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INVESTIGATION OF ESSENTIAL OIL COMPOSITION IN EUCALYPTUS OIL BY GC-MS METHOD

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ABSTRACT: Nowadays, the use of essential oils as alternative therapies has gained worldwide concern, owing to their various biological activities. Considerable attention has been devoted to essential oil, which is widely used for its important properties, including antimicrobial, anti-inflammatory, antispasmodic, cytoprotective, hepatoprotective, with strong antioxidant actions. Eucalyptol is an essential oil present in large amounts in a variety of plants, which is frequently used in the manufacture of cosmetics, to increase percutaneous penetration of drugs, as a nasal decongestant and anti-cough agent, and in aromatherapy. GC analysis of the essential oil was carried out on the Agilent 7820A system with a mass detector (MS) and a HP-5 MS column (30 m \times 0.25 mm, 0.25 μ m film thickness). Nineteen essential chemical constituents were identified based on GC-MS in Eucalyptus oil supplied from the pharmacy. The main constituents of Eucalyptus oil are eucalyptol (76.81%), γ -terpinene (7.75), α -pinene (6.62), and α -terpineol (1.44%). These four components account for 92.6% of the total relative content of the Eucalyptol oil. This research also demonstrated that eucalyptol oil is rich in eucalyptol -terpinene, and α -pinene could is a good source for these compounds.

INTRODUCTION: Eucalyptus **Fig. 1** belongs to the Myrtaceae family and comprises about 900 species¹. More than 300 species of this genus contain volatile oils in their leaves. Fewer than 20, within these species, known for their high content of eucalyptus (More than 70%), have been commercially used for the production of essential oils in pharmaceutical and cosmetic industries². Over the past few years, the interest in natural medicine has been increasing in industrialized society's particularly against microbial agents, because of the ever-growing problem of antibiotic resistance³. Consequently, the scientific interest in this field has been expanding.

Some researchers have demonstrated some efficacy of eucalyptus globulus essential oil against haemophilus influenza and stentrophomonas maltophilia³⁻⁵. Many studies reported the antifungal propriety of plant extracts and essential oils against dermatophytes, filamentous and candida albicans^{6, 7}. Herbal medicine, as a major part of traditional medicine, has been used in medical practice since antiquity and is a common element of ayurvedic, homeopathic, and naturopathic medicine.

World health organization notes that 74% of the plant-derived medicines are used in modern medicine, in a way that their modern application directly correlates with their traditional use as herbal medicines by native cultures^{8, 9}. Mass spectrometry coupled with chromatographic separations such as gas chromatography (GC-MS) is normally used for direct analysis of components existing in traditional medicines and medicinal plants.

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In this study, the nineteen chemical content of the essential oil composition in eucalyptus oil was determined by the GS-MS method.



FIG. 1: EUCALYPTUS LEAVES

MATERIALS AND METHOD:

Chemicals and Reagents: Analytical purity chemicals were used in the study. Eucalyptol, γ -terpinene, α -pinene, α -terpineol, β -pinene, α -terpinolen, linalyl acetate, linalool oxide, β -myrcene, p-cymenene, viridiflorol, linalool, caryophyllene, ledene, cis-sabinene hydrate, 4-terpineol, α -terpinyl acetate, ledol, and nerolidol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Eucalyptus oil was obtained from the pharmacy (Erzurum, Turkey).

GC-MS System: Chromatographic analysis was carried out on an Agilent 7820A gas chromatography system equipped with 5977 series mass selective detector, 7673 series autosampler and chemstation (Agilent Technologies, Palo Alto, CA). HP-5 MS column with 0.25 μ m film thickness (30 m \times 0.25 mm I. D., USA) was used for separation. The temperatures of the inlet, transfer line, and detector were 250, 250, and 300 $^{\circ}$ C, respectively.

GC-MS Conditions: Different temperature programs were investigated for GC-MS method. The end of this investigation, the temperature program of the GC/MS was as follows: initial temperature was 60 $^{\circ}$ C, held for 10 min, increased to 220 $^{\circ}$ C at a rate of 4.0 $^{\circ}$ C / min held for 10 min, increased to 240 $^{\circ}$ C at a rate of 1.0 $^{\circ}$ C / min and held for 1 min.

Identification of Components: Identification was based on the molecular structure, molecular mass, and calculated fragments.

Interpretation of mass spectrum GC-MS was conducted using the database on the National Institute of Standard and Technology (NIST), having more than 62,000 patterns. The name, molecular weight, and structure of the components of the test materials were ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The spectrum of the unknown component was compared with the spectrum of the component stored in the NISTL library Version (2005), Software, Turbo mass.

RESULTS AND DISCUSSION:

Method Development and Optimization: In this study, the GC-MS method was developed for the analysis of essential constituent sineucalyptu soil. The capillary column coated with 5% phenyl, 95% dimethylpolysiloxane is a good choice for separation of these analytes since they elute as symmetrical peaks at a wide range of concentrations. Different temperature programs were investigated for the GC oven. The end of this investigation, the best temperature program was selected for good separation. The temperature programs of the GC oven were as follows: the initial temperature was 60 $^{\circ}$ C, held for 10 min, increased to 220 $^{\circ}$ C at a rate of 4.0 $^{\circ}$ C/min held for 10 min, increased to 240 $^{\circ}$ C at a rate of 1.0 $^{\circ}$ C / min and held for 1 min. The split injection mode was chosen. The injector volume was 1 μ l in split (1:40) mode, and the carrier gas was helium at a flow rate of 0.8 ml/min.

GC-MS Analysis: The more precise information in qualitative analysis can be obtained by GC-MS. For quantitative determination, gas chromatography with flame ionization detector (GC-FID) and GC-MS are preferred. GC-MS is one of the best techniques to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols, acids, esters *etc.* The GC-MS analysis of Eucalyptu soil revealed the presence of nineteen compounds identification of the essential oil compounds.

The essential oil compounds were confirmed based on the retention time, molecular formula, compound name and mass spectrum. The essential oil compound sineucalyptu soil are presented as compound chromatogram is in **Table 1**.

Nineteen essential chemical constituents were identified based on GC-MS in eucalyptus oil supplied from the pharmacy. The identification of the main constituents eucalyptol (76.81), γ -terpinene (7.75%), α -pinene (6.62%) and α -terpineol (1.44%). These components account for 92.6% of the total relative content of the eucalyptus oil. This research also demonstrated that Eucalyptus oil is rich in eucalyptol could be a good source for these compounds.

TABLE 1: CHEMICAL COMPOSITION OF THE ESSENTIAL OIL IN EUCALYPTUS SOIL

| Peak | Retention Time (min) | Compound | % of Total |
|------|----------------------|----------------------------|------------|
| 1 | 3.24 | β -pinene | 0.99 |
| 2 | 3.54 | α -pinene | 6.62 |
| 3 | 3.60 | eucalyptol | 76.81 |
| 4 | 3.61 | cis-sabinene hidrate | 0.69 |
| 5 | 4.13 | γ -terpinene | 7.75 |
| 6 | 4.88 | linalool | 0.29 |
| 7 | 7.21 | 4-terpineol | 0.57 |
| 8 | 7.68 | α -terpineol | 1.44 |
| 9 | 9.34 | linalyl acetate | 0.15 |
| 10 | 11.45 | β -myrcene | 1.17 |
| 11 | 14.87 | caryophyllene | 0.09 |
| 12 | 16.35 | linalool oxide | 0.07 |
| 13 | 17.26 | α -terpinolen | 0.37 |
| 14 | 17.46 | p-cymenene | 0.08 |
| 15 | 20.45 | viridiflorol | 0.21 |
| 16 | 28.06 | α -terpinyl acetate | 0.08 |
| 17 | 32.97 | ledene | 0.05 |
| 18 | 36.50 | ledol | 0.04 |
| 19 | 37.80 | nerolidol | 0.03 |
| | | Total | 97.5 |

Eucalyptol is an essential oil present in large amounts in a variety of plants, which is frequently used in the manufacture of cosmetics to increase percutaneous penetration of drugs, as a nasal decongestant and anti-cough agent, in aromatherapy, and in dentistry^{10, 11}.

Eucalyptol has been used to treat bronchitis, sinusitis, and chronic rhinitis and also for the treatment of asthma¹². These actions seem to be related to an anti-inflammatory action¹³ inhibiting the production of tumor necrosis factor-alpha¹⁴. γ -Terpinene is a volatile essential oil. It shows antimicrobial properties against various human pathogens. It is also known as antioxidant, anti-inflammatory, and anti-proliferative activities. α -pinene is the major monoterpene of essential pine oils and a hydrocarbon group of bicyclic terpenes with a strong turpentine odor.

It has been widely used as a food flavoring ingredient¹⁶. In addition, a number of studies have attributed biological properties, including antimicrobial¹⁷, hypertensive¹⁸, and anti-inflammatory¹⁹ effects to α -pinene.

CONCLUSION: The main components of oils can often be identified from the peak pattern of the chromatograms obtained directly from GC-MS analysis. Similarly, unique qualitative and quantitative patterns from a GC analysis will often help identify the source of many oils. The construction of chromatographic fingerprints aims at evaluating the quality of essential oil.

The importance of the study is due to the biological activity of some of these compounds. The present study, which reveals the presence of essential components in eucalyptus soil, suggests that the contribution of these compounds on the pharmacological activity should be evaluated

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CONFLICTS OF INTEREST: Nil

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