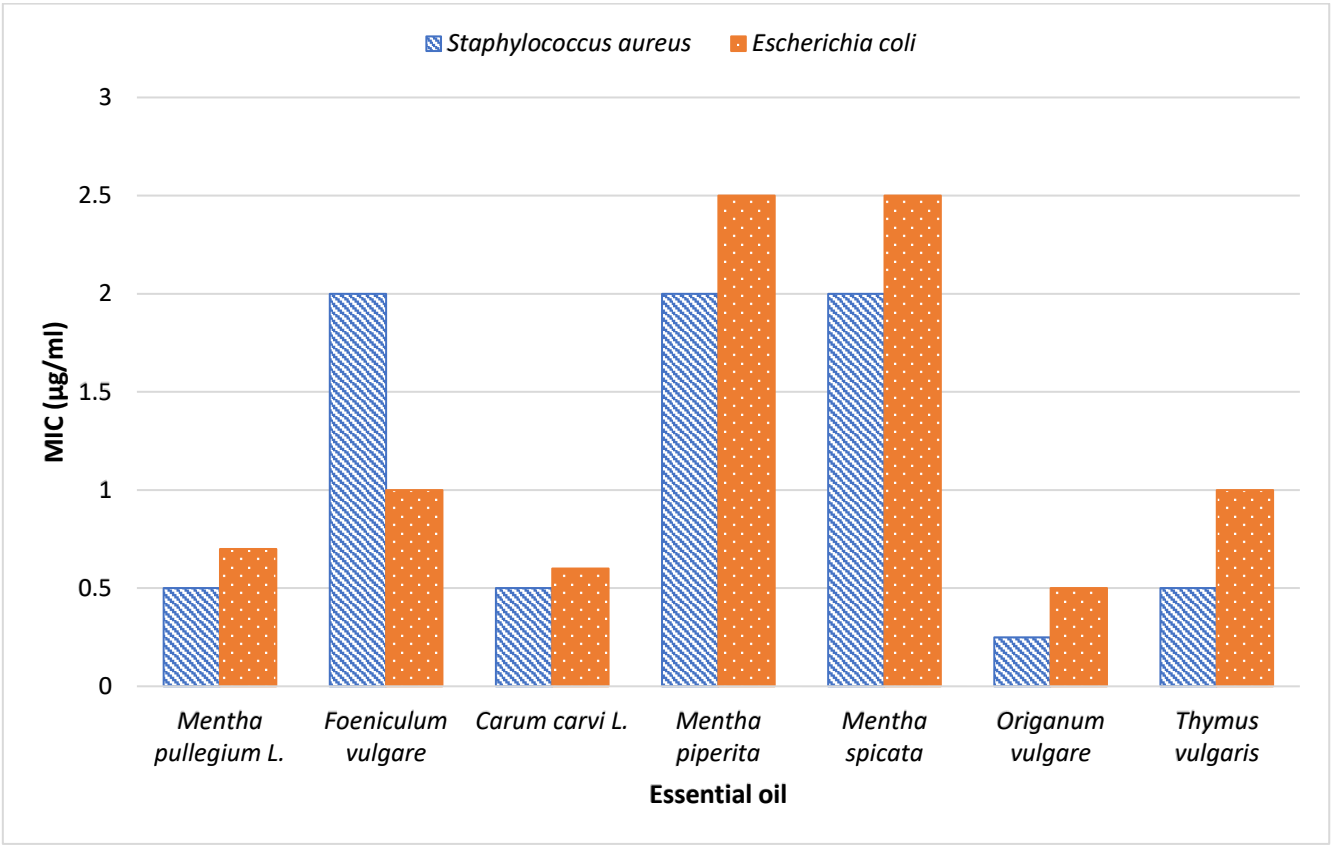

Figure 4. MIC of various essential oils against *S. aureus* and *E. coli* by microdilution method [64-65].

The ABTS technique, also known as Trolox equivalent antioxidant capacity is frequently used to assay the antioxidant activity of essential oils. This process depends on the reduction of $ABTS^+$, a blue-green radical. The degree of decolorization is measured as a percentage inhibition of $ABTS^+$ and is calibrated against Trolox which is taken as the reference standard, based on concentration and duration. The reduction capacity of metallic elements like Cu(II) is also used for antioxidant assay reduction. It is predicated on the antioxidant in the sample reducing Cu(II) to Cu(I). Bathocuproin Cu(I) and (2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline) combine to create a complex. this complex has maximum absorption at 490 nm [69]. DPPH (2,2-diphenyl-1-picryl-hydrazyl, λ_{max} 517 nm) assay is another frequently used method to access the antioxidant potential of natural products. Any molecule that has a weak X-H bond or an antioxidant reacts with the colorful and persistent radical DPPH, this discolorizes the DPPH solution. A common approach to represent results is half-maximal inhibitory concentration (IC50), which is the concentration of the potential antioxidant needed to lower the initial absorbance of the colored radical by 50%. The relationship between antioxidant activity and IC50 is inversely proportional. An antioxidant-active sample has a greater level when its IC50 value is lower. A comparison of certain essential oils that are most well-known for their antioxidant capabilities is shown in Figures 2(a) and 2(b) [70].

7.2 Antibacterial activity

The two main types of bacteria, Gram-positive and Gram-negative, show susceptibility to essential oils and their components. Typically, the techniques employed for the antibacterial assay are agar or broth dilution procedures or disc diffusion methods. In disc-diffusion methods, an essential oil-impregnated paper disc is placed on an infected agar medium. After a certain period of time, diameter of the region surrounding the disc, where bacteria were unable to proliferate, is determined after the disc is incubated. Despite their popularity, disc-diffusion methods yield fewer valuable data than agar and broth dilution procedures due to the zones of inhibition they produce. The hydrophobic nature and restricted water solubility of essential oils significantly impair the diffusion of EOs through the agar which is a crucial component of disc-diffusion studies. Minimum inhibitory concentrations (MICs) can be found using agar dilution or broth dilution techniques. These techniques involve serial dilutions of the test oil on agar or broth media that are infected with a known quantity of test organism. The minimum essential oil concentration (MIC) is commonly understood to be the concentration at which the test organism cannot grow. Since MICs can assist in establishing safe and effective final doses in formulated goods, they are more beneficial than zones of growth inhibition. It is important to note that this level of activity is approximately 1000 times lower than that of traditional antibiotics, for which the MICs of sensitive bacteria are given in µg/ml levels. Nevertheless, this level of activity is still potentially beneficial. Figure 3 shows comparison between the antibacterial activity of various essential oils [71].

7.3 Anticarcinogenic Activity

The formation of tumors is a multiphase process that starts with transformation in a cell, continues via hyperproliferation, ends with development of metastatic lesions, invasive potential, and angiogenic qualities. Cigarette smoking, industrial pollutants, fuel fumes, inflammation and infection, nutrition, and dietary carcinogens are the primary causes of human cancer. Prevention of cancer will eventually result from research on nutrition and dietary conditions [72]. Natural substances extracted from therapeutic plants are abundant sources of innovative anticancer medications. East Asian countries such as China, Japan, India, and Thailand have utilized traditional medicinal herbs for pharmacological and nutritional purposes for centuries. These days, these herbs are extensively employed in cancer treatment [73]. Essential oils of aromatic plants contain non-nutritive dietary substances called monoterpenes. Numerous experimental works and population-based research suggests that dietary isoprenoids are important in preventing cancer [74]. Mechanistic investigations reveal that limonene inhibits posttranslational isoprenylation and decreases cyclin D1 messenger RNA levels before it inhibits cholesterol production and promotes cell proliferation and cell cycle advancement. Protein isoprenylation is inhibited by alcohol perillyl, limonene, and their active serum metabolites [75].

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test is one frequently used technique for evaluating the anticancer potential of essential oils. This test is based on the process by which living cells' mitochondrial dehydrogenases convert the yellow tetrazolium salt MTT into purple formazan crystals. A measure of cell viability and cytotoxicity is provided by the direct proportionality between the amount of formazan generated and the number of live cells. The basis of the brine shrimp lethality test (BSLT) assay: it is the idea that a material's lethality may be ascertained by tracking how it affects the death of brine shrimp larvae. The cytotoxic potential of the test material is determined by counting the number of larvae that survive after being exposed to it. Cell cycle analysis: This technique uses flow cytometry to examine the distribution of cells in the G0, G1, S, G2, and M stages of the cell cycle. Apoptosis or cell cycle arrest may be induced by the test chemical, as shown by changes in the cell cycle profile. Apoptosis assay: This assesses the pro-apoptotic effects of test material by looking for particular indicators of apoptosis, such as caspase activation, DNA fragmentation, and phosphatidylserine externalization (Annexin V-FITC/PI staining) [76-77].

7.4 Other biological activities possessed by essential oils

The property of being harmful to cells is known as cytotoxicity. Toxic agents include things like immune cells and certain venoms. Essential oils are thought to be cytotoxic because of their effects on cellular safety, which can result in illness and apoptosis. Essential oils can induce depolarization in the mitochondrial membranes of eukaryotic cells by depressing the membrane potential, affecting ionic Ca^{++} cycling and other ionic channels, and reducing the pH gradient. Utilizing plant extracts with known antibacterial

qualities can have a major impact on medical interventions. Numerous research demonstrating this effectiveness has been carried out in various nations in the past several years. Due to substances produced in the plant's secondary metabolism, several plants have been utilized for their antibacterial properties [78]. Some viral diseases in humans like Herpes simplex virus (HSV; types 1, 2), has been treated with synthetic antiviral medications. Certain essential oils have been used to treat certain viral illnesses. HSV-2 replication was suppressed by the citral and citronellal-containing essential oil of *M. officinalis* [79]. The central nervous system can be greatly regulated by inhaling essential oils or the volatile parts of them. For instance, storax essential oil and *Acorus gramineus* rhizome essential oil inhalations are used in Chinese traditional medicine to treat epilepsy [80].

8. Conclusions

Essential oils are complex mixtures of various biosynthesized components including monoterpenes, sesquiterpenes, phenols, terpenoids, aldehydes, ketones, esters, and oxides which are responsible for their diverse applications in cosmetics, industries, and biological systems. conventional essential oil extraction techniques in common use are solvent extraction, distillation, and pressing however modern techniques like Ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), pressurized liquid extraction (PLE), and supercritical fluid extraction (SFE) are used to attain increased yield and good quality product. After the extraction of essential oil from plant material, components of oil are isolated using isolation techniques. Advanced isolation techniques of bioactive components from essential oils isolation based on partition coefficient are high-speed countercurrent chromatography (HSCCC), High-Performance Counter-Current Chromatography (HPCCC), and centrifugal partition chromatography (CPC). These techniques reduce the disadvantages of conventional isolation methods like the use of solid support, irreversible adsorption of solute, impurities, and constraints related to pH. Membrane filtration and gel filtration chromatography are novel technologies for separation, purification, and concentration of bioactive compounds based on molecular size. Microfiltration, ultrafiltration, and nanofiltration can filter particles of size range 0.2 µm, 5 kDa, and 300Da respectively. Supercritical fluid chromatography combines the advantages of GC and HPLC hence giving an effective separation. Simulated moving bed chromatography is a continuous process, that isolates natural products with less solvent consumption and a short time. Prep-GC uses packed or capillary columns for large sample mass and improved component separation. In case all components are not separated by a single column multi-dimensional chromatographic separation is employed which uses a second column for absolute isolation. Bioactive components isolated from essential oils possess antibacterial, antifungal, antiviral, anti-inflammatory, anticancer, and antioxidant activities.

References

[1] U.N.I.D. Organization, S.S. Handa, S.P.S. Khanuja, G. Longo, D.D. Rakesh. (2008). Extraction technologies for medicinal and aromatic plants. Earth, Environmental and Marine Sciences and Technologies: pp.

[2] K. Hüsnü, C. Başer, F. Demirci, Chemistry of essential oils. In *Flavours and fragrances: chemistry, bioprocessing and sustainability*, Springer: 2007; pp 43-86.

[3] J. Janick, J.E. Simon In *Advances in New Crops: Proceedings of the First National Symposium NEW CROPS, Research, Development, Economics, Indianapolis, Indiana, October 23-26, 1988*, 1st National Symposium NEW CROPS: Research, Development, Economics, Indianapolis, Ind.(USA), 1988, 1990; Timber Press: 1990.

[4] Z.A. Aziz, A. Ahmad, S.H.M. Setapar, A. Karakucuk, M.M. Azim, D. Lokhat, M. Rafatullah, M. Ganash, M.A. Kamal, G.M. Ashraf. (2018). Essential oils: extraction techniques, pharmaceutical and therapeutic potential-a review. Current drug metabolism. 19(13): 1100-1110.

[5] A. Sarkic, I. Stappen. (2018). Essential oils and their single compounds in cosmetics—A critical review. Cosmetics. 5(1): 11.

[6] B.S. Jugreet, S. Suroowan, R.K. Rengasamy, M.F. Mahomoodally. (2020). Chemistry, bioactivities, mode of action and industrial applications of essential oils. Trends in Food Science & Technology. 101: 89-105.

[7] J. Fern, M. Viuda-Martos. (2018). Application of essential oils in food systems. MDPI: pp.

[8] B. Ali, N.A. Al-Wabel, S. Shams, A. Ahamad, S.A. Khan, F. Anwar. (2015). Essential oils used in aromatherapy: A systemic review. Asian Pacific Journal of Tropical Biomedicine. 5(8): 601-611.

[9] J.S. Raut, S.M. Karuppayil. (2014). A status review on the medicinal properties of essential oils. Industrial Crops and Products. 62: 250-264.

[10] M.N. Aljaafari, A.O. AlAli, L. Baqais, M. Alqubaisy, M. AlAli, A. Molouki, J. Ong-Abdullah, A. Abushelaibi, K.-S. Lai, S.-H.E. Lim. (2021). An overview of the potential therapeutic applications of essential oils. Molecules. 26(3): 628.

[11] W. Dhifi, S. Bellili, S. Jazi, N. Bahloul, W. Mnif. (2016). Essential oils' chemical characterization and investigation of some biological activities: A critical review. Medicines. 3(4): 25.

[12] E. Roselló-Soto, M. Koubaa, A. Moubarik, R.P. Lopes, J.A. Saraiva, N. Boussetta, N. Grimi, F.J. Barba. (2015). Emerging opportunities for the effective valorization of wastes and by-products generated during olive oil production process: Non-conventional methods for the recovery of high-added value compounds. Trends in Food Science & Technology. 45(2): 296-310.

[13] J. Giacometti, D.B. Kovačević, P. Putnik, D. Gabrić, T. Bilušić, G. Krešić, V. Stulić, F.J. Barba, F. Chemat, G. Barbosa-Cánovas. (2018). Extraction of bioactive compounds and essential oils from mediterranean herbs by conventional and green innovative techniques: A review. Food research international. 113: 245-262.

[14] Q.-W. Zhang, L.-G. Lin, W.-C. Ye. (2018). Techniques for extraction and isolation of natural

products: A comprehensive review. Chinese medicine. 13: 1-26.

[15] J.B. Sharmeen, F.M. Mahomoodally, G. Zengin, F. Maggi. (2021). Essential oils as natural sources of fragrance compounds for cosmetics and cosmeceuticals. Molecules. 26(3): 666.

[16] M. Chávez, R. Rodríguez, C. Aguilar, Chapter 11-Essential Oils: A Natural Alternative to Combat Antibiotics Resistance. In Kateryna Kon, Mahendra Rai,. Academic Press: 2016.

[17] P. Bolouri, R. Salami, S. Kouhi, M. Kordi, B. Asgari Lajayer, J. Hadian, T. Astatkie. (2022). Applications of essential oils and plant extracts in different industries. Molecules. 27(24): 8999.

[18] N.Z. Mamadalieva, D.K. Akramov, E. Ovidi, A. Tiezzi, L. Nahar, S.S. Azimova, S.D. Sarker. (2017). Aromatic medicinal plants of the Lamiaceae family from Uzbekistan: ethnopharmacology, essential oils composition, and biological activities. Medicines. 4(1): 8.

[19] A. Berthod, M. Ruiz-Ángel, S. Carda-Broch. (2009). Countercurrent chromatography: People and applications. Journal of Chromatography A. 1216(19): 4206-4217.

[20] D. Gutzeit, P. Winterhalter, G. Jerz. (2007). Application of preparative high-speed counter-current chromatography/electrospray ionization mass spectrometry for a fast screening and fractionation of polyphenols. Journal of Chromatography A. 1172(1): 40-46.

[21] H. Song, J. Lin, X. Zhu, Q. Chen. (2016). Developments in high-speed countercurrent chromatography and its applications in the separation of terpenoids and saponins. Journal of separation science. 39(8): 1574-1591.

[22] S.M. Colegate. (2007). 21 Plant-Associated Toxins: Bioactivity-Guided Isolation, ELISA, and LC-MS Detection. Bioactive Natural Products. 555.

[23] C.-x. Wang, L.-x. Wang, C.-y. Li, C. Hu, S.-h. Zhao. (2020). Anti-proliferation activities of three bioactive components purified by high-speed counter-current chromatography in essential oil from ginger. European Food Research and Technology. 246: 795-805.

[24] Y. Yang, B.M. Khan, X. Zhang, Y. Zhao, K.-L. Cheong, Y. Liu. (2020). Advances in separation and purification of bioactive polysaccharides through high-speed counter-current chromatography. Journal of chromatographic science. 58(10): 992-1000.

[25] I. Sutherland, P. Hewitson, S. Ignatova. (2009). Scale-up of counter-current chromatography: Demonstration of predictable isocratic and quasi-continuous operating modes from the test tube to pilot/process scale. Journal of Chromatography A. 1216(50): 8787-8792.

[26] H. Guzlek, P.L. Wood, L. Janaway. (2009). Performance comparison using the GUESS mixture to evaluate counter-current chromatography instruments. Journal of Chromatography A. 1216(19): 4181-4186.

[27] K. Skalicka-Woźniak, M. Walasek, A. Ludwiczuk, K. Głowniak. (2013). Isolation of terpenoids from P impinella anisum essential oil by high-performance counter-current chromatography. Journal of separation science. 36(16): 2611-2614.

[28] Y. Wei, Q. Xie, D. Fisher, I.A. Sutherland. (2011). Separation of patuletin-3-O-glucoside, astragalin, quercetin, kaempferol and isorhamnetin from Flaveria bidentis (L.) Kuntze by elution-pump-out high-performance counter-current chromatography. Journal of Chromatography A. 1218(36): 6206-6211.

[29] Y.Y. Dang, X.C. Li, Q.W. Zhang, S.P. Li, Y.T. Wang. (2010). Preparative isolation and purification of six volatile compounds from essential oil of Curcuma wenyujin using high-performance centrifugal partition chromatography. Journal of separation science. 33(11): 1658-1664.

[30] I. Khemakhem, O.D. Gargouri, A. Dhouib, M.A. Ayadi, M. Bouaziz. (2017). Oleuropein rich extract from olive leaves by combining microfiltration, ultrafiltration and nanofiltration. Separation and Purification Technology. 172: 310-317.

[31] D. Peshev, L. Peeva, G. Peev, I. Baptista, A. Boam. (2011). Application of organic solvent nanofiltration for concentration of antioxidant extracts of rosemary (Rosmarinus officiallis L.). Chemical engineering research and design. 89(3): 318-327.

[32] C. Ó'Fágáin, P.M. Cummins, B.F. O'Connor. (2017). Gel-filtration chromatography. Protein Chromatography: Methods and Protocols. 15-25.

[33] L. Shi. (2016). Bioactivities, isolation and purification methods of polysaccharides from natural products: A review. International journal of biological macromolecules. 92: 37-48.

[34] R.R. Schendel, A. Becker, C.E. Tyl, M. Bunzel. (2015). Isolation and characterization of feruloylated arabinoxylan oligosaccharides from the perennial cereal grain intermediate wheat grass (Thinopyrum intermedium). Carbohydrate research. 407: 16-25.

[35] H. Hu, J. Huang, S. Wu, P. Yu. (2013). Simulation of vapor flows in short path distillation. Computers & Chemical Engineering. 49: 127-135.

[36] A.V. Borgarello, G.N. Mezza, M.C. Pramparo, M.F. Gayol. (2015). Thymol enrichment from oregano essential oil by molecular distillation. Separation and Purification Technology. 153: 60-66.

[37] J.A. García-Fajardo, D.A. Flores-Méndez, Á. Suárez-Jacobo, L.G. Torres-Martínez, M. Granados-Vallejo, R.I. Corona-González, G.M. Guatemala-Morales, E. Arriola-Guevara. (2023). Separation of D-Limonene and Other Oxygenated Compounds from Orange Essential Oil by Molecular Distillation and Fractional Distillation with a Wiped Film Evaporator. Processes. 11(4): 991.

[38] R.K. Hofstetter, M. Hasan, C. Eckert, A. Link. (2019). Supercritical fluid chromatography: from science fiction to scientific fact. ChemTexts. 5(3): 13.

[39] P. Dugo, L. Mondello, G. Dugo, D.M. Heaton, K.D. Bartle, A.A. Clifford, P. Myers. (1996). Rapid analysis of polymethoxylated flavones from citrus oils by supercritical fluid chromatography. Journal of agricultural and food chemistry. 44(12): 3900-3905.

[40] P. Ramírez, M.R. García-Risco, S. Santoyo, F.J. Señoráns, E. Ibáñez, G. Reglero. (2006). Isolation of functional ingredients from rosemary by preparative-supercritical fluid chromatography (Prep-SFC). Journal of Pharmaceutical and Biomedical Analysis. 41(5): 1606-1613.

[41] C.-K. Wu, Y.-Z. Xu, Z.-Y. Bai, C.-Y. Shi, J.-Z. Wang, C.-H. Yan, L.-C. Gong, S. You, J. Wang. (2023). High-purity tri-alpha-linolenin isolation from silkworm pupae oil using sequential simulated moving bed chromatography. Biomass Conversion and Biorefinery. 1-14.

[42] L. Kim, P.J. Marriott, Preparative gas chromatography. In *Gas Chromatography*, Elsevier: 2021; pp 487-504.

[43] G. Özek, M. Ishmuratova, N. Tabanca, M.M. Radwan, F. Göger, T. Özek, D.E. Wedge, J.J. Becnel, S.J. Cutler, K.H. Can Başer. (2012). One-step multiple component isolation from the oil of C rinitaria tatarica (Less.) S ojak by preparative capillary gas chromatography with characterization by spectroscopic and spectrometric techniques and evaluation of biological activity. Journal of separation science. 35(5-6): 650-660.

[44] C. Rühle, G.T. Eyres, S. Urban, J.-P. Dufour, P.D. Morrison, P.J. Marriott. (2009). Multiple component isolation in preparative multidimensional gas chromatography with characterisation by mass spectrometry and nuclear magnetic resonance spectroscopy. Journal of Chromatography A. 1216(30): 5740-5747.

[45] J.V. Seeley. (2012). Recent advances in flow-controlled multidimensional gas chromatography. Journal of Chromatography A. 1255: 24-37.

[46] J.M.D. Dimandja, S.B. Stanfill, J. Grainger, D.G. Patterson Jr. (2000). Application of comprehensive two-dimensional gas chromatography (GC× GC) to the qualitative analysis of essential oils. Journal of High Resolution Chromatography. 23(3): 208-214.

[47] W. Wen, P. Xu, H. Xiang, M. Wen, X. Ye, C. Chu, S. Tong. (2023). Comprehensive two-dimensional countercurrent chromatography× gas chromatography characterization of Artemisia argyi essential oil. Analytica Chimica Acta. 1237: 340614.

[48] Y. Wei, J. Du, Y. Lu. (2012). Preparative separation of bioactive compounds from essential oil of F laveria bidentis (L.) K untze using steam distillation extraction and one step high-speed counter-current chromatography. Journal of separation science. 35(19): 2608-2614.

[49] E. Sieniawska, R. Sawicki, J. Golus, M. Swatko-Ossor, G. Ginalska, K. Skalicka-Wozniak. (2018). Nigella damascena L. essential oil—a valuable source of β-elemene for antimicrobial testing. Molecules. 23(2): 256.

[50] P. Szczeblewski, M. Wróblewski, J. Borzyszkowska-Bukowska, T. Bairamova, J. Górska, T. Laskowski, A. Samulewicz, M. Kosno, Ł. Sobiech, J.T. Polit. (2022). The role of centrifugal partition chromatography in the removal of β-asarone from Acorus calamus essential oil. Scientific Reports. 12(1): 22217.

[51] G.R. Pettit, Y. Tang, Q. Zhang, G.T. Bourne, C.A. Arm, J.E. Leet, J.C. Knight, R.K. Pettit, J.-C. Chapuis, D.L. Doubek. (2013). Isolation and structures of axistatins 1–3 from the republic of palau marine sponge Agelas axifera hentschel. Journal of natural products. 76(3): 420-424.

[52] I.A. Ogunwande, N.O. Olawore, K.A. Adeleke, W.A. Konig. (2003). Chemical composition of the essential oils from the leaves of three Eucalyptus species growing in Nigeria. Journal of Essential Oil Research. 15(5): 297-301.

[53] J. Zhang, M. An, H. Wu, R. Stanton, D. Lemerle. (2010). Chemistry and bioactivity of Eucalyptus essential oils. Allelopathy Journal. 25(2).

[54] J.D. Gbenou, J.F. Ahounou, H.B. Akakpo, A. Laleye, E. Yayi, F. Gbaguidi, L. Baba-Moussa, R. Darboux, P. Dansou, M. Moudachirou. (2013). Phytochemical composition of Cymbopogon citratus and Eucalyptus citriodora essential oils and their anti-inflammatory and analgesic properties on Wistar rats. Molecular biology reports. 40: 1127-1134.

[55] S. Mandal, M. Mandal. (2015). Coriander (Coriandrum sativum L.) essential oil: Chemistry and biological activity. Asian Pacific Journal of Tropical Biomedicine. 5(6): 421-428.

[56] E.-J. Song, M.-J. Ko. (2022). Extraction of monoterpenes from coriander (Coriandrum sativum L.) seeds using subcritical water extraction (SWE) technique. The Journal of Supercritical Fluids. 188: 105668.

[57] K. Pramote, S. Nucha, S. Suched, P. Parinda, S. Prasong, A. Shuji. (2012). Subcritical water extraction of flavoring and phenolic compounds from cinnamon bark (Cinnamomum zeylanicum). Journal of oleo science. 61(6): 349-355.

[58] R. Budiarto, R. Poerwanto, E. Santosa, D. Efendi, A. Agusta. (2021). Preliminary study on antioxidant and antibacterial activity of kaffir lime (Citrus hystrix DC) leaf essential oil. Applied Research in Science and Technology. 1(2): 58-65.

[59] F. Yang, H. Zhang, G. Tian, W. Ren, J. Li, H. Xiao, J. Zheng. (2021). Effects of molecular distillation on the chemical components, cleaning, and antibacterial abilities of four different citrus oils. Frontiers in Nutrition. 8: 731724.

[60] S.-A. Yang, S.-K. Jeon, E.-J. Lee, C.-H. Shim, I.-S. Lee. (2010). Comparative study of the chemical composition and antioxidant activity of six essential oils and their components. Natural Product Research. 24(2): 140-151.

[61] B. Boulanouar, G. Abdelaziz, S. Aazza, C. Gago, M.G. Miguel. (2013). Antioxidant activities of eight Algerian plant extracts and two essential oils. Industrial Crops and Products. 46: 85-96.

[62] W. Wang, Y. Zhang, Z. Yang, Q. He. (2021). Effects of incorporation with clove (Eugenia caryophyllata) essential oil (CEO) on overall performance of chitosan as active coating. International Journal of Biological Macromolecules. 166: 578-586.

[63] M. Olszowy, A.L. Dawidowicz. (2016). Essential oils as antioxidants: Their evaluation by DPPH, ABTS, FRAP, CUPRAC, and β-carotene bleaching methods. Monatshefte für Chemie-Chemical Monthly. 147: 2083-2091.

[64] M. Mohsenzadeh. (2007). Evaluation of antibacterial activity of selected Iranian essential oils against Staphylococcus aureus and Escherichia coli in nutrient broth medium. Pak J Biol Sci. 10(20): 3693-7.

[65] M. Soković, J. Glamočlija, P.D. Marin, D. Brkić, L.J. Van Griensven. (2010). Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an in vitro model. Molecules. 15(11): 7532-7546.

[66] A.L. Dawidowicz, M. Olszowy. (2014). Does antioxidant properties of the main component of essential oil reflect its antioxidant properties? The comparison of antioxidant properties of essential oils and their main components. Natural Product Research. 28(22): 1952-1963.

[67] L. Valgimigli. (2012). Essential oils: an overview on origins, chemistry, properties and uses. Essential Oils as Natural Food Additives: Composition, Applications, Antioxidant and Antimicrobial Properties.: 1-24.

[68] H.A. Shaaban, A.H. El-Ghorab, T. Shibamoto. (2012). Bioactivity of essential oils and their volatile aroma components. Journal of Essential Oil Research. 24(2): 203-212.

[69] M.G. Miguel. (2010). Antioxidant activity of medicinal and aromatic plants. A review. Flavour and Fragrance Journal. 25(5): 291-312.

[70] B. Bozin, N. Mimica-Dukic, I. Samojlik, E. Jovin. (2007). Antimicrobial and antioxidant properties of rosemary and sage (Rosmarinus officinalis L. and Salvia officinalis L., Lamiaceae) essential oils. Journal of agricultural and food chemistry. 55(19): 7879-7885.

[71] C.F. Carson, K.A. Hammer. (2011). Chemistry and bioactivity of essential oils. Lipids and essential oils as antimicrobial agents. 203-238.

[72] H.L. Bradlow, D.W. Sepkovic. (2002). Diet and breast cancer. Annals of the New York Academy of Sciences. 963(1): 247-267.

[73] Y. Cai, Q. Luo, M. Sun, H. Corke. (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life sciences. 74(17): 2157-2184.

[74] P.L. Crowell. (1999). Prevention and therapy of cancer by dietary monoterpenes. The Journal of nutrition. 129(3): 775S-778S.

[75] A.R. Koroch, H. Rodolfo Juliani, J.A. Zygadlo. (2007). Bioactivity of essential oils and their components. Flavours and fragrances: chemistry, bioprocessing and sustainability. 87-115.

[76] R. Amorati, L. Valgimigli. (2018). Methods to measure the antioxidant activity of phytochemicals and plant extracts. Journal of agricultural and food chemistry. 66(13): 3324-3329.

[77] M. Antolovich, P.D. Prenzler, E. Patsalides, S. McDonald, K. Robards. (2002). Methods for testing antioxidant activity. Analyst. 127(1): 183-198.

[78] H.H. Rassem, A.H. Nour, R.M. Yunus. (2018). Biological activities of essential oils–A review. Pacific International Journal. 1(2): 1-14.

[79] A. Allahverdiyev, N. Duran, M. Ozguven, S. Koltas. (2004). Antiviral activity of the volatile oils of Melissa officinalis L. against Herpes simplex virus type-2. Phytomedicine. 11(7-8): 657-661.

[80] B.-S. Koo, S.-I. Lee, J.-H. Ha, D.-U. Lee. (2004). Inhibitory effects of the essential oil from SuHeXiang Wan on the central nervous system after inhalation. Biological and Pharmaceutical Bulletin. 27(4): 515-519.