

RESEARCH ARTICLE

EXTRACTION OF ESSENTIAL OIL FROM HOLY BASIL (OCIMUM SANCTUM) BY STEAM DISTILLATION AND ITS CHEMICAL AND BIOLOGICAL PROFILING

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ABSTRACT

The research and analysis of bioactive components from aromatic plants, specifically Holy basil, which has demonstrated significant medicinal benefits, were conducted. To examine the existence of these therapeutic compounds, three distinct methods for solvent extraction were utilized: distillation, soxhlet extraction, and expressiveness (petrissage). A total of 56 chemicals were identified after GC-MS (gas chromatography-mass spectrometry) investigation, the majority of which were terpenoids and hydrocarbons. Significant components within the essential oil included estragole, licorice extract, geraniol, dimethyl citronella, luteolin, and licorice extract. The experimental outcomes suggest that Holy basil demonstrates rapid leaf growth, with each stem producing an additional four to five leaves capable of mobilizing sucrose, potentially contributing to the development of monoterpenes. The presence of oxygenated components in the oil, known for their flavoring and therapeutic properties, underscores its potential as an economically viable product, especially in the production of aromatic compounds, owing to its low proportion of oxidized constituents. These findings emphasize the significance of Holy basil's essential oil as a valuable source of medicinal compounds.

KEYWORDS

Aromatic, Essential oil, Gas Chromatography, Terpene

1. INTRODUCTION

Holy basil, scientifically known as *Ocimum Sanctum*, holds great global significance and is often referred to as the "Queen of plants" or "Mother Medica of Creation." This plant originates from Asia and has been cultivated since 3000 B.C. It is prominently grown in all provinces of Pakistan. Terpenoids and other chemical substances, such as flavonoids, estragole, geraniol, dimethyl citronello, luteolin, and licorice extract, are widely present in holy basil essential oils. Holy basil is a popular culinary plant that is also well known for having powerful antibacterial and antioxidant qualities. (Coker et al. 2008).

The demand for essential oils derived from holy basil, a rich source of phytochemicals, is on the rise. These essential oils are helpful for food preservation since studies have shown that even little amounts of them can effectively prevent microbial development and prevent food from spoiling. (Dutta et al. 2013).

In traditional medicine, holy basil is used to treat a wide range of ailments, including cancer, convulsions, diarrhea, headaches, and asthma, among others. It is also used in cooking to enhance the flavor of dishes such as sauces, roast beef, and various traditional foods (Knobloch et al. 1990). Holy basil essential oil holds global importance due to its diverse benefits. Its components have various medicinal properties, including anti-inflammatory, pain-relieving, antidepressant, anti-mutagenic, hypoglycemic, cardio-protective, and hypocholesterolemic effects.

Holy basil essential oil has a long history of use in Ayurvedic procedures

in South Asia. Eugenol, which gives it its characteristic aroma and makes it valuable in perfumes, aerosols, and other aromas, is present in substantial amounts in it. The components of holy basil essential oil may be useful in treating a variety of skin disorders including colonization ulcers, according to pharmacological research, potentially obviating the need for multiple pharmaceutical therapies.

Additionally, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* have been shown to be resistant to holy basil essential oil's antimicrobial effects. These properties make it a valuable asset for wound care and infection control (Raina et al., 2013).

Due to its numerous applications in the treatment of infectious diseases and the preservation of food from spoilage, essential oils (EOs) have gained significant significance in modern society. These EOs are known for their therapeutic properties, leading to extensive research efforts to explore their antibacterial compositions. Apart from their medical applications, therapists also use the natural ingredients in essential oils to treat various psychological disorders (Wan-Nurdiyana et al. 2014).

Beyond their aroma, essential oils have numerous uses in the culinary, pharmaceutical, and cosmetics sectors. Researchers are continuously working to develop more potent EO-based solutions, and these natural extracts are integrated into various forms of medications, including pills, sauces, balms, lotions, and aerosols. As a result, their production volumes have consistently increased, particularly in the pharmaceutical sector. Additionally, some therapeutic products derived from essential oils are employed as antibiotics, particularly against influenza, and as fungal

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medicines by pharmaceutical companies (Benyoussef et al. 2020).

Steam distillation is used to extract essential oils, also known as volatile oils, from the various components of fragrant plants, including the flowers, leaves, twigs, stems, and seeds (Taherzadeh et al. 2018). These essential oils are in liquid form and are known for their pleasant fragrances and delightful flavors. The origins of modern essential oil extraction can be traced back to the eighth century when an Italian Dominican priest named Thomas Quinas pioneered the distillation technique to isolate active plant constituents. His initial extraction involved capturing the aroma from rose blossoms in the form of rosewater. This innovation marked the beginning of a journey that would uncover the vast potential of essential oils across various industries and applications.

Distillation stands out among the many extraction techniques since it is a crucial step in the process of obtaining rich essential oils from diverse aromatic plant parts. From less than 1% to as much as 15%, the oil outputs can vary. These aromatic extracts are valued for their pleasant scents and are often used to make phytochemicals, oxygenated derivatives, terpenoids, various hydrocarbons, air fresheners, perfumes, and other household products (Dellacassa et al. 2009).

In Pakistan, essential oils are utilized in aromatherapy, but the country relies on imports to meet its essential oil requirements. Pakistani companies import raw materials like oils, fragrances, drink extracts, and ice cream formulations from Western countries. Pakistan's agricultural landscape is well-suited for cultivating various plants that produce essential oils due to its diverse range of antimicrobial and commercially valuable flora. Aromatic plants naturally thrive in both mountainous and plain regions in Pakistan (Sultan et al., 2014). However, the essential oil industry in Pakistan faces challenges, including the lack of modern agricultural practices and limited knowledge about the conversion of raw materials into finished products. While certain plants like basil, peppermint, and orange are grown for economic purposes in Pakistan, the sector has not fully realized its potential (Ahmed et al., 2019).

Essential oils share several common physico-chemical characteristics. Firstly, they exhibit high solubility in alcohol and ethanol, making them

easy to dissolve. Secondly, these oils are known for their volatility; they are typically colorless and exist in liquid form at typical room temperatures. Additionally, essential oils are recognized for their distinct and often pleasant aromas, which contribute to their aromatic properties. Lastly, these oils possess specific optical qualities, including a refractive index and notable optical activity, which can be important in various applications.

Terpene-based hydrocarbons and oxygenated chemicals make up the majority of the components in essential oils. Aromatic chemicals like limonene, 1,8 citronellol, tocopherol, dimethyl cinnamaldehyde, eugenol, methoxy carvacrol, caryophyllene, and curcumin are among the most prevalent substances in essential oils (Ganjewala, 2009). The volatile fractions of these oils contain components like methyl esters, aromatic alkynes, and phthalates, while the non-volatile fractions contain substances such as carotene, essential fats, polyphenols, and waxes, making up a significant portion (1-10%) of the separated essential oil. Terpenoids, a group of hydrocarbons, are crucial to the chemical composition of essential oils (Setzer et al. 2018).

Essential oils are made from a variety of aromatic plants that can be found in hot, humid countries, such as Pakistan. Flowers, buds, stalks, fruits, and leaves are just a few of the plant parts that can be used to extract these oils. The rate at which the oil moves from the plant's leaf tissue to its outer surface, where it may be collected using a variety of techniques, determines how quickly essential oils are produced. Steam distillation and hydro distillation are the two most popular methods for obtaining essential oils. The oil is also separated using a separating funnel and solvent extraction. In both food and medicine, essential oils derived through distillation are frequently employed. The essential oils that are produced can vary in amount, quality, and biochemical makeup depending on the extraction technique used (Goodarznia, 2015). The genetic make-up and metabolic traits of the plants, along with the environmental and developmental circumstances, are all very important factors in defining the chemical composition of natural oils. Variations in the characteristics of essential oils might result from different plant species and their developmental stages (Hepokur, 2016).

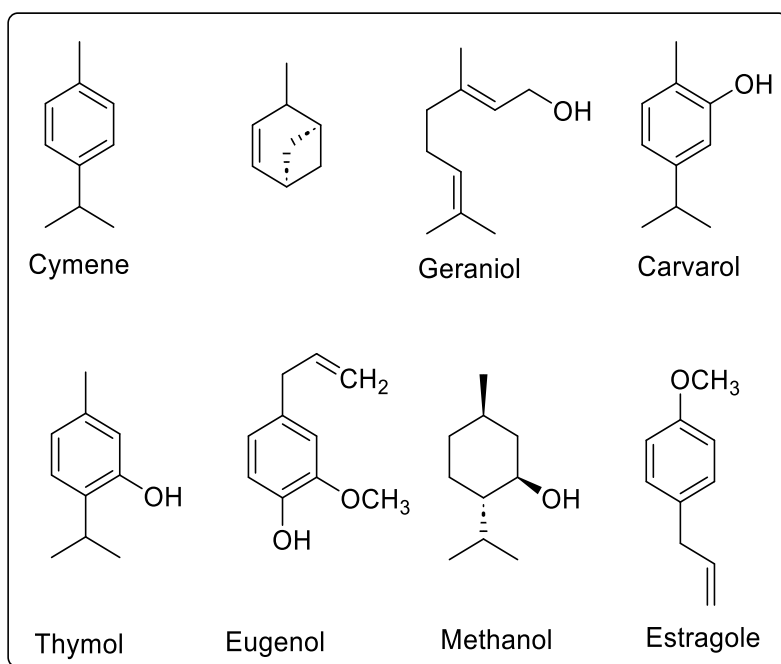


Figure 1: The elemental analysis of several essential oil constituents

Essential oils extracted from aromatic plants have garnered economic attention because of their wide-ranging applications and unique biological properties. These oils are utilized in various industries, including skincare, fragrances, beverages, confectionery, and pharmaceuticals. Among the approximately 3,000 essential oils, around 300 have economic significance for different sectors. Some essential oils or their active components, such as terpenes, geraniol, or citronella, are used in hygiene products.

Traditionally, essential oils have been employed in indigenous healthcare systems to treat various ailments. Aloe Vera-based essential oils, for instance, are used in massages due to their perceived medicinal effects in addressing organ dysfunction. Essential oils possess antimicrobial,

antifungal, medicinal, and dietary applications and are considered environmentally friendly alternatives to synthetic pharmaceuticals. They also hold potential in food preservation and as natural antioxidants (Hashemi et al. 2020).

2. EXPERIMENTAL WORK

For that research, the plant material was gathered from the Jampur area in Punjab, Pakistan, where Holy Basil is plentiful. Afterward, a substantial quantity of the plant material was obtained, and it was processed by cutting the entire plant into small pieces suitable for the biomass flask used in steam distillation. The heating mantle was subsequently activated, initiating the boiling of water in the lower flask, generating steam that

gradually transferred into the biomass flask. Approximately 40 minutes into the heating process, condensation of steam commenced within the Clevenger apparatus. Throughout this procedure, a minor quantity of water condensed in the biomass flask was added and flowed back into the boiling flask, causing a modification in the water's color. Detailed records were diligently maintained, including the instance when the initial droplets passed through the condenser and into the receiver.

Most of the essential oils were gathered within the initial 1 to 3 hours of distillation, and further oils were obtained in the subsequent hour. The distillation process was sustained until either the essential oil layer had not shown any increase for the preceding half-hour or the newly forming hydrosol in the Clevenger had lost its fragrance.

After the distillation process was completed, the remaining hydrosol was carefully drained into an overflow beaker, leaving behind the layer of volatile oils. Subsequently, the Clevenger apparatus was removed from the condenser, and, if desired, some of the hydrosol was preserved in a labeled bottle. The weights for empty essential oil vials were recorded, and once a vial was filled, it was weighed again before sealing it.

In July and August, the aerial parts of cultivated *Ocimum sanctum* (Holy Basil) were harvested from three different locations near the D.G. Khan region. During these months, the average minimum and maximum temperatures in the D.G. Khan area were documented at $28.1^{\circ}\text{C} \pm 2.6^{\circ}\text{C}$ and $38.1^{\circ}\text{C} \pm 3.4^{\circ}\text{C}$, respectively, with an average of 33.1°C . The typical moisture content in the region during July and August was around $46.6\% \pm 14.6\%$, and the total rainfall measured 79.2 millimeters.

Oils from plants that originated in different parts of the D.G. Khan region were included in the analysis of oil content, antioxidants, toxicity, and antibacterial activity. These plant samples were delivered to the lab in spotless polythene bags, washed in tap water, and then dried in a drying oven at a temperature of 30°C to determine their weight.

Subsequent to the collection of the dried leaf pieces, they underwent an additional washing with distilled water and were subsequently cut into smaller segments. These smaller leaf pieces were then introduced into the upper round-bottom flask, referred to as the biomass flask, while 300ml of distilled water was introduced into the lower round-bottom flask. The entire steam distillation apparatus was assembled and positioned on a heating mantle. After approximately 40 minutes, steam was generated, which traversed through the plant leaves to facilitate the extraction of essential oil, and subsequently passed through the condenser. The condensed liquid, consisting of both water and organic essential oil, was collected in a beaker.

In spite of diligent efforts, involving magnetic stirring, difficulties were encountered in effectively collecting the essential oil due to its volatile nature, which led to its vaporization along with n-hexane. As a result, the entire steam distillation process was repeated the following day using fresh leaves, and enhancements were made by incorporating a chiller and a Clevenger apparatus to improve the essential oil collection.

Air-dried organic material, finely ground to a size of 80 mesh, was subjected to steam distillation using a Clevenger-type apparatus for a duration of 4 hours. High-pressure steam was generated by heating water to a temperature exceeding 100°C . This generated steam was passed through the lower part of the apparatus to maximize the release of oil without introducing contamination.

In assessing the final quality of the extracts, several crucial factors were taken into account, including the transportation of raw materials, the cultivation location, the climate of the area, and the timing of harvesting.

In the solvent extraction process, the initial step involved transferring the solution obtained from steam distillation, typically amounting to 200 milliliters, into a separating funnel. Subsequently, 20 milliliters of hexane were added to the separating funnel, and the two liquids were thoroughly mixed by gently shaking the funnel. Once the mixing was complete, the separating funnel was securely fastened onto an iron stand and left undisturbed for a duration of 4 to 5 minutes. During this time, a distinct separation occurred within the funnel, resulting in two clearly defined layers. The upper layer was identified as the organic layer, while the lower layer settled as the aqueous layer. This process of layer separation was repeated three times to ensure the comprehensive extraction of the desired components.

Upon completing these repetitions, the outcome was a mixture comprising hexane and an oil layer. To obtain the final product, which is the essential oil, the hexane was carefully evaporated from the mixture, leaving behind

the valuable essential oil as the end result of the solvent extraction process.

2.1 Analysis of Essential Oil

The refractive index and density of Holy basil essential oils were determined using established procedures. To ascertain the refractive index of the essential oils, we utilized a digital refractometer RX-7000a. In the course of chemical analysis, Gas Chromatography-Mass Spectrometry (GC-MS) was employed. Specifically, a gas chromatograph equipped with a flame ionization detector and an HP-5MS capillary column, measuring 30 meters in length and 0.25 millimeters in diameter, was utilized. The injector and detector were maintained at temperatures of 220°C and 290°C , respectively. The column oven was programmed to gradually increase the temperature from 80°C to 220°C at a rate of 4°C per minute. The initial and final temperatures were maintained for 3 and 10 minutes, respectively. Hydrogen was used as the carrier gas, flowing at a rate of 1.5 milliliters per minute. We employed a split mode to inject $1.0\ \mu\text{L}$ of the sample with a split ratio of 1:100. For analysis, we utilized the built-in data application of the gas chromatograph. The composition results were presented as a percentage of the total peak area.

The analysis of the essential oils was conducted using a GC/MS system equipped with an HP-5 MS capillary column measuring 30 meters in length and 0.25 millimeters in diameter, featuring a film thickness of $0.25\ \mu\text{m}$. A sample of $1.0\ \mu\text{L}$ was injected using the split option, employing a split ratio of 1:100. For the GC/MS analysis, an electron impact instrument with an ionization energy of 70 electronvolts (eV) was employed. Helium was used as the carrier gas with a flow rate of 1.5 mL per minute. The injector and MS sample injection temperatures were set at 220°C and 290°C , respectively. To determine the compounds, mass spectra were compared with those present in the NIST mass spectral database, and retention times were also compared to reference values.

3. RESULTS AND DISCUSSION

The goal of this study was to extract and examine the elements found in Holy basil essential oil. The constituents within the essential oil of Holy basil (*Ocimum sanctum*) are significant for assessing its quality. We conducted steam distillation and GC-MS analysis, revealing a variety of chemical compounds within the plant samples. The key compounds identified encompassed estragole, licorice extract, geraniol, dimethyl citronella, luteolin, and licorice extract.

The study observed that the period of production spanning from July to August, marked by fluctuating temperatures, resulted in a greater yield of essential oil compared to the later stages of leaf development, typically occurring between 40 to 50 days. The analysis of essential oil quantity and characteristics unveiled a noteworthy correlation between the age of Holy basil (*Ocimum sanctum*) leaves and the production of essential oil. These findings indicate that only actively growing Holy basil leaves possess the capacity for rapid synthesis.

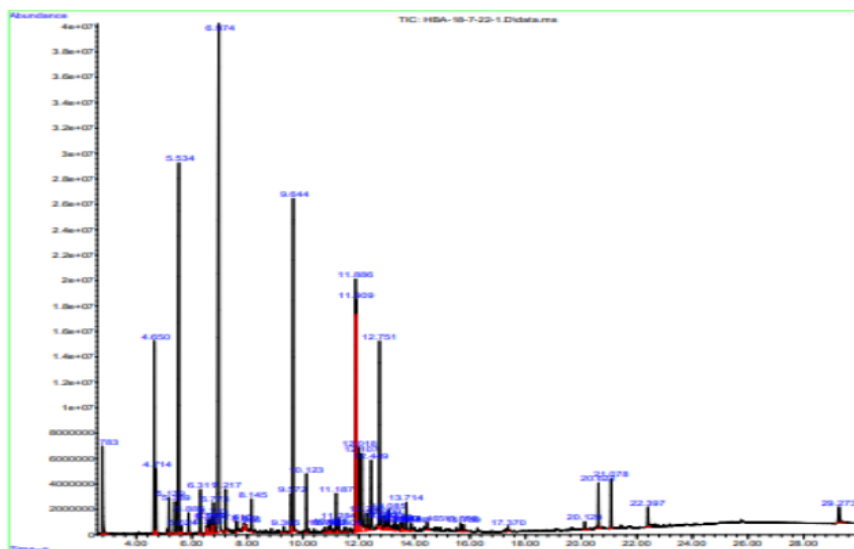
Additional observations were conducted in accordance with the prevailing circumstances. Holy basil clusters had naturally separated into slips, with each slip comprising 2 to 3 leaves, merely 5.5 months after planting. This phenomenon signified an ongoing production cycle. Each stem yielded four to five fresh leaves, demonstrating the capacity to mobilize sucrose and transform it into monoterpenes.

3.1 Chemical Composition of Holy Basil (*Ocimum sanctum*) Essential Oil:

The content of Holy basil (*Ocimum sanctum*) oil displayed notable variations depending on the final harvest. In this research, a total of 56 essential oil components were detected. Between July and August, the D.G. Khan region recorded average minimum and maximum temperatures of 28.1 ± 2.6 and 38.1 ± 3.4 Celsius, respectively, with an overall average temperature of 33.1°C . Additionally, the average relative humidity and total rainfall in the D.G. Khan region for the months of July and August were 46.6 ± 14.6 percent and 79.2 millimeters, respectively. We conducted Gas Chromatography-Mass Spectrometry (GC-MS) analysis to investigate the essential oil of Holy basil (*Ocimum sanctum*), which resulted in the identification of 56 components during the analysis.

3.2 Gas Chromatography-Mass Spectrometry Analysis

In the GC-MS analysis of Holy basil essential oil (*Ocimum sanctum*), a total of 56 distinct components with characteristic peaks were identified. Graph 1 displays the calibration curve for total ions.

Graph 1: Total ion Holy basil (*O. Sanctum*)Table 1: Total ion in Holy basil (*O. Sanctum*)

Peak#	Component Name	Fragments	R. Time (minutes)	Chemical Formula	Area %
1	Chlorobenzene	51, 77 & 112	2.783	C ₆ H ₅ Cl	2.38
2	D-Limonene	53, 68, 93, 107, 121 & 136	4.650	C ₁₀ H ₁₆	4.44
3	Eucalyptol	43, 63, 81, 108 & 125	4.714	C ₁₀ H ₁₈ O	1.45
4	2-Furanmethanol, 5-ethenyltetrahydro- $\alpha,\alpha,\alpha,5$ - trimethyl-, cis-	41, 59, 77, 94 & 111	5.179	C ₁₀ H ₁₈ O ₂	0.89
5	Ethyl 2-(5-methyl-5- vinyltetrahydrofuran-2-yl)propan-2-yl carbonate	43, 59, 79, 94, 111, 137 & 155	5.389	C ₁₃ H ₂₂ O ₄	0.76
6	Linalool	43, 71, 93, 121 & 139	5.534	C ₁₀ H ₁₈ O	12.95
7	Fenchol	43, 63, 81, 111 & 139	5.885	C ₁₀ H ₁₈ O	0.47
8	(+)-2-Bornanone	43, 68, 81 & 94	6.311	C ₁₀ H ₁₆ O	1.03
9	2H-Pyran-3-ol, 6-ethenyltetrahydro-2,2,6-trimethyl	43, 59, 68, 81 & 94	6.548	C ₁₀ H ₁₈ O ₂	0.20
10	(3R,6S)-2,2,6-Trimethyl-6-vinyltetrahydro-2H-pyran-3-ol	43, 59, 68, 81 & 94	6.584	C ₁₀ H ₁₈ O ₂	0.37
11	Bicyclo [2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-	41, 55, 69, 95, 110 & 139	6.639	C ₁₀ H ₁₈ O	0.48
12	Terpinen-4-ol	43, 57, 71, 93, 111 & 136	6.733	C ₁₀ H ₁₈ O	0.38
13	3,7-Octadiene-2,6-diol, 2,6-dimethyl	43, 59, 82 & 109	6.773	C ₁₀ H ₁₈ O	0.94
14	Estragole	51, 77, 91, 121 & 148	6.974	C ₁₀ H ₁₂ O	21.59
15	Fenchyl acetate	43, 67, 81, 107, 121 & 136	7.217	C ₁₂ H ₂₀ O ₂	0.95
16	2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-	41, 69, 93 & 123	7.610	C ₁₀ H ₁₈ O	0.39
17	2,6-Octadienal, 3,7-dimethyl-, (E)-	41, 55, 69, 84, 107, 121 & 136	7.872	C ₁₀ H ₁₆ O	0.29
18	1,7-Octadiene-3,6-diol, 2,6-dimethyl	43, 67, 82, 107, 121 & 135	7.925	C ₁₀ H ₁₈ O ₂	0.17
19	Bornyl acetate	43, 69, 95, 121 & 154	8.145	C ₁₂ H ₂₀ O ₂	0.77
20	Glutaric acid, 2,2-dichloroethyl geranyl ester	41, 69, 93, 121 & 136	9.306	C ₁₇ H ₂₆ Cl ₂ O ₄	0.19
21	Cyclohexane, 1-ethenyl-1-methyl- 2,4-bis(1-methylethenyl)-	41, 55, 68, 81, 94, 107, 121, 134 & 147	9.572	C ₁₅ H ₂₄	0.89
22	Methyleugenol	37, 51, 77, 91, 107, 137, 147, 163 & 178	9.644	C ₁₁ H ₁₄ O ₂	10.06
23	Bicyclo[3.1.1] hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	41, 69, 93, 119 & 161	10.123	C ₁₅ H ₂₄	1.35
24	(1S,5S)-4-Methylene-1-((R)-6-methylhept-5-en-2-yl)bicyclo[3.1.0]hexane	41, 55, 69, 93, 107, 120, 133, 147 & 166	10.794	C ₁₅ H ₂₄	0.16
25	(1S,2S,4S)-Trihydroxy-p-menthane	43, 65, 81, 109, 127, 145 & 166	10.923	C ₁₀ H ₂₀ O ₃	0.22
26	Phenol, 2,6-bis(1,1-dimethylethyl)-	40, 57, 74, 91, 121, 145, 163 & 191	10.985	C ₁₄ H ₂₂ O	0.23
27	Naphthalene, 1,2,4a,5,6,8a- hexahydro-4,7-dimethyl-1-(1-methylethyl)	41, 55, 77, 91, 105, 119, 133, 148 & 161	11.187	C ₁₅ H ₂₄	0.97
28	4-isopropyl-1,6-dimethyl-1,2,3,4-Tetrahydronaphthalene	43, 59, 79, 93, 119, 132, 145 & 159	11.284	C ₁₅ H ₂₂	0.32
29	(Z,Z)- α -Farnesene	43, 69, 93, 119, 135 & 161	11.509	C ₁₅ H ₂₄	0.22
30	trans-4-Methoxycinnamaldehyde	39, 63, 77, 91, 105, 121 & 147	11.886	C ₁₀ H ₁₀ O ₂	7.09

31	4-Methoxycinnamaldehyde	39, 63, 77, 91, 105, 121 & 147	11.909	C ₁₀ H ₁₀ O ₂	2.67
32	Benzeneacetonitrile, 3,4-diethoxy-	43, 57, 77, 91, 105, 119, 133, 149 & 177	12.018	C ₁₂ H ₁₅ NO ₂	2.32
33	Bicyclo [3.1.1] heptane,6,6-dimethyl- 3-methylene	43, 58, 79, 109 & 121	12.107	C ₁₀ H ₁₆	3.05
34	Camphene	41, 68, 93 & 121	12.291	C ₁₀ H ₁₆	0.45
35	1-(4-Methoxyphenyl)-1- cyclopropane carboxylic acid	43, 69, 91, 119, 147 & 164	12.449	C ₁₁ H ₁₂ O ₃	2.67
36	(1R,2S,6S,7S,8S)-8-Isopropyl-1- methyl-3-methyl enetricyclo [4.4.0.02,7]decane-rel-	43, 65, 81, 105, 134 & 172	12.751	C ₁₅ H ₂₄	4.74
37	1-Formyl-2,2-dimethyl-3-trans-(3- methyl-but-2-enyl)-6-methylidene- cyclohexane	41, 69, 91, 109, 135, 159 & 177	12.801	C ₁₅ H ₂₄ O	0.32
38	2-Cyclopenten-1-one, 3-methyl-2-(2- pentenyl)-, (Z)	41, 55, 79, 93, 110, 135, 149 & 164	9.17	C ₁₁ H ₁₆ O	0.26
39	Alloaromadendrene oxide-(1)	43, 55, 81, 95, 109, 122, 135 & 151	10.032	C ₁₅ H ₂₄ O	0.26
40	Geranyl isobutyrate	41, 69, 93, 121,136 & 154	9.306	C ₁₄ H ₂₄ O ₂	0.19
41	Limonene oxide, cis	43, 67, 91, 109 & 137	10.085	C ₁₀ H ₁₆ O	0.59
42	Oxiranecarboxaldehyde, 3-methyl-3-(4-methyl-3-pentenyl)-	41, 69, 91, 107, 135 & 169	13.348	C ₁₀ H ₁₆ O ₂	0.35
43	Murol-5-en-4-one	41, 55, 77, 91, 107, 121, 135, 149, 161, 191 & 206	9.581	C ₁₄ H ₂₂ O	0.43
44	1,1,4,7-Tetramethyldecahydro-1H-cyclopropa[e]azulene-4,7-diol 43.0	43, 74, 91, 121, 147, 164, 187 & 206	13.520	C ₁₅ H ₂₆ O ₂	0.40
45	(3S,4R,5R,6R)-4,5-Bis(hydroxymethyl)-3,6- dimethylcyclohexene	43, 65, 83, 103, 121 & 147	13.593	C ₁₀ H ₁₈ O ₂	0.24
46	Bergamotol, Z-.alpha.-trans	43, 71, 93, 119 & 136	13.714	C ₁₅ H ₂₄ O	0.88
47	1-Methylene-2-vinylcyclopentane	43, 69, 111 & 134	13.889	C ₈ H ₁₂	0.23
48	benzenamine, 4-(1H-imidazol-1-yl)-	39, 65, 105, 132 & 159	14.465	C ₉ H ₉ N ₃	0.17
49	Hexadecanoic acid, methyl ester	43, 74, 97, 121, 143, 167, 185, 207 & 227	15.658	C ₁₇ H ₃₄ O ₂	0.20
50	Ethyl geranyl acetate	43, 67, 81, 109 & 125	15.759	C ₁₄ H ₂₄ O ₂	0.22
51	11-Octadecenoic acid, methyl ester	38, 55, 79, 97, 112 & ,147	17.370	C ₁₉ H ₃₆ O ₂	0.12
52	2'-Ethoxyacetophenone	44, 69, 91, 121, 147 & 177	20.129	C ₁₀ H ₁₂ O ₂	0.17
53	Bibenzyl, 2,3,4,6-tetramethyl-	41, 65, 91, 115, 147 & 238	17.370	C ₁₈ H ₂₂	0.12
54	Bis(2-ethylhexyl) phthalate	35, 57, 83, 113, 149 & 180	21.078	C ₂₄ H ₃₈ O ₄	1.15
55	Benzoic acid, 2,4,6-trimethyl-, 2,4,6-trimethylphenyl ester	44, 91, 115, 147, 173 & 207	22.397	C ₁₉ H ₂₂ O ₂	0.58
56	4-(4-Hydroxyphenyl)-4-methyl-2- pentanone, TMS derivative	57, 91, 117, 147, 175, 207, 253 & 316	29.273	C ₁₂ H ₁₆ O ₂	0.95

Different elements present in the essential oil of Holy Basil (*Ocimum sanctum*) were identified and described through the application of steam distillation, gas chromatography, and mass spectrometry. The outcomes of this examination, along with the chemical profile, are succinctly outlined in Graph 1.

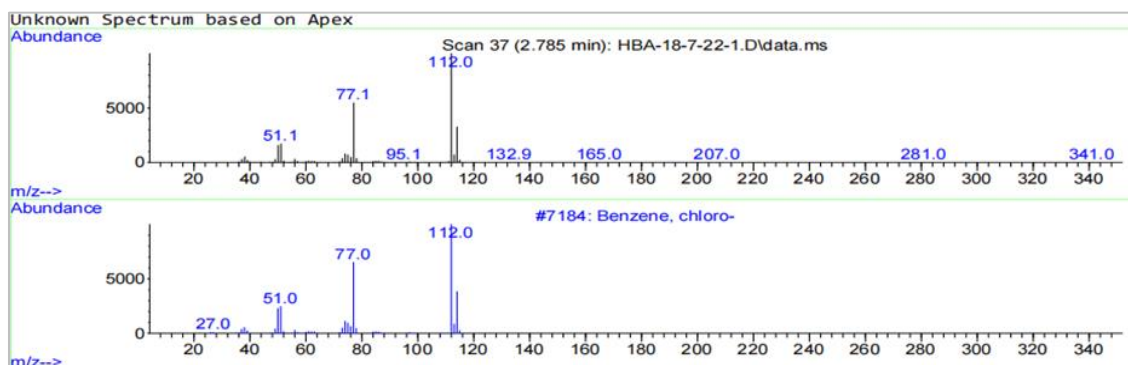
The Graph 1. includes the identification of 56 distinct components present in the essential oil, each with specific fragment ions, retention times, chemical formulas, and corresponding area percentages. These constituents include various chemical classes such as benzenes, terpenes, aldehydes, esters, and phenolic compounds, among others. These results offer valuable information about the chemical makeup of Holy Basil Essential Oil, which is crucial for comprehending its possible biological

and medicinal characteristics.

4. MAJOR CHEMICAL COMPONENTS OF THE OIL

4.1 Chlorobenzene

The Electron Impact (EI) mass spectrum of Chlorobenzene (#7184) with the formula C₆H₅Cl exhibits peaks at m/z (77, 112, 51), respectively, indicating the most prominent mass-to-charge ratios observed in the spectrum **Graph 2**. This emerged at retention time (R.T) 2.783 min in the total ion chromatography paper. The presence of these characteristic fragments provides valuable insights into the molecular composition and fragmentation pattern of Chlorobenzene, aiding in its chemical identification and analysis. (**Table 2**)



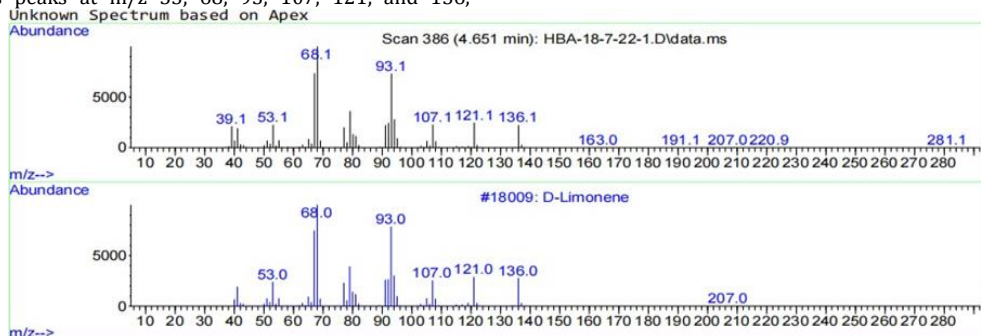
Graph 2: Mass spectrum of Chlorobenzene

Table 2: Fragmentation of Chlorobenzene

m/z value of [fragment] ⁺	112	77	51
[molecular fragment] ⁺	[C ₆ H ₅ Cl] ⁺	[C ₆ H ₅] ⁺	[C ₄ H ₃] ⁺

4.2 D-Limonene

The EI mass spectrum of D-Limonene exhibits distinct molecular fragments associated with specific m/z values (**Graph 3**). The spectrum prominently displays peaks at m/z 53, 68, 93, 107, 121, and 136,

**Graph 3: Mass spectrum of D-Limonene****Table 3: Fragmentation Pattern of D-Limonene**

m/z value of [fragment] ⁺	136	121	107	93	68	53
[molecular fragment] ⁺	[C ₁₀ H ₁₆] ⁺	[C ₉ H ₁₃] ⁺	[C ₈ H ₁₁] ⁺	[C ₇ H ₉] ⁺	[C ₅ H ₈] ⁺	[C ₄ H ₅] ⁺

4.3 Eucalyptol

The EI mass spectrum of Eucalyptol reveals characteristic m/z values that represent distinct molecular fragments (**Graph 4**). The spectrum prominently features peaks at m/z 43, 63, 81, 108, and 125, corresponding to specific molecular fragments. These fragments are denoted as [C₃H₇]⁺, [C₅H₃]⁺, [C₆H₉]⁺, [C₈H₁₂]⁺, and [C₉H₁₇]⁺, respectively. This spectral information was obtained at R.T 4.714 minutes in the total ion chromatography paper (**Table 4**).

4.4 Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl) propan-2-yl carbonate

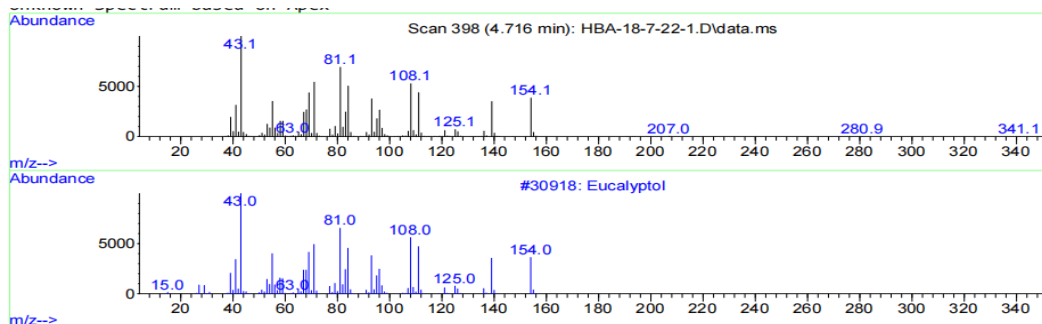
The EI mass spectrum of Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl) propan-2-yl carbonate exhibits prominent peaks at specific m/z values, indicating distinct molecular fragments (**Graph 5**). These characteristic

representing distinct molecular fragments. These fragments correspond to [C₄H₅]⁺, [C₅H₈]⁺, [C₇H₉]⁺, [C₈H₁₁]⁺, [C₉H₁₃]⁺, and [C₁₀H₁₆]⁺, respectively. This spectral information was recorded at R.T 4.650 minutes in the total ion chromatography paper. (**Table 3**)

peaks are observed at m/z 41, 59, 77, 94, 111, 137, and 155. They correspond to molecular fragments denoted as [C₃H₅]⁺, [C₃H₇O]⁺, [C₅H₅]⁺, [C₇H₁₁O]⁺, [C₉H₁₃O]⁺, and [C₁₀H₁₉O]⁺, respectively. This mass spectral information was obtained R.T 5.389 minutes in the total ion chromatography paper (**Table 5**).

4.5 Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl) propan-2-yl carbonate

The EI mass spectrum of Fenchol reveals distinct peaks at specific m/z values, signifying characteristic molecular fragments (**Graph 6**). These significant peaks are observed at m/z 43, 63, 81, 111, and 139, corresponding to molecular fragments denoted as [C₃H₇]⁺, [C₅H₃]⁺, [C₆H₉]⁺, [C₈H₁₅]⁺, and [C₉H₁₅O]⁺, respectively. This mass spectral data was obtained at R.T 5.885 minutes in the total ion chromatography paper. (**Table 6**)

**Graph 4: Mass spectrum of Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl) propan-2-yl carbonate****Table 4: Fragmentation Pattern of Eucalyptol**

m/z value of [fragment] ⁺	125	108	81	63	43
[molecular fragment] ⁺	[C ₉ H ₁₇] ⁺	[C ₈ H ₁₂] ⁺	[C ₆ H ₉] ⁺	[C ₅ H ₃] ⁺	[C ₃ H ₇] ⁺

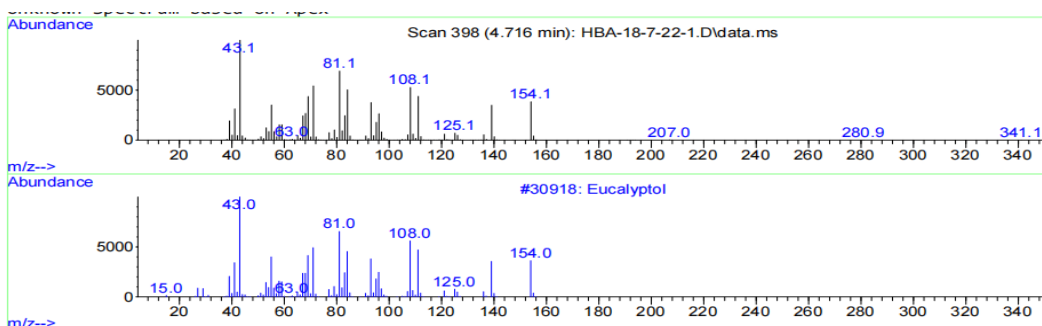
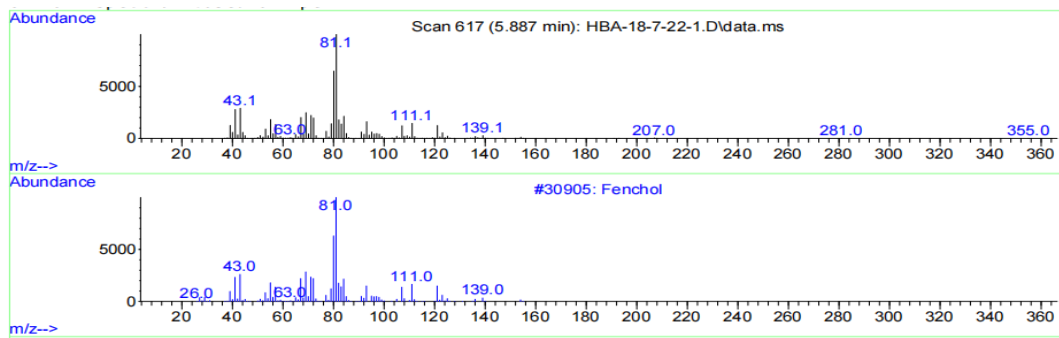
**Graph 5: Mass spectrum of Eucalyptol**

Table 5: Fragmentation Pattern of Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl) propan-2-yl carbonate

m/z value of [fragment] ⁺	155	137	111	77	59	41
[molecular fragment] ⁺	[C ₁₀ H ₁₉ O] ⁺	[C ₉ H ₁₃ O] ⁺	[C ₇ H ₁₁ O] ⁺	[C ₅ H ₅] ⁺	[C ₃ H ₇ O] ⁺	[C ₃ H ₅] ⁺

**Graph 6:** Mass spectrum of Fenchol**Table 6:** Fragmentation Pattern of Fenchol

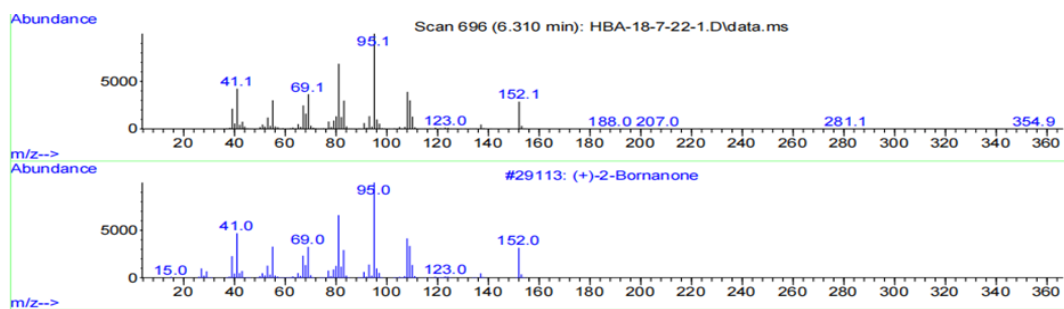
m/z value of [fragment] ⁺	139	111	81	63	43
[molecular fragment] ⁺	[C ₉ H ₁₅ O] ⁺	[C ₈ H ₁₅] ⁺	[C ₆ H ₉] ⁺	[C ₅ H ₃] ⁺	[C ₃ H ₇] ⁺

4.6 (+)-2-Bornanone

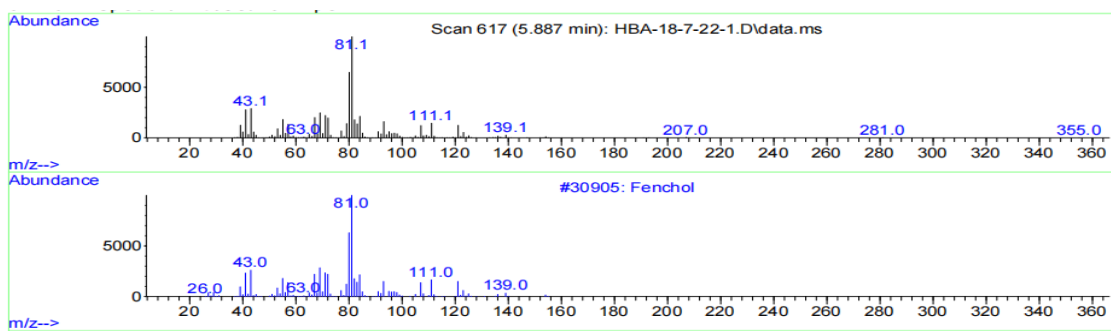
The EI mass spectrum of (+)-2-Bornanone displays major peaks at specific m/z values, indicating distinct molecular fragments (**Graph 7**). These significant peaks are observed at m/z 41, 69, 95, 123, and 152, corresponding to molecular fragments denoted as [C₃H₅]⁺, [C₄H₅O]⁺, [C₇H₁₁]⁺, [C₉H₁₅]⁺, and [C₁₀H₁₆O]⁺, respectively. This mass spectral data was obtained at an R.T of 6.311 minutes in the total ion chromatography paper. (**Table 7**)

4.7 Fenchol, exo

The EI mass spectrum of Fenchol, exo reveals significant peaks at specific m/z values, indicating distinctive molecular fragments (**Graph 8**). The major peaks in this spectrum are observed at m/z 43, 63, 81, 111, and 139, corresponding to molecular fragments denoted as [C₃H₇]⁺, [C₅H₃]⁺, [C₆H₉]⁺, [C₈H₁₅]⁺, and [C₉H₁₅O]⁺, respectively. This mass spectral data was obtained at an R.T (retention time) of 5.885 minutes in the total ion chromatography paper (**Table 8**).

**Graph 7:** Mass spectrum of (+)-2-Bornanone**Table 7:** Fragmentation Pattern of (+)-2-Bornanone

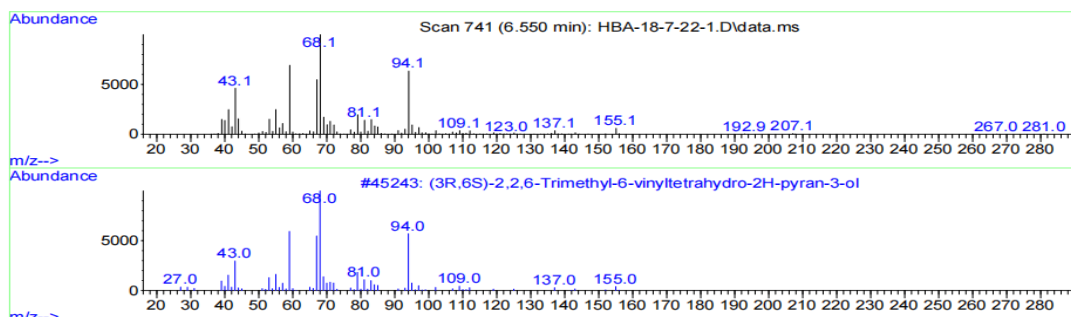
m/z value of [fragment] ⁺	152	123	95	69	41
[molecular fragment] ⁺	[C ₁₀ H ₁₆ O] ⁺	[C ₉ H ₁₅] ⁺	[C ₇ H ₁₁] ⁺	[C ₄ H ₅ O] ⁺	[C ₃ H ₅] ⁺

**Graph 8:** Mass spectrum of Fenchol, exo**Table 8:** Fragmentation Pattern of Fenchol, exo

m/z value of [fragment] ⁺	139	111	81	63	43
[molecular fragment] ⁺	[C ₉ H ₁₅ O] ⁺	[C ₈ H ₁₅] ⁺	[C ₆ H ₉] ⁺	[C ₅ H ₃] ⁺	[C ₃ H ₇] ⁺

4.8 (3R,6S)-2,2,6-Trimethyl-6-vinyltetrahydro-2H-pyran-3-ol

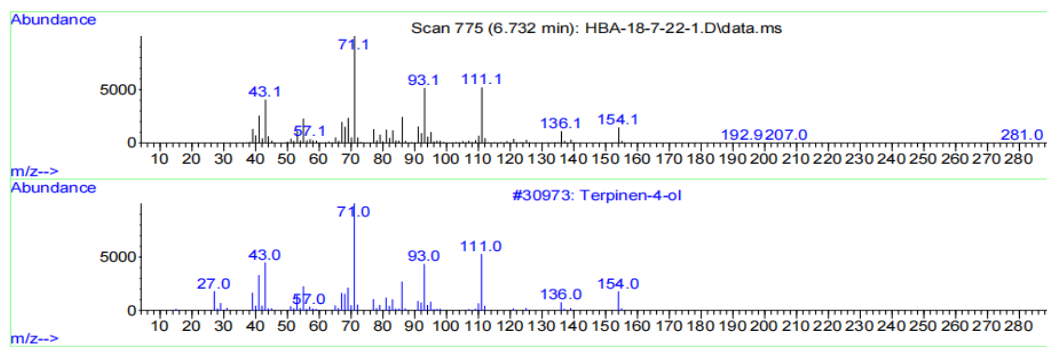
The EI mass spectrum of (3R,6S)-2,2,6-Trimethyl-6-vinyltetrahydro-2H-pyran-3-ol exhibits prominent peaks at specific m/z values, indicating distinct molecular fragments (**Graph 9**). The primary peaks in this spectrum are observed at m/z 43, 68, 81, and 94, corresponding to molecular fragments represented as $[C_2H_3O]^+$, $[C_4H_4O]^+$, $[C_5H_5O]^+$, and $[C_6H_6O]^+$, respectively. This mass spectral data was obtained at R.T 6.548 minutes in the total ion chromatography paper (**Table 9**).



Graph 9: Mass spectrum of (3R,6S)-2,2,6-Trimethyl-6-vinyltetrahydro-2H-pyran-3-ol

Table 9: Fragmentation Pattern of (3R,6S)-2,2,6-Trimethyl-6-vinyltetrahydro-2H-pyran-3-ol

m/z value of [fragment] ⁺	94	81	68	43
[molecular fragment] ⁺	$[C_6H_6O]^+$	$[C_5H_5O]^+$	$[C_4H_4O]^+$	$[C_2H_3O]^+$



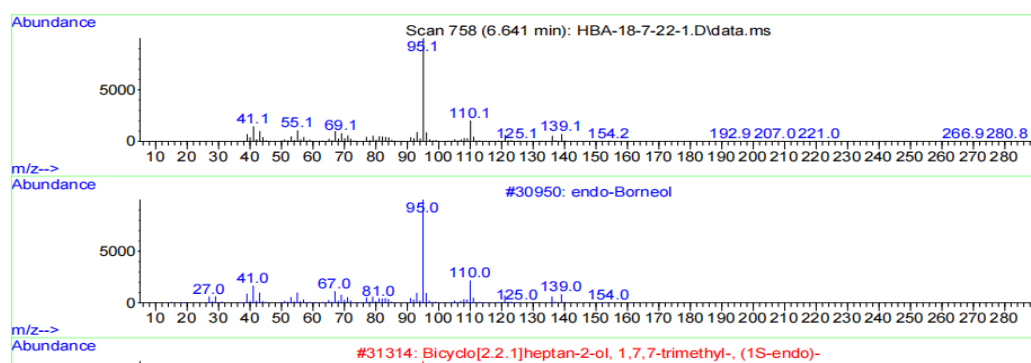
Graph 10: Mass spectrum of Terpinen-4-ol

Table 10: Fragmentation Pattern of Terpinen-4-ol

m/z value of [fragment] ⁺	136	111	93	71	57	43
[molecular fragment] ⁺	$[C_{10}H_{16}]^+$	$[C_7H_{11}O]^+$	$[C_7H_9]^+$	$[C_4H_7O]^+$	$[C_4H_9]^+$	$[C_3H_7]^+$

4.10 Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)

The EI mass spectrum of Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo) displays prominent peaks at specific m/z values, indicative of distinct molecular fragment (**Graph 11**). The primary peaks in this spectrum are observed at m/z 41, 55, 69, 95, and 110, corresponding to molecular fragments denoted as $[C_3H_5]^+$, $[C_4H_7]^+$, $[C_4H_5O]^+$, $[C_7H_{11}]^+$, and $[C_8H_{14}]^+$, respectively. This mass spectral data was obtained at R.T 6.639 minutes in the total ion chromatography paper (**Table 11**).



Graph 11: Mass spectrum of Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)

4.9 Terpinen-4-ol

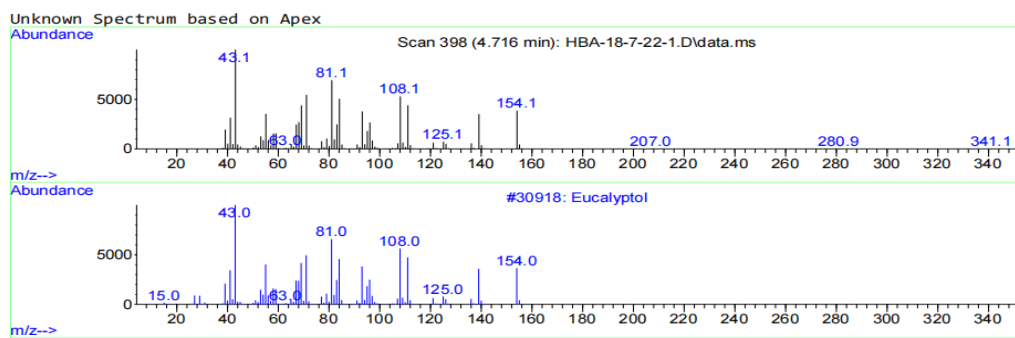
The EI mass spectrum of Terpinen-4-ol displays distinct peaks at specific m/z values, revealing characteristic molecular fragments (**Graph 10**). The primary peaks in this spectrum are observed at m/z 43, 57, 71, 93, 111, and 136, corresponding to molecular fragments denoted as $[C_3H_7]^+$, $[C_4H_9]^+$, $[C_4H_7O]^+$, $[C_7H_9]^+$, $[C_7H_{11}O]^+$, and $[C_{10}H_{16}]^+$, respectively. This mass spectral data was obtained at R.T 6.733 minutes in the total ion chromatography paper (**Table 10**).

4.11 Estragole

The EI mass spectrum of Estragole displays prominent peaks at specific m/z values, revealing characteristic molecular fragments (**Graph 13**). The major peaks in the spectrum correspond to m/z 43, 51, 63, 81, and 108, representing molecular fragments denoted as $[C_4H_3]^+$, $[C_6H_5]^+$, $[C_5H_3]^+$, $[C_6H_9]^+$, and $[C_8H_{12}O]^+$, respectively. This mass spectral data was obtained at an R.T of 6.974 minutes during total ion chromatography (**Table 14**).

Table 11: Fragmentation Pattern of Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)

m/z value of [fragment] ⁺	139	110	95	69	55	41
[molecular fragment] ⁺	[C ₉ H ₁₅ O] ⁺	[C ₈ H ₁₄] ⁺	[C ₇ H ₁₁] ⁺	[C ₄ H ₅ O] ⁺	[C ₄ H ₇] ⁺	[C ₃ H ₅] ⁺

**Graph 12: Mass spectrum of Estragole****Table 12: Fragmentation Pattern of Estragole**

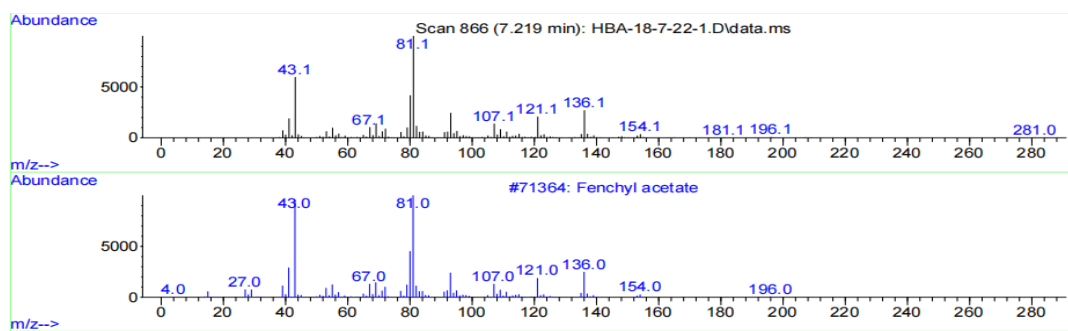
m/z value of [fragment] ⁺	148	121	91	77	51
[molecular fragment] ⁺	[C ₁₀ H ₁₂ O] ⁺	[C ₈ H ₉ O] ⁺	[C ₇ H ₇] ⁺	[C ₆ H ₅] ⁺	[C ₄ H ₃] ⁺

4.12 Fenchyl Acetate

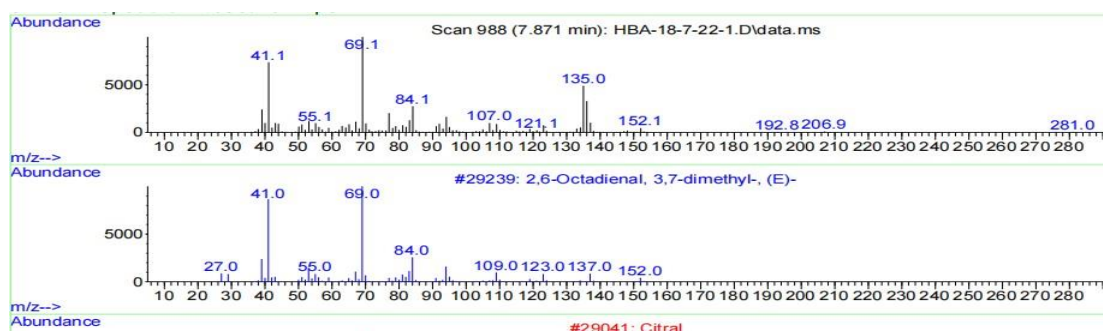
The EI mass spectrum of Fenchyl acetate exhibits distinct peaks at specific m/z values, indicating characteristic molecular fragments (**Graph 13**). The primary peaks in the spectrum correspond to m/z 43, 67, 81, 107, 121, and 136, representing molecular fragments denoted as [C₂H₃O]⁺, [C₄H₃O]⁺, [C₆H₉]⁺, [C₈H₁₁]⁺, [C₈H₉O]⁺, and [C₁₀H₁₆]⁺, respectively. This mass spectral data was obtained at an R.T of 7.217 minutes during total ion chromatography (**Table 13**).

4.13 Citral

The EI mass spectrum of Citral D-Limonene reveals prominent peaks at specific m/z values, indicating characteristic molecular fragments (**Graph 14**). The primary peaks in the spectrum correspond to m/z 41, 55, 69, 84, 107, and 135, representing molecular fragments denoted as [C₃H₅]⁺, [C₄H₇]⁺, [C₄H₅O]⁺, [C₆H₁₂]⁺, [C₈H₁₁]⁺, and [C₁₀H₁₅]⁺, respectively. This mass spectral data was obtained at an R.T of 7.872 minutes during total ion chromatography (**Table 14**).

**Graph 13: Mass spectrum of Fenchyl acetate****Table 13: Fragmentation Pattern of Fenchyl Acetate**

m/z value of [fragment] ⁺	136	121	107	81	67	43
[molecular fragment] ⁺	[C ₁₀ H ₁₆] ⁺	[C ₈ H ₉ O] ⁺	[C ₈ H ₁₁] ⁺	[C ₆ H ₉] ⁺	[C ₄ H ₃ O] ⁺	[C ₂ H ₃ O] ⁺

**Graph 14: Mass spectrum of Citral****Table 14: Fragmentation Pattern of Citral**

m/z value of [fragment] ⁺	135	107	84	69	55	41
[molecular fragment] ⁺	[C ₁₀ H ₁₅] ⁺	[C ₈ H ₁₁] ⁺	[C ₆ H ₁₂] ⁺	[C ₄ H ₅ O] ⁺	[C ₄ H ₇] ⁺	[C ₃ H ₅] ⁺

4.14 1,7-Octadiene-3,6-diol, 2,6-dimethyl

The EI mass spectrum of 1,7-Octadiene-3,6-diol, 2,6-dimethyl exhibits prominent peaks at specific m/z values, signifying characteristic molecular fragments (**Graph 15**). The major peaks in the spectrum correspond to m/z 43, 67, 82, 107, 121, and 135, representing molecular fragments designated as $[C_3H_7]^+$, $[C_4H_3O]^+$, $[C_5H_6O]^+$, $[C_6H_9O]^+$, $[C_8H_{11}]^+$, and $[C_9H_{11}O]^+$, respectively. This mass spectral data was obtained at an R.T of 7.925 minutes during total ion chromatography (**Table 15**).

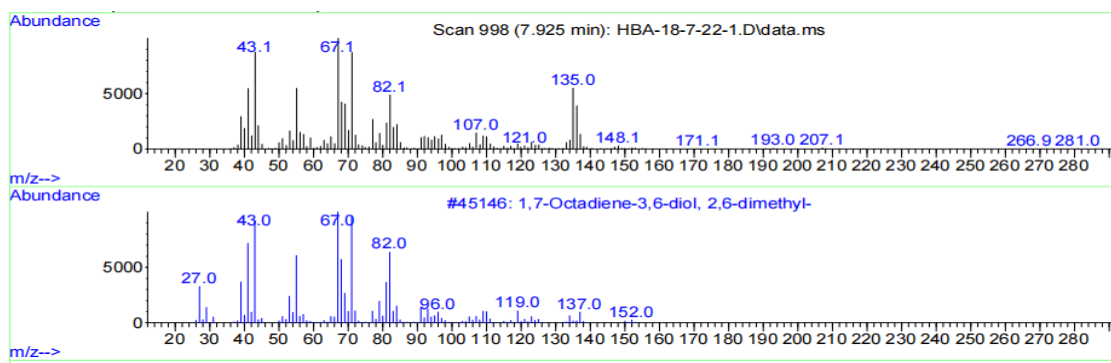
4.15 Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, (1S-endo)

The EI mass spectrum of Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, (1S-endo) exhibits distinct peaks at m/z values of 43, 69, 95, 121,

and 154, (**Graph 16**) representing corresponding molecular fragments designated as $[C_2H_3O]^+$, $[C_5H_9]^+$, $[C_7H_{11}]^+$, $[C_9H_{13}]^+$, and $[C_{10}H_{18}O]^+$, respectively. This mass spectral data was obtained at an R.T of 6.225 minutes during total ion chromatography (**Table 16**).

4.16 Glutaric acid, 2,2-dichloroethyl geranyl ester :

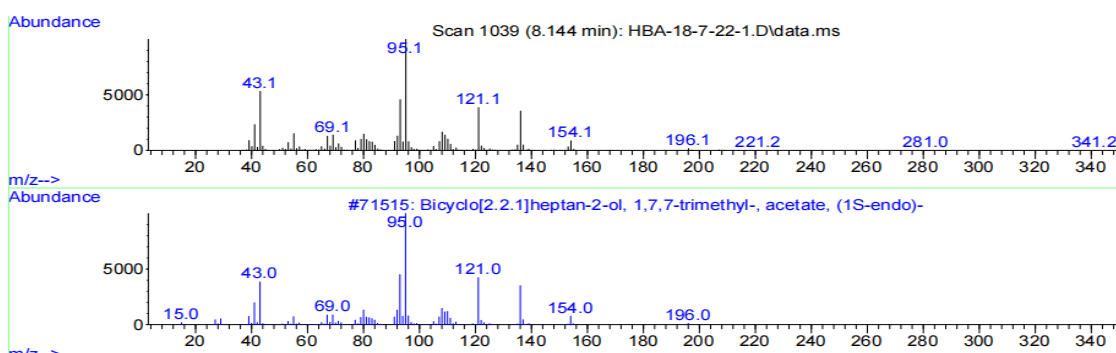
The EI mass spectrum of Glutaric acid, 2,2-dichloroethyl geranyl ester displayed prominent peaks at m/z values of 43, 69, 93, 121, and 136, (**Graph 17**) representing corresponding molecular fragments designated as $[C_3H_7]^+$, $[C_5H_9]^+$, $[C_7H_9]^+$, $[C_9H_{13}]^+$, and $[C_{11}H_4]^+$, respectively. This mass spectral data was recorded at an R.T of 9.306 minutes during total ion chromatography (**Table 17**).



Graph 15: Mass spectrum of 1,7-Octadiene-3,6-diol, 2,6-dimethyl

Table 15: Fragmentation Pattern of 1,7-Octadiene-3,6-diol, 2,6-dimethyl

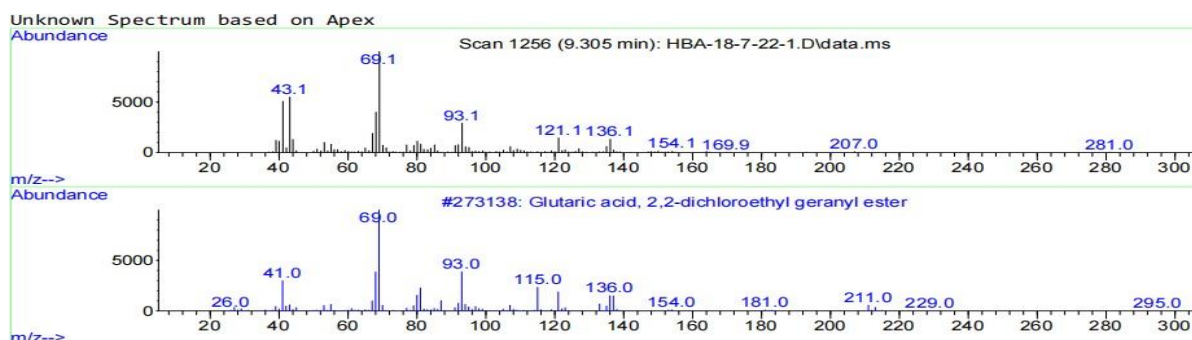
m/z value of [fragment] ⁺	135	121	107	82	67	43
[molecular fragment] ⁺	$[C_9H_{11}O]^+$	$[C_8H_9O]^+$	$[C_8H_{11}]^+$	$[C_5H_6O]^+$	$[C_4H_3O]^+$	$[C_3H_7]^+$



Graph 16: Mass spectrum of Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, (1S-endo)

Table 16: Fragmentation Pattern of Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, (1S-endo)

m/z value of [fragment] ⁺	154	121	95	69	43
[molecular fragment] ⁺	$[C_{10}H_{18}O]^+$	$[C_9H_{13}]^+$	$[C_7H_{11}]^+$	$[C_5H_9]^+$	$[C_2H_3O]^+$



Graph 17: Mass spectrum of Glutaric acid, 2,2-dichloroethyl geranyl ester

Table 17: Fragmentation Pattern of Glutaric Acid, 2,2-Dichloroethyl Geranyl Ester

m/z value of [fragment] ⁺	136	121	93	69	43
[molecular fragment] ⁺	$[C_{11}H_4]^+$	$[C_9H_{13}]^+$	$[C_7H_9]^+$	$[C_5H_9]^+$	$[C_3H_7]^+$

4.17 Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)

The EI mass spectrum of Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl), displayed significant peaks at m/z values of 41, 55, 107, 121, 134, and 147, (**Graph 18**) corresponding to molecular fragments denoted as $[C_3H_5]^+$, $[C_4H_7]^+$, $[C_8H_{11}]^+$, $[C_9H_{13}]^+$, $[C_{10}H_{14}]^+$, and $[C_{11}H_{15}]^+$, respectively. This mass spectral data was recorded at different relative intensities (R.I) of 46.36%, 54.75%, and 78.06% during total ion chromatography (**Table 18**).

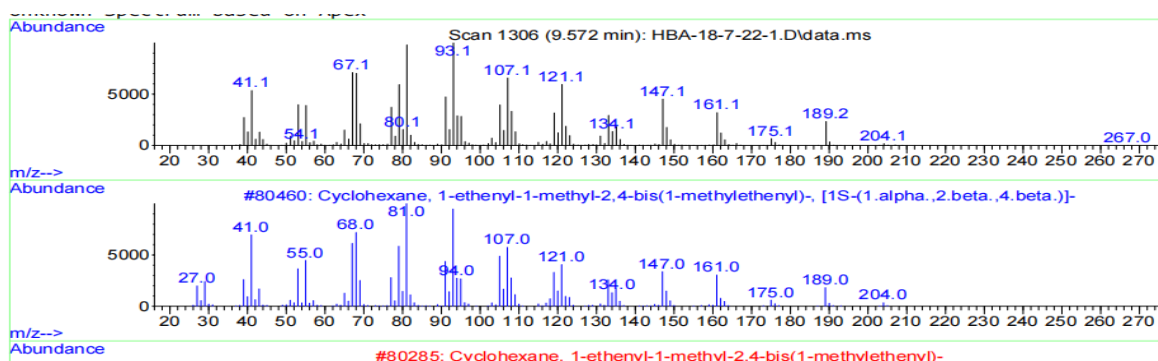
Holy basil (*Ocimum sanctum*) essential oil can be classified into several groups, each characterized by specific mass-to-charge ratios (m/z) and retention times (R.T):

- Benzen Chloro (51, 77 & 112) m/z , (2.783) min
- D-Limonene (53, 68, 93, 107, 121 & 136) m/z , (4.650) min
- Eucalyptol (43, 63, 81, 108 & 125) m/z , (4.714) min
- 2-Furanmethanol, 5-ethenyltetrahydro- α,α , 5-trimethyl-, cis (41, 59, 77, 94 & 111) m/z , (5.179) min
- Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl) propan-2-yl carbonate (43, 59, 79, 94, 111, 137 & 155) m/z , (5.389) min
- Linalool (43, 71, 93, 121 & 139) m/z , (5.534) min
- Fenchol (43, 63, 81, 111 & 139) m/z (5.885) min
- (+)-2-Bornanone (43, 68, 81 & 94) m/z , (6.311) min
- 2H-Pyran-3-ol, 6-ethenyltetrahydro-2,2,6-trimethyl (43, 59, 68, 81 & 94) m/z (6.548) min
- (3R,6S)-2,2,6-Trimethyl-6-vinyltetrahydro-2H-pyran-3-ol (43,59,68,81 & 94) m/z (6.584) min
- Terpinen-4-ol (43,57,71,93,111 & 136) m/z , (6.733) min
- 3,7-Octadiene-2,6-diol, 2,6-dimethyl (43,59,82 & 109) m/z , (7.925) min

- Estragole (51,77,91,121 & 148) m/z , (6.974) min
- Fenchyl acetate (43,67,81,107,121 & 136) m/z (7.217) min
- 2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)- (41, 69, 93 & 123) m/z , (7.610) min
- 2,6-Octadienal, 3,7-dimethyl-, (E)- (41, 55, 69, 84, 107, 121 & 136) m/z , (7.872) min
- 1,7-Octadiene-3,6-diol, 2,6-dimethyl (43,67,82,107,121 & 135) m/z , (7.925) min
- Bornyl acetate (43, 69, 95, 121 & 154) m/z , 8.145 min
- Glutaric acid, 2,2-dichloroethyl geranyl ester (41, 69,93, 121 & 136) m/z (9.306) min
- Cyclohexane,methyl2,4bis(1methylethenyl) (41, 55,68, 81,94, 107, 121, 134 & 147) m/z , (9.572) min
- Methyl eugenol (37, 51, 77, 91,107, 137, 147, 163 & 178) m/z , (9.644) min

These compounds, which are contained in essential oils, are used for a variety of purposes, including nutritional and medicinal ones, and they support the general health of the entire plant. They can also interact synergistically due to the presence of different functional polyphenols. Some essential oils, particularly those with sweetening properties, are commonly used in a variety of products, including beverages, pills, baked goods, confectionery, and cosmetics.

Throughout history, plant essential oils and extracts have been employed for various purposes such as preservation, medicinal use, homeopathy, and ecological remedies. It is crucial to carefully study the plant species used in medicinal herbs to enhance patient outcomes. In this context, the analysis of Holy basil (*Ocimum sanctum*) essential oil using GC-MS revealed the presence of 240 components, with 56 of them identified. Essential oils have been utilized throughout history for religious, aromatic, and medicinal purposes, underscoring their enduring significance in human civilization.



Graph 18: Mass spectrum of Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)

Table 18: Fragmentation Pattern of Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)

m/z value of [fragment] ⁺	147	134	121	107	55	41
[molecular fragmen] ⁺	$[C_{11}H_{15}]^+$	$[C_{10}H_{14}]^+$	$[C_9H_{13}]^+$	$[C_8H_{11}]^+$	$[C_4H_7]^+$	$[C_3H_5]^+$

5. CONCLUSION

Holy basil (*Ocimum sanctum*) leaves were used to extract the essential oil, which was then dried using sodium sulfate dehydrate. This essential oil had a yield of 0.21 percent, and it had a distinct scent and appeared as a pale yellow liquid. It was found to be a complex mixture comprising 240 constituents, with 56 of them successfully identified. These constituents were grouped based on their mass-to-charge ratios (m/z) and retention times (R.T). Notable groups among these constituents included Benzen Chloro, D-Limonene, Eucalyptol, 2-Furanmethanol, Linalool, Fenchol, (+)-2-Bornanone, 2H-Pyran-3-ol, Terpinen-4-ol, 3,7-Octadiene-2,6-diol, Estragole, Fenchyl acetate, 2,6-Octadien-1-ol, 2,6-Octadienal, 1,7-Octadiene-3,6-diol, Bornyl acetate, Glutaric acid, Cyclohexane, and Methyl eugenol. Many of these oxygenated compounds found in the oil are recognized for their flavor-enhancing and therapeutic properties. Consequently, this essential oil holds economic potential as a valuable

product. Additionally, its low percentage of oxidized constituents makes it suitable for use in fragrance compound production.

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