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# Bioactivities of essential oils: a review of biosynthesis, analysis, and chemistry

*Hamad Raza*[1], Awais Altaf[2]*

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

[2]*Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan.*

**Abstract**

The present article offers a thorough overview of essential oils, including their biosynthesis, analysis, and chemistry. It also rapidly elevates the reader to a proficient level in identifying essential oils and their constituents and imparts knowledge on the biological functions of oils. Vital essential oils are blends of volatile organic compounds that are extracted through distillation from unprocessed plant materials, condensed into an oil with a potent scent, and collected as the upper (rarely lower) layer of separated liquids in a container. in two stages: water and oil. Components of essential oils are typically generated from two biosynthetic groups: phenylpropanoids (an aromatic ring with a propene tail) and terpenes (monoterpenes, sesquiterpenes, and their derivatives). Essential oils can be extracted using a variety of techniques, including solvent extraction, steam distillation, hydrodistillation, and hydrodiffusion. They can also be evaluated using gas chromatography and GC-MS mass spectrometry. People from a variety of disciplines, including aromatherapy, pharmacy, synthetic and analytical chemistry, and hobbyists, congregate in the scientific niche of essential oils. It was required to develop this introduction to the basics of essential oil chemistry in order to make science more approachable for the interested student or researcher. As concerned to the biological activities of the essential oils, these play an important role as Anticancerous, antiviral, anti-Inflammatory, Antinociceptive, penetration enhancement, antioxidant activity, antimicrobial activity, hormonal and endocrine activity, Antiparasitic, allergen, antiulcer, Pesticidal activity and aromatherapy.

**Keywords:** biological activities, essential oil

**Full-length article** **Hamad Raza*, e-mail: hraza4657@gmail.com

## 1. Introduction

Aromatic plants release complex, natural, volatile chemicals known as essential oils, which have a unique smell. These substances are created as secondary metabolites. Usually, steam or hydrodistillation—which the Arabs devised in the middleages—is used to manufacture them. They are applied as antibacterial, analgesic, sedative, anti-inflammatory, spasmolytic, and local anesthetic therapies in addition to embalming and food preservation. In addition to their medicinal benefits and scent, they are also known for their antiseptic or bactericidal, virucidal, and fungicidal characteristics [1]. These characteristics have essentially not changed throughout time, with the exception that some of its modes of action—primarily at the antimicrobial level—are now better recognized. Plants may synthesize them in all of their parts, including their leaves, stems, flowers, fruits, shoots, seeds, roots, and bark. After that, they are kept in glandular trichomes, secretory cells, epidermal cells, cavities, or canals [2].

The primary families that generate essential oils are Anneliceae, Apiaceae, Araceae, Asteraceae, Ericaceae, Burseraceae, Cistaceae, Cupressaceae, Geraniaceae, Gramineae, Lamiaceae, Lauraceae, Malvaceae, Myristicaceae, Myrtaceae, Oleaceae, Pinaceae, Piperaceae, Rosaceae, Rutaceae, and Valerianaceae [3]. Environmental factors and systemic factors contribute to the formation of EOs. EOs give plants volatile chemicals that support photosynthesis in the face of oxidative and heat stress. By preventing germination, chemicals included in essential oils operate as allopathic agents, influencing how plants interact with their surroundings. EOs function in plant-animal interactions by discouraging predators [4-6].

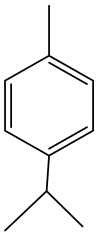
Many fragrant plants, mostly found in temperate to warm climates like the Mediterranean and tropical regions, are the source of essential oils. The ancient pharmacopoeias make extensive use of these oils. They are volatile, fat-soluble, transparent, and infrequently colored. Additionally, they dissolve in organic solvents, the densities of which are typically lower than those of water. They can be synthesized by any part of the plant, including the buds, flowers, stems, leaves, branches, fruits, seeds, roots, wood, and bark. Subsequently, they gather in glandular or epidermal cells, cavities, canals, and secretory cells [2].

## 2. **Composition**

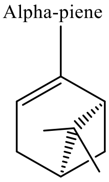
Essential oils are intricate blends of natural ingredients that come in a variety of strengths, ranging from 20 to 60 components. They have two or three main components in quite high quantities (20–70%) as compared to others. In the case of Origanum compactum, Coriandrum sativum, 1,8-cineole (50%) of Cinnamomum camphora, a-phellandrene (36%) and limonene (31%) of leaf and carvone (58%), limonene (37%) from the seed of Anethum graveolens, menthol (59%) and menthone (19%) from the essential oil of Mentha piperita for instance, are the principal components of these plants. These main components usually dictate the biological properties of essential oils [7-8].There are two main categories of biosynthetic origin among the components. Terpenes and terpenoids make up the primary group, while aromatic and aliphatic elements make up the other group. All of these constituents have low molecular weight [9].

**A.Terpenes**
-Monoterpenes

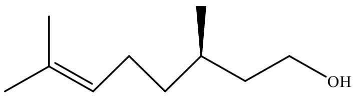
**Carbure monocyclic**



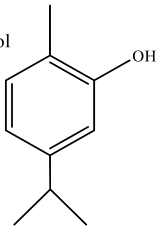
**Carbure bicyclic**
Alpha-piene



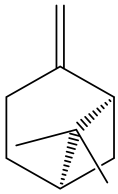
**Alcohol acyclic**
Citronellol



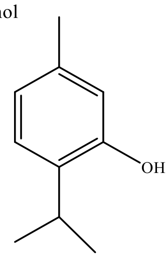

**Phenol**
Carvacol



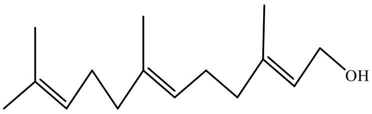

Beta-piene



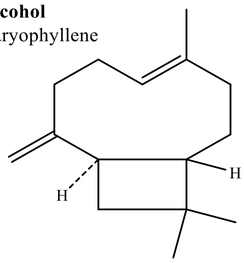
Thymol




Carbure
Farnesol

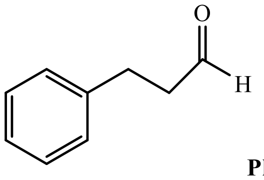



**Alcohol**
Caryophyllene

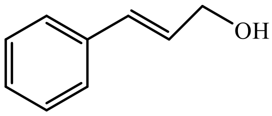



**B**. **Aromatic compound**

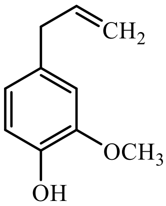
**Aldehyde**
Cinnamaldehyde



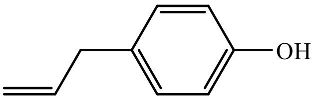

**Alcohol**
Cinnamyl alcohol



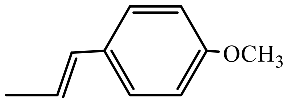

**Phenol**
Eugenol



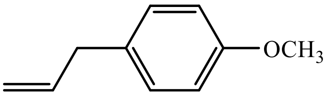

**Phenol**
Chavicol



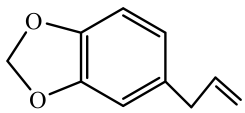

**Methoxy derivatives**
Anethole




**Methoxy derivatives**
Estragole

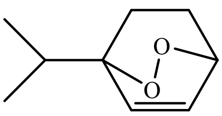



**Methylene dioxy compound**
Safrole

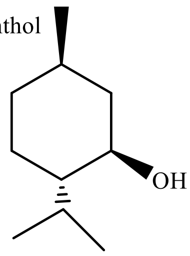



**C. Trepenoides (Isoprenoides)**

Ascaridole




Menthol




## 3. Extraction method for essential oil

Distinct extraction techniques may be used to extract essential oils from a variety of plants with distinct components. The type of plant material utilized dictates how essential oils are extracted and produced. Additional aspects considered are the content's format and condition.

### 3.1. Steam distillation

The most popular technique for obtaining essential oils is steam distillation, which is simply heating raw plant materials and passing them through hot steam to extract the volatile components of the plant material, which evaporates with the heat of the steam and causes the vaporized substances to rise and enter a condensing unit, which is a cooling chamber, where the vapor condenses back to a liquid state as they cool. Ninety-three percent of essential oils are extracted through steam distillation, and the remaining seven percent are extracted through other methods [10]. The water-soluble parts of the plant that include water and essential oils are called hydrosols, or floral water. After condensation, these are combined in a receiver with two distinct outputs. Since oil and water don't mix, essential oil floats on water. Consequently, the upper flow will extract essential oil while the lower flow will extract water [11].

### 3.2. Hydrodistillation

When it comes to extracting essential oils from plant materials like wood or flowers, HD has become the industry standard. These products are frequently used to separate high-boiling-point, non-water-soluble natural substances. As part of the process, the plant materials are submerged entirely in water and then boiled. This process preserves the extracted oils to some degree since the surrounding water serves as an impediment to keep them from burning [12]. Condensing the heated vapor and essential oil vapor together yields an aqueous fraction. This method has the benefit of allowing for the distillation of the necessary substance at a temperature below than $100^0$C.

### 3.3. Hydrodiffusion

The only way that Hydrodiffusion extraction differs from steam distillation is in how steam is introduced into the still container. This process is applied when the plant material has dried and is not damaged by boiling temperatures. Steam is administered from the very top of the plant component during hydrodiffusion, while steam is introduced from the bottom during the steam distillation process [13]. The procedure lowers the temperature of the steam to less than 100 °C and can also be run under vacuum or low pressure. Because it requires less steam and produces more oil with a shorter processing time, the hydrodiffusion technique is better than steam distillation

### 3.4. Solvent extraction

In order to separate the oils, this contemporary technique uses food-grade solvents such ethanol, benzene, dimethyl, or hexane. It is frequently employed to extract essential oils from delicate fragrant plants that are typically unable to tolerate the pressure of steam distillation, such rose or jasmine. Furthermore, compared to most other extraction techniques, solvent extraction typically yields a finer scent, which adds to its allure in some applications. Plant materials are dipped in a solution and allowed to dissolve in order to extract essential oils. Only the vegetable oil is left after the solvent has absorbed the essential oil and the resultant extract has evaporated. The residual oil is referred to as absolute, non-essential oil in technical terms. A highly concentrated aromatic material that closely mimics a plant's inherent scent is called an absolute. Its color is also more vibrant than that of an essential oil [14]. As a result, extracts for cosmetic or scent purposes are frequently made using solvent extraction. This kind of extraction method's primary drawback is the possibility that some solvent residue may remain after it evaporates. As a result, the finished absolute product can include trace levels of harsh chemicals that, when applied, could irritate skin [15].

### 3.5. $CO_2$ extraction

Pressurizing the CO2 until it liquefies is the first step in the extraction process. Utilizing liquid CO2 as a solvent allows for the extraction of oils from plants. The extract returns to normal pressure once the CO2 has absorbed the essential oil, which causes the CO2 to transform back into a gas. Consequently, the extracted oil contains no more solvent. Moreover, CO2 has no taste, smell, colour, or toxicity and has no effect on the final oil [16]. However, all of the oil from the plant material—including pesticide residues—is recovered because CO2 extraction is carried out in a fully sealed chamber. As a result, compared to other standard extraction techniques, the final oil may include more pesticides.

### 3.6 Supercritical fluid extraction

For the purpose of extracting and separating essential oils (EOs) from aromatic plants, supercritical fluid extraction (SFE) has gained widespread usage. This method avoids the use of hazardous organic solvents, offers quick and efficient extraction, and only needs moderate temperatures and cleaning processes. Since carbon dioxide (CO2) is non-explosive, non-toxic, cheaply obtainable, and easily separated from the extracted products, it is the perfect solvent for extracting and isolating essential oil (EO) from plants[17] . Experimental data on the supercritical CO2 (SC-CO2) extraction of essential oil (EO) from Algerian rosemary leaves was presented by Zermane, Meniai, and Barth. Per gramme of dried rosemary, they were able to extract 0.95 to 3.52 g of essential oil. Moreover, analyses using gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) showed that the primary component in the EO was camphor (at 48.89 weight percent). [18].

## 4. Analysis of the essential oils

Historically, the primary technical goal of essential oil analysis has been to maximize separation performance through the application of cutting-edge technologies. The analysis's outcome can be applied to address the research or industrial analysis questions that prompted it. When comparing one oil to another for quality control or adulteration research, or when identifying novel constituents or characterizing the chemical classes of compounds present, this can be done for comparison purposes [19]. It is evident that today's analyst uses chromatography to achieve separation and can supplement it with mass spectrometry to help with identification. Because of the power of GC-MS, developments in mass spectrometry detection and separation technology, as well as better data management tools, will have an immediate impact on the essential oil industry. The current study outlines the evolution of instrumental methods for gas chromatography analysis of essential oils [20]. Insofar as mass spectrometry is the separation that most analysts select for essential oil analysis, it will be included. Thus, sample introduction or handling approaches before the analysis stage, if these techniques provide some level of separation, will be explored, along with multidimensional and single column analysis. We'll talk about the recent evidence of comprehensive gas chromatography as the most effective possible technique for separating essential oils.

Essential oils have been utilised in folk medicine, the food industry, and cosmetics from the beginning of human history in many different parts of the world. The most popular analytical technique for qualitative analysis is GC-MS, but the conventional approach for quantifying essential oils is GC-FID. Many chiral components of essential oils could be identified thanks to chiral GC. GC analysis can be substituted with HPLC chromatography. Important details regarding the composition of essential oil constituents can be obtained by the application of hyphenated techniques, such as LC-MS-MS. Nonetheless, the literature has a very small number of papers on the HPLC analysis of essential oils. Multidimensional chromatography is a method that can yield more precise results [21]. An additional instrument for the examination of essential oils is 13C-NMR spectroscopy. This technique has the advantage of being able to detect stereoisomers and thermally unstable chemicals over mass spectrometry, but it has the drawback of not being able to distinguish smaller components of oils. Promising findings were obtained using 1H-NMR as an online tool for GC analysis; however, more research is needed to adapt this technique to essential oil analysis. The range of data obtained from essential oil analysis enables the application of chemometric techniques [22].

### 4.1 Gass Chromatography

The analysis of volatile organic substances is done with gas chromatography (GC) connected to several types of detection devices. The first stage in the analysis of essential oils is gas chromatography, which separates the mixture into its constituent parts. A "column" is used in this method to accomplish this division. At the beginning of the column, a little amount of essential oil is introduced. A gas then forces the mixture through to the other side, where each separated component comes into contact with a detector, which is typically a flame ionization detector or a mass detector (MS). The column is gradually heated as the components pass through it. It starts off at a low temperature, usually between 40 and 60 °C, and is subsequently elevated at a rate of 3 to 5 °C per minute until it reaches its maximum temperature, which is between 280 and 300 °C. The essential oil components move through the column as vapors in the direction of gas flow when an inert gas, such as nitrogen or helium, is forced through it. The term "gas chromatography" may have contributed to the widespread misconception that the constituents of essential oils flow through the column in a gaseous state. However, the name "gas chromatography" actually refers to the process of using helium or nitrogen gas, not the physical state of the constituents of essential oils as they pass through the column. The components of essential oils typically elute before they reach their boiling point because they reside in the column as liquids or vapors. For instance, spatulanol has a boiling point of 297 °C at atmospheric pressure. Though it typically elutes between 150 and 200 °C, thermodynamic theory states that spatulanol has a substantially higher boiling point in a pressurized column. Furthermore, limonene has a boiling point of 176°C, however it usually elutes about 105–115°C. Greater quantities of components, like volatile diterpenes and coumarins, have boiling values that are greater than those of the apparatus. For example, incensol acetate has a boiling point of 420°C, although it is still eluted in gas chromatography like other components, but it needs a longer retention period [23]. The physical state of essential oil components (and volatile derivatives) as they pass through the column is influenced by various factors such as temperature, the concentration and vapor pressure of individual molecules, the intermolecular interactions with the stationary phase (the column), and the inlet gas pressure (helium or nitrogen). Essential oil components are in a vapor phase when they are moving through the column; but, when they are stationary, they are absorbed as a liquid into the column matrix [24]. When the column's temperature rises to a point where the intermolecular interactions with the stationary phase of the column are disrupted, the majority of the constituents of essential oils evaporate. All of the liquid turns into vapor when that temperature is attained since there are very few molecules in the column. The vapor then exits the column and comes into contact with the detector.

### 4.2 Mass Spectrometric Identification by Comparing to a Mass Spectral Library

There are two primary diagnostic techniques used to identify the constituents of essential oils. The mass spectrometer's "fingerprint" or fragment pattern is the first (Figure 4). Gas chromatography-mass spectrometry (GC-MS) is the term used to describe the combination of a mass spectrometer and a gas chromatograph [24]. Electron impact ionization is a technique used by the mass spectrometer in gas chromatography to ionize molecules using an electron beam. The compound is therefore subjected to a precisely calibrated electron charge, usually 70 millivolts, after which the masses of the fragments are identified. The entire molecule at 210 Da, represented by the molecular ion peak (M+) in Figure 4, is also included in the mass spectral pattern. Next, in order to find a match, this fragmentation pattern is compared to a library of spectra. Because it is formed from an uncommon compound, the enormous fragmentation pattern in Figure 4 could not be found in the NIST commercial library. It was separated and recognised as 1-acetoximiodesert-3-ene, an uncommon monoterpene iridoid, using nuclear magnetic resonance spectroscopy.

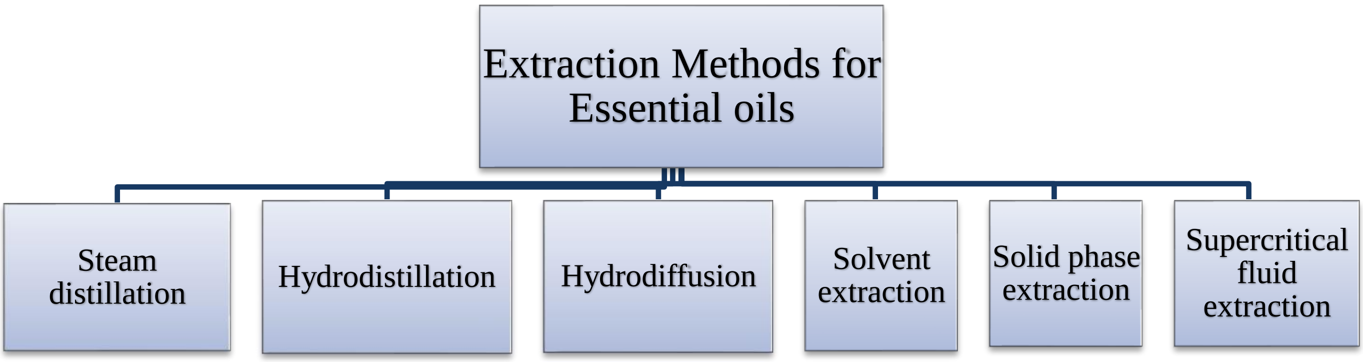



**Figure 1.** Commonly used methods for essential oil extraction.

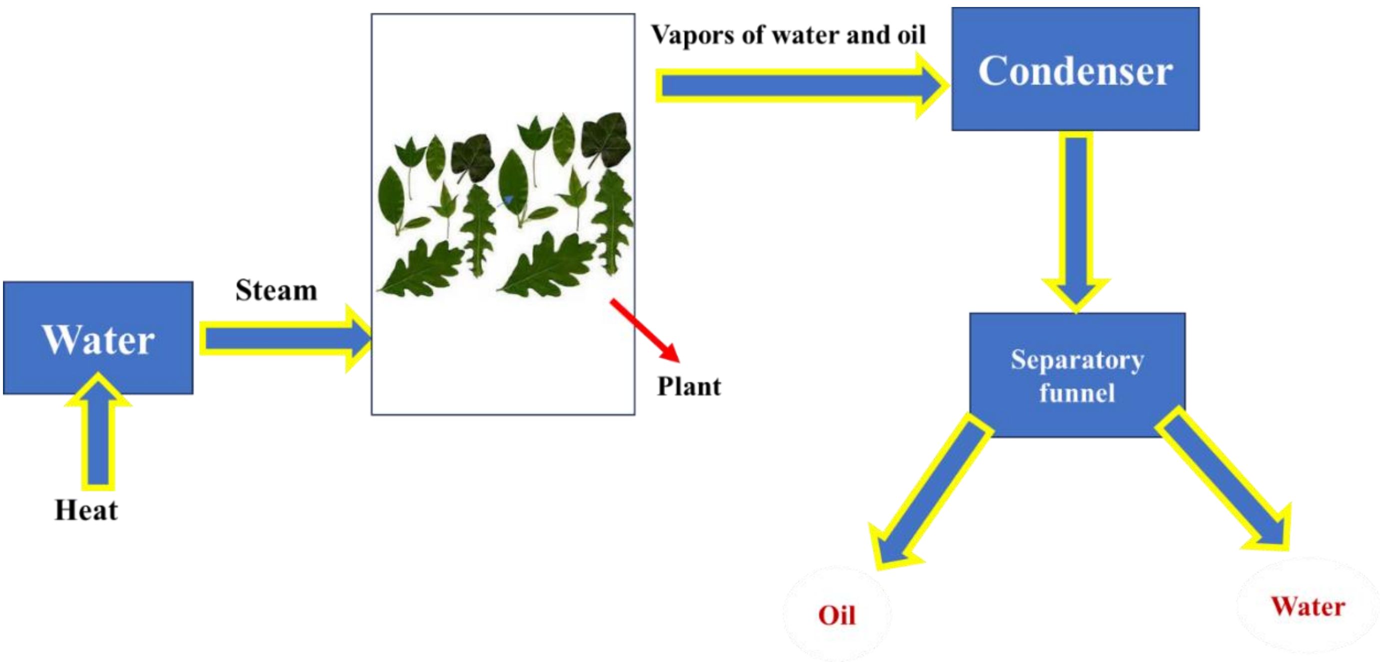



**Figure 2.** Extraction mechanism of essential oil by steam distillation.

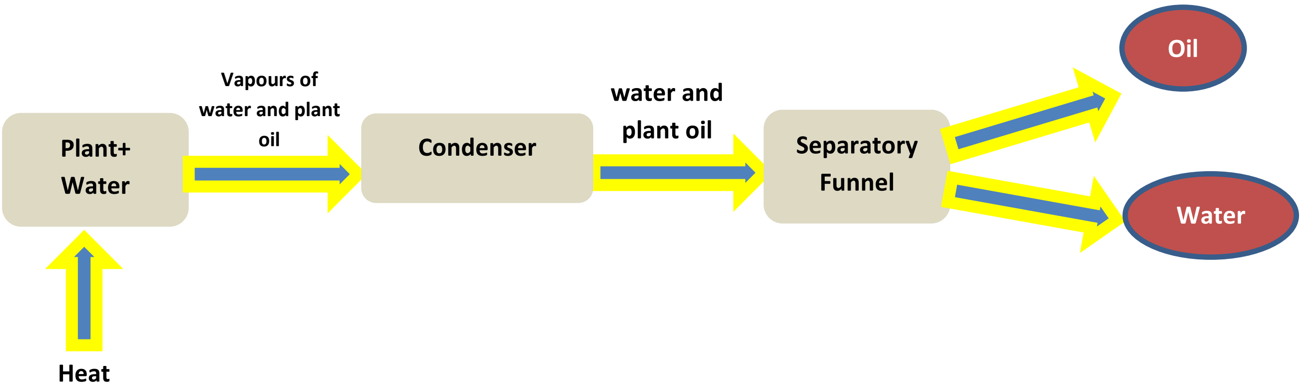



**Figure 3.** Extraction mechanism of essential oil by steam/hydrodistillation.

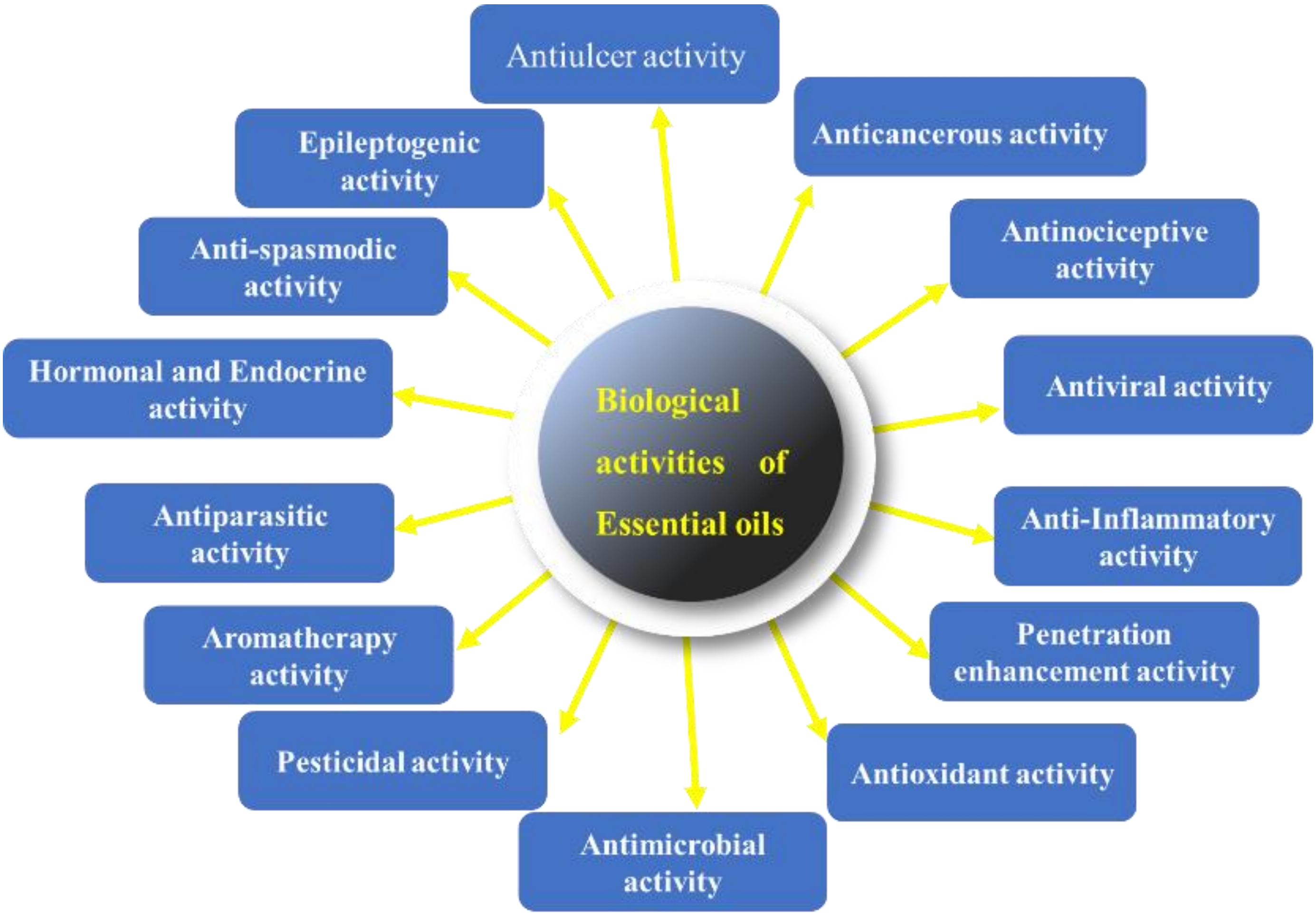



**Figure 4.** Major bioactivities of essential oils.

**Table 1.** List of essential oils and their biological activities

| Essential oil | Plant part used | Biological activities |
|---|---|---|
| Anise oil | Ripe fruit | Antioxidant [25]<br>Insecticidal activity [26]<br>Antimicrobial activity [27] |
| Bitter-fennel fruit oil | Ripe fruit | Antithrombotic [28]<br>Insecticidal [29]<br>Antioxidant [30] |
| Bitter-fennel herb oil | Aerial parts | Antifungal activity [31]<br>Antimycobacterial and anticandidal activity [32] |
| Caraway oil | Dry fruit | Antimicrobial activity [33] |
| Cardamom oil | Seeds | Anticarcinogenic [34]<br>Antioxidant [35]<br>Antimicrobial |
| Cassia oil | Leaves and young branches | Antifungal and antibacterial activity [36] |
| Cinnamon leaf oil, Ceylon | Leaves | Antidiabetic [37]<br>Antioxidant<br>Antimicrobial |
| Citronella oil | Fresh aerial part | Antimicrobial anthelmintic, Antioxidant, [38] anticonvulsant |
| Clary sage oil | Fresh flowering stem | Analgesic, anti-stress, anti-depression [39], cytotoxic, antioxidant |
| Clove oil | Dried flower buds | Antioxidant, Antifungal activity [40], Insecticidal activity, antiseptic [41] |
| Coriander oil | Fruits | Antidiabetic, anticancer, antimutagenic, free radical [42] |
| Dwarf pine oil | Fresh leaves and twigs | Antioxidant activity,<br>Antidyslipidemic [43] |
| Eucalyptus oil | Fresh leaves and fresh terminal branches | antibacterial activity [44], anticancer, anti-inflammatory, antiseptic |
| Juniper oil | Ripe, non-fermented berry cones | Memory enhancing effect [45], antimicrobial, antioxidant [46] |
| Lavender oil | Flowering tops | Anti-depressive, carminatives [47], antifungal, antibacterial |
| Lemon oil | Fresh peel | Cell Cytotoxicity [48], anti-inflammatory, antioxidant, antibacterial |
| Mandarin oil | Fresh peel | Anti- proliferative [49]<br>schistosomicidal effects, antioxidant [50], antibacterial |
| Matricaria oil | Fresh flower-head, flower tops | Anti-parasitic, anti-cancer, anti-inflammatory [51], antidiabetic, |
| Mint oil | Fresh, flowering aerial part | Antifatigue, neuroprotective, antiviral, scolicidal, immunomodulatory [52], antitumor |
| Neroli oil | Fresh flower | Antibacterial activity, antifungal activity [53] |
| Nutmeg oil | Dried and crushed kernels | antifungal activity, antimicrobial, antimalarial activity, fumigant [54] |
| Peppermint oil | Fresh aerial parts of the flowering plant | antiviral, scolicidal, immunomodulatory, neuroprotective, Antifatigue [52] |
| Pine sylvestris oil | Fresh leaves and branches | Antiseptic, antioxidant, antifungal, anti-inflammatory [55] |
| Rose oil | Fresh flowers | Genotoxic activity, memory enhancing, Antibacterial activity, antifungal activity, teratogenic, antifertility [56] |
| Rosemary oil | Flowering aerial parts | Antibacterial activity, antifungal activity, antioxidant, hepatoprotective [57] |
| Tea tree oil | Foliage and terminal branches | Antibacterial activity, Antibacterial activity, antifungal activity [58] |

**Table 2.** Biological activities of key components of essential oils

| Compound | Biological activity | References |
|---|---|---|
| Curdione | Anticancer | [59] |
| | Antibacterial | [60] |
| | Antifungal | [60] |
| | Anti-inflammatory | [61] |
| 1,8-Cineole | Antioxidant | [62] [63] |
| | Anticarcinogenic | [64] |
| β-Caryophyllene | Antitumor | [65] [66] |
| | Antileishmanial | [67] |
| | Antitrypanosomal | [68] |
| Myrcene | Antimutagenic | [69] |
| | Chemopreventive | [70] [71] |
| | Antiproliferative | [72] |
| | Antioxidant | [73] |
| ar-Turmerone | Antimutagenic | [74] |
| | Hypoglycemic | [75] |
| | Neuroprotective | [76] |
| | Insect repellent | [77] |
| | Antivenom | [78] |
| | antibacterial | [79] |
| Germacrone | Skin-penetration enhancer | [80] |
| | Antitumor | |
| | Antibacterial | [81] |
| | Antioxidant | [82] |
| | | [83] |
| Xanthorrhizol | Estrogenic | [82] |
| | Nephroprotective | [84] |
| | Neuroprotective | [85] |
| | Chemoprotective | [86] |
| | Hepatoprotective | [85] |
| | Antibacterial | [85] |
| B-Elemene | Antiangionenic | [87] |
| | Hepatoprotective | [88] |
| | Antitumor | [89] |
| Terpinolene | Antioxidant | [90] |
| | Chemoprotective | [73] |
| | Anti-inflammatory | [91] |
| Curzerene | Antioxidant, Anticancer | [92] [93] |

## 5. Authentication of Essential Oils

Essential oil adulteration and counterfeiting is dishonest, unethical, and potentially harmful to consumers. [94]. On the other hand, customers' desire for and motivation to engage in counterfeiting will decline if they are aware of the warning indications of dishonest marketing. [95].

### 5.1 Simplistic Methods for Authentication of Essential Oils

A thorough analysis that was released in 2015 included a crucial synopsis of the adulteration strategies frequently used in the essential oil sector. It is implied that there isn't a set of guidelines that apply to every kind of adulteration. Every natural substance that has the potential to be tampered with contains indicators that are distinctive to each instance and context-specific analysis techniques. For instance, adulterating an essential oil with synthetic linalool will infect it with dihydro and dehydrolinalool, byproducts of the synthetic process [96]. It is possible to authenticate essential oils using a number of simple techniques, without resorting to "sophistication" as a cover for dishonest merchants or traders trying to undercut their prices. In these kinds of situations, figuring out whether the goods is a fake requires simply basic techniques. Basic laboratory equipment, however rudimentary, is generally required (apart from evaporation tests) [95]. Thin layer chromatography, ultraviolet absorbance (UV spectrophotometry), and infrared determination can all yield a wealth of information. These components are regarded as unsophisticated since they represent the most fundamental laboratory procedures.

#### 5.1.1 **UV Absorbance Determination Using Spectrophotometry**

Another example is that the UV active ingredients, the coumarins, are diluted when citrus pressed oils are diluted with cottonseed oil. UV spectrophotometry can be used to determine the concentration difference. Similarly, a UV spectrophotometer can be used to determine the concentration difference when any essential oil containing phenylpropanoids is diluted with a carrier oil. There is a UV maximum (peak absorption wavelength, λmax) that can be used for any component that has delocalized electrons. This method allows for the examination of benzene-containing components found in essential oils, such as p-cymene, thymol, carvacrol, cumin aldehyde, and α-turmerone, as well as terpenoid components. Since minor variations may be caused by variations in the relative amount of the constituents occurring naturally, the UV absorption value must be many orders of magnitude different from the corresponding authentic natural essential oil [97]. Anethole, eugenol, safrole, elemicin, and cinnamonaldehyde are examples of common phenylpropanoids. They all exhibit UV chromophores that absorb at wavelengths (eugenol = 280.9 nm), which are comparable to those of most aromatic terpenes (thymol = 275 nm). Alternatively, the spectrophotometer can be used to find out if the oil has fixed components like flavonoids or coumarins, which will show that the product is not an essential oil but rather an absolute or pressing oil since essential oils are not expected to have a UV chromophore.

#### 5.1.2 Evaporation Ability

Testing a product's ability to evaporate is another method of figuring out if it is or is not an essential oil. For instance, an essential oil, pressed oil, or absolute mixed with a carrier oil won't evaporate entirely. This is because if the product is not an essential oil, it will contain non-volatile components. Therefore, the authenticity may be determined by applying a few drops of the product on a piece of paper and heating it with a hair dryer for five minutes [27]. A portion of the oil that is still on the paper indicates that it is either contaminated or not an essential oil because it is not volatile. Nonetheless, even in the absence of hydro distillation, an oil that is pressed from citrus peels—like bergamot—is classified as essential according to the International Standards Organization. Bergamot is therefore still genuine even if it turns out to be pressed oil rather than hydro distilled.

#### 5.1.2 Thin Layer Chromatography

As an alternative to spectrophotometry, thin layer chromatography can be used (TLC) can be used to authenticate essential oils. Crude extracts often contain components that are more polar than essential oil components, so TLC can be used to reveal these. Any component that stays at the baseline of a TLC plate using 10% ethyl acetate in 90% hexane, is too polar to be an essential oil component, and must, therefore, be from extraction, not hydro distillation. If the TLC mobile phase is changed to 20% ethyl acetate, 80% hexane, then components below an Rf value of 0.4 are impossible in an essential oil [98]. Common authentication methods, however, can also identify components that have a vapor pressure too low to be volatile but are nonetheless volatile or somewhat polar. In these situations, the authenticator must be aware of what to look for in order to spot instances of tampering. Understanding the adulteration strategies employed by counterfeiters is necessary for this. For example, if the authenticator is aware that oil produced from grapefruit peel is often used to tamper with lavender essential oil, TLC can identify the presence of coumarins. [99]. A UV indicator on the TLC plate can be used to assess the presence of coumarin auraptene (F254). As an alternative, the standard silica gel plate can be fumigated with ammonia gas, which will make the component glow when exposed to ultraviolet light. The result is more believable if the authenticator possesses a sample of aurapten, which may be utilized as an authentic standard for comparison utilizing TLC migration (Rf value) [99].

## 6. Biological activities of essential oil

### 6.1 Anticancer activity of EOs

Numerous tumor cell lines reveal the anticancer activities of essential oils (EOs). EOs boost anti-oxidant, anti-mutagenic, and antiproliferative properties in cancer cells. EOs in aromatherapy boost immunity and reduce cancer-related discomfort [100]. Essential oils are cytotoxic by nature. Through the induction of necrosis, apoptosis, cell cycle arrest, and major organelle dysfunction, essential oils (EOs) induce programmed cell death in cancer cells. Target-oriented medication administration can be guided via EO nanoemulsions or nanoencapsulation. Anticancer medication resistance may be addressed by using EOs in more recent drug formulations [101].

### 6.2 Antinociceptive activity of EOs

For ages, folk medicine has employed plants and essential oils (EOs) to treat a wide range of illnesses, from pain management to analgesia. In this regard, the Antinociceptive action of EOs has garnered interest since pain management remains a significant medical concern [102]. There is substantial data supporting the pharmacological Antinociceptive efficacy of bergamot essential oil (BEO) in treating both neuropathic and nociceptive pain [103].

### 6.3 Antiviral activity of EOs

It is well known that the chemical components of essential oils (EOs) and their efficacy against various viruses is broad. The antiviral properties of EOs are attributed to the presence of oxygenated monoterpenes and sesquiterpenes. It is currently impossible to determine which EOs will provide the highest level of protection because to the lack of comprehensive understanding around the new coronavirus strain, now known as SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus). It is reasonable to anticipate, though, that some of the EOs and related terpenes will provide a detectable amount of protection, just as they do for a large number of other known viruses [104].

### 6.4 Anti-Inflammatory activity of EOs

Numerous illnesses, including infections caused by bacteria, viruses, and protozoa, autoimmune conditions including diabetes and arthritis, Alzheimer's disease, and cancer, are linked to inflammation. Non-steroidal anti-inflammatory medicines (NSAIDs) and corticosteroids are two of the numerous treatments that are available to stop or slow the course of inflammation, although they have certain side effects. Conventional medicine has served the public's health needs for a long time and today offers a wide range of medical care options. This medication uses essential oils to treat a wide range of illnesses [105]. According to research, essential oils including thyme, chamomile, and eucalyptus can reduce inflammation by modulating the NFκB signaling system, which is the primary regulator of the generation of proinflammatory cytokines. Many essential oils, such as eucalyptus, lavender, and tea tree oils, have immunomodulatory properties that might change inflammatory processes. As a result, they may be used as an alternative therapy for a variety of immunological or infectious illness [106].

### 6.5 Penetration enhancement activity of EOs

Transdermal medication delivery is a delivery method in which active molecules are transferred through the skin. Its advantages include safety, steady plasma drug concentration, and a decrease in the hepatic first-pass effect. Since the skin serves as a barrier to both dangerous chemicals and pharmaceuticals, permeability enhancers/enhancers are used to increase the permeability of medications through the skin. This brief overview covers recent research on essential oils. Through their interactions with the stratum corneum, essential oils improve skin penetration (SC). It was discovered that they were effective in boosting the skin penetration of both hydrophilic and lipophilic medications. Furthermore, because essential oils are volatile and readily expelled from the body through urine and feces, they do not collect in the body [107]. Essential oils are natural, less poisonous, less allergic, and generally do not harm skin while boosting skin penetration.

### 6.6 Antioxidant activity of EOs

Substances known as antioxidants have the capacity to shield living things from oxidative stress. Liquid mixes of volatile substances derived from aromatic plants are known as essential oils (EOs). Numerous essential oils (EOs) possess antioxidant qualities, and the application of EOs as natural antioxidants is gaining attention due to the recent suspicions regarding the possible health risks of some synthetic antioxidants, such BHA and BHT. Therefore, adding EOs to edible products—either directly by mixing or through active packaging and edible coatings may be a viable way to stop autoxidation and extend shelf life [108]. However, assessing the antioxidant efficacy of essential oils (EOs) is a critical matter, as most widely employed “tests” are unsuitable and yield inconsistent outcomes that might potentially skew future investigations. An examination of the potential in food protection is presented together with the chemistry elucidating the antioxidant activity of EO. A comprehensive evaluation is conducted on literature approaches to evaluate the antioxidant efficacy of EOs [109].

### 6.7 Antimicrobial Activity of EOs

It has been documented that plant essential oils can shield food against pathogenic and spoiling microbes. Carvacrol is one of the chemical constituents of a number of essential oils that has been demonstrated to have a particular antibacterial effect. Carvacrol is the primary constituent of thyme essential oil (45% carvacrol) and oregano essential oil (60% to 74% carvacrol). It works effectively against a broad variety of microbiological agents including the majority of gram-positive and gram-negative bacteria. Carvacrol breaks down the outer membrane of gram-negative bacteria, releasing lipopolysaccharides and increasing the permeability of the cytoplasmic membrane to ATP. In Gram-positive bacteria, it can alter the permeability of cations like $H^+$ and $K^+$ by interacting with their membranes [110]. Gram-positive bacteria are more vulnerable to the antibacterial properties of essential oils than gram-negative bacteria, as numerous studies have demonstrated. Gram-positive bacteria can more readily absorb the hydrophobic components of essential oils because the lipophilic ends of their lipoteichoic acid peptides are lipophilic [111].

### 6.7 Pesticidal activity of EOs

Certain essential oils may be effective as a natural pesticide, according to studies. Case studies have demonstrated the various deterring effects of different oils on pests, especially insects and specific arthropods. The special effects of the oil on pests might be repellent, hinder digestive processes, restrict development, decrease the rate of reproduction, or even cause the bugs to die [112]. But generally, the chemicals in the oils that have these actions are not harmful to animals. Because of the molecules' unique properties, these "green" insecticides may be widely used without endangering anything but bugs. Research has been done on the following essential oils: eucalyptus, rose, peppermint, basil, lemon grass, lavender, and thyme [113].

### 6.8 Aromatherapy activity of EOs

Aromatherapy is a type of complementary medicine wherein the therapeutic properties of essential oils and other plant extracts are attributed to their aromatic constituents. While aromatherapy can help promote relaxation, there isn't enough proof to say that essential oils can successfully treat any kind of illness. According to scientific studies, essential oils are not able to treat or prevent any kind of sickness or chronic condition [114]. There are significant methodological flaws in the use of essential oils for medicinal reasons. It was discovered that the essential oils' efficacy as complementary therapies was within acceptable methodological bounds. There has been at least one incidence of mortality linked to the usage of essential oils, which can also result in allergic responses and skin irritation [115].

### 6.9 Hormonal and Endocrine activity of EOs

Korach claims that ingredients in essential oils have never before been categorized as endocrine disruptors. These substances either replicate or counteract the effects of androgens, or male sex hormones, such testosterone, as well as estrogen, the female sex hormone [116]. Lead researcher at the NIEHS Receptor Biology Group and senior author Kenneth Korach, Ph.D., stated, "These results showed that the two oils can have hormonal-like effects for estrogen and testosterone in the body." "There may be health risks associated with lavender and tea tree oil exposure [117]." Some essential oils have the potential to influence the hormonal and endocrine systems, thereby balancing hormone levels in the body. This could have an impact on reproductive health and overall well-being [118].

### 6.10 Anti-spasmodic activity of EOs

The antispasmodic action of drugs is utilized in a variety of clinical contexts to treat pain and cramping that affects the smooth muscles of the genitourinary, biliary, or gastrointestinal tracts. [119] [120].Since the synthetic antispasmodic medications that are currently on the market have a number of unpleasant side effects, the pharmaceutical industry has made finding new molecules with a natural origin one of its top priorities. An increasing number of patients are using herbal remedies with antispasmodic properties to treat symptoms of functional dyspepsia, intestinal, colonic, or ureteral spasms, gallbladder hyperactivity, and uterine cramps [121]. Within the broad category of medicinal plants, aromatic plants with high essential oil content are regarded as a valuable and readily available natural resource for the synthesis of novel compounds that may prove to be therapeutic candidates. Essential oils are complex mixtures that mostly consist of aromatic terpenes, which are divided into monoterpenes and sesquiterpenes based on how many isoprene units they contain. They also contain phenylpropanoid compounds [122]. Muscle cramps and spasms can be lessened by essential oils' anti-spasmodic properties. Because of this, they can be helpful in the treatment of ailments like irritable bowel syndrome (IBS) [123].

### 6.11 Antiparasitic Activity of EOs

The secondary metabolites of medicinal plants are called essential oils (EOs), and they contain bioactive substances including terpenes, terpenoids, phenylpropenes, and isothiocyanates that work in concert with one another. The hydrophobic chemicals included in EOs have the ability to enter the cells of bacteria and parasites, resulting in cell distortions and organelle failure. In aquatic creatures, dietary supplements of essential oils (EOs) also influence growth, immunity, and resistance to infectious diseases [124]. Because EOs are hydrophobic and permeabilic, they cause potassium ions and the cytoplasmic substance of parasitic cells to leak out, changing the shape of the cells and stopping parasitic activity [125] [126]. Because of lipoteichoic acids in cell membranes that may make it easier for EOs' hydrophobic components to penetrate, Gram-positive bacteria are often more sensitive to EOs than Gram-negative bacteria [127]. EO is made up of several substances that don't target any particular cell in parasites. The EOs' monoterpenes, α-pinene and sabinene, have demonstrated noteworthy antiprotozoal action. Another important aspect of EOs that demonstrated a greater mode of action than individual compounds is the synergistic effects of several chemicals [128].

### 6.12 Epileptogenic activity of EOs

Due to the presence of highly reactive monoterpenes such pilocamate, thujone, camphor, 1,8 cineole, pilgene, and sabinylacetate, tansy has long been known to possess epileptogenic properties, or strong convulsants [129].

### 6.13 Anthelmintic activity of EOs

Anti-anthelmintic action has been observed in β-thujone, essential oils, and ether extracts that were extracted from T. vulgare [129].

### 6.14 Allergen activity of EOs

Gardeners and florists who have been exposed to parts of Compositae plants have reported developing contact dermatitis, commonly referred to as "chrysanthemum allergy" or "compositae dermatitis [130]**."** The main component of Compositae thought to be accountable for contact dermatitis is thought to be sesquiterpene lactones [131]. Reports of cross-sensitivities have also been made with plant species that are phytochemically similar [132]. There are several clinical research on T. parthenium, T. vulgare, and Chrysanthemum cinerariaefolium (T. cinerariaefolium)-related contact dermatitis [133].

### 6.15 Anticoagulant and Antifibrinolytic activity of EOs

The anticoagulant and antifibrinolytic properties of chloroform extracts, aqueous extracts, and essential oils of T.cilicium, T. corymbosum, and T. macrophyllum were investigated [40]. It was discovered that each extract had strong anticoagulant and antifibrinolytic properties. This illustrates how tanacetum extracts have anticoagulant and antifibrinolytic qualities [133].

### 6.16 Antiulcer activity of EOs

Additionally, 8-β-hydroxyaquiline, which was extracted from T. microphyllum's aerial portion, has demonstrated antiulcer properties [64]. T. ferulaceum flowers have been treated with antiulcer treatment. T. vulgare and parthenolide have been demonstrated to be beneficial in rats with stomach ulcers. [134].

### 6.17 Phytotoxic activity of EOs

Every chemical that was separated from T. cinerariaefolium, including α-lactone, has prevented Chinese cabbage seedlings' roots from growing [135].

### 6.18 Cognitive activity of EOs

Because of their many biological actions, essential oils have been utilized as treatments for a variety of ailments since ancient times. Different pharmacological responses in the nervous system have been observed in recent preclinical and clinical research, resulting in anxiolytic, antidepressant, sedative, and anticonvulsant effects. Research conducted on animal models has demonstrated that many neurotransmitter systems are involved in the mechanism of action of essential oils, leading to quantifiable physiological impacts on the brain [136] [137]. There is proof that exposing oneself to a variety of scents, or olfactory enrichment, enhances brain function in both humans and lab animals. Thus, the theory that inhaling various essential oils before bedtime could serve as a kind of brain tonic to prevent memory loss [138]. It is thought that certain essential oils can help with attention, concentration, and memory, among other cognitive benefits.

In aromatherapy, these oils are frequently used to improve mental clarity.

### 6.19 Allelopathic Activity

The creation of secondary metabolites is the source of allelopathic interactions, according to the International Allelopathy Society (IAS), which defined allelopathy as "the science that studies any process involving secondary metabolites produced by plants, algae, bacteria and fungi that influences the growth and development of agricultural systems and biological systems" in 1996. Plants and microbes produce secondary metabolites for a variety of defense mechanisms. Allelochemicals are the name for the secondary metabolites that are involved. [139].

Considered a major source of lead compounds in agriculture, volatile oils and their constituents are being investigated for weed and insect control [71]. Many species' defense mechanisms include bioactive terpenoids, which are a relatively unexplored source of active chemicals with potential applications in both the agricultural and pharmaceutical fields [140]. In actuality, the terpenoid route yields a wide variety of very phytotoxic allelochemicals, and studies have been conducted on the phytotoxicity of essential oils. Angelini examined the allelopathic action of the tea tree's essential oil, Melaleuca alternifolia (Maiden and Betche) Cheel, against the contaminating fungus Trichoderma harzianum, which results in significant losses while cultivating Pleurotus species. Trichoderma harzianum can be controlled allelopathically by this essential oil in vitro [141].

### 6.20 Cytotoxicity

Essential oils lack cellular ligands because of their intricate molecular makeup [74]. Because they are lipophilic mixes, they can pierce the cell membrane, break down the phospholipid, fatty acid, and polysaccharide layers, and cause permeabilization. It seems that this cytotoxicity involves this kind of membrane damage. Membrane permeabilization in bacteria is linked to ATP pool depletion, proton pump collapse, and ion loss and decreased membrane potential [75]. Lipids and proteins can be harmed by essential oils and can clog the cytoplasm. Lysis and the release of macromolecules can result from damage to the cell wall and membrane [142]. Additionally, as in the case of oxidative stress, essential oils change the fluidity of the membrane, making it unusually porous and allowing proteins, cytochrome C, radicals, and Ca2+ ions to leak out. Apoptosis and necrosis are the two ways that this permeabilization of the outer and inner membranes results in cell death. There are multiple compartments where the cell's ultrastructural modification is evident. Electron microscopy can also show that essential oils disrupt the viral membrane of the herpes simplex virus (HSV) [143]. Analysis of Saccharomyces cerevisiae microtubule genes involved in ergosterol production, sterol absorption, lipid metabolism, and the structure and function of cell detoxification further supported the induction of membrane degradation. Alpha therapy affects transport and the cell wall. -terpine [144].

## 7. Conclusions

Essential oils are one category of natural plant products that merits special consideration because of their widespread application in traditional healing practices. Moreover, the procedure of distilling essential oils from plant organs is today quite dependable and reasonably priced. Many preclinical studies have demonstrated the biological actions of essential oils, clarifying their pharmacological objectives and mode of action, and proving their effectiveness. More specifically, antibacterial, antioxidant, anti-inflammatory, and anticancer effects have been demonstrated in a range of cellular and animal models. The benefits of essential oils for dental health have been demonstrated, however the lack of human studies relative to in vivo/in vitro research limits their potential as safe and effective phototherapeutic agents. Therefore, more meticulously designed clinical trials are needed to establish a high quality of scientific proof and validate the true efficacy and safety of plant-based medicines that people have been using since prehistoric times. Essential oils should be valued in a number of fields, primarily human health, green chemistry, and sustainable agriculture, because of their many biological functions. To maximize its potential use, however, much study on its mechanism of action and likely toxicological effects needs to be done.

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