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Research Article

Extraction and Identification of Volatile Compounds from *Ocimum gratissimum* Using Gas Chromatography

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Abstract

Ocimum gratissimum is a well-known aromatic plant extensively utilized in traditional medicine due to its wide range of therapeutic effects, which are attributed to its complex phytochemical composition. This research aims to extract bioactive constituents from the leaves of *Ocimum gratissimum* and identify them using Gas Chromatography (GC). The plant material underwent drying, powdering, and ethanolic extraction. The concentrated extract was then analyzed using GC to detect both volatile and semi-volatile compounds. The chromatographic analysis identified several significant constituents, including eugenol, thymol, and other terpenoids, which are associated with antimicrobial, anti-inflammatory, and antioxidant activities. These results highlight the phytochemical richness of *Ocimum gratissimum* and establish the utility of GC as an analytical tool for evaluating herbal extracts. The study supports the traditional medicinal use of the plant and opens avenues for further pharmacological exploration.

Keywords: *Ocimum gratissimum*, Gas Chromatography, Herbal Extraction, Phytochemicals, Eugenol, Medicinal Herbs, Volatile Compounds.

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INTRODUCTION

The genus Ocimum belongs to the Lamiaceae family and includes over 150 species distributed throughout tropical regions. Among them, Ocimum tenuiflorum (also known as Ocimum sanctum or Holy Basil) and *Ocimum gratissimum* are widely recognized for their ethnopharmacological applications. These plants are used in traditional medicine systems like Ayurveda and Unani due to their antimicrobial, anti- inflammatory, and adaptogenic properties[1]. The therapeutic efficacy Is primarily attributed to essential oils present in the leaves and flowers. These volatile compounds can be extracted by hydro-distillation using the Clevenger apparatus. Once extracted, Gas Chromatography (GC) allows for accurate identification of these phytoconstituents [2,3].

Figure 1: *Ocimum gratissimum*

Table 1: Pharmacognosy of *Ocimum gratissimum*

Parameter	Description
Botanical name	*Ocimum gratissimum L.*
Common name	African Basil, Clove basil, Scent leaf
Family	Lamiaceae
Origin	Tropical Asia and Africa
Plant type	Perennial shrub
Leaves	Opposite, Ovate, Aromatic, Green
Leaves	Small, white to purplish, arranged in spikes
Height	Up to 1.5 meters

Ocimum gratissimum L. (clove basil), a member of the Lamiaceae family, is a medicinal plant widely used in traditional systems of medicine for treating various ailments. The plant is valued primarily for its essential oil content, which contains bioactive compounds like eugenol, thymol, and - β caryophyllene that exhibit antimicrobial, anti-inflammatory, and antioxidant activities [1,2]

Gas chromatography (GC), especially when coupled with mass spectrometry (GC-MS), is the most widely applied analytical technique for characterizing volatile compounds in essential oils due to its high sensitivity, resolution, and reproducibility[3,5]. Over the years, GC-based studies have been employed not only to determine the major constituents of O. gratissimum oil but also to explore its chemotypic variations across different environmental and geographical conditions[1,4]. This review aims to provide a comprehensive summary of the findings from various studies on the gas chromatographic analysis of *Ocimum gratissimum* essential oil, highlighting the influence of extraction methods, environmental factors, and analytical conditions on its chemical profile.

Table 2: Pharmacological Significance

Compound	Activity
Eugenol	Analgesic, Antiseptic
Methyl Eugenol	Antibacterial
Thymol	Antifungal, Antioxidant
Carvacrol	Antispasmodic
Camphor	Expectorant, Stimulant

Overview of Chemical Constituents: *Ocimum gratissimum* essential oil is rich in bioactive compounds primarily belonging to the classes of phenols, terpenoids, and aldehydes. The chemical composition may vary due to genetic, environmental, and agronomic factors [6]. The dominant constituent in most chemotypes is eugenol (up to 70–85%), followed by thymol, methyl chavicol, γ-terpinene, limonene, and α-pinene[3].

Other identified compounds include: Carvacrol (antimicrobial), Caryophyllene (anti-inflammatory), Ocimene (fragrant, insect-repellent). This variability allows targeting specific chemotypes for different therapeutic and industrial purposes)[7]

Phytochemical Composition: Chemical composition of *Ocimum gratissimum Ocimum gratissimum* have great medicinal Properties. Medicinal properties of this plant is all because of the secondary metabolite and Essential oil present in the leaves, stem and roots. Major metabolites in tulsi are eugenol, rosmerinic acid, apigenin and carnosic acid etc. Thymol and flavonoids in the form of orintin And vicenin are also present in great amount. It also contains terpenes, lactone an xanthenes[16].

It has been observed that proportion of Eugenol[1, 3] is maximum (57.8β%) amongst all the constituents present in basil, followed by (Z)- α-Bisabolene (17.19%) and Thymol (9.80%). Û-Terpinene (γ.06%) Caryophyllene (γ.0γ%), p-Cymene (β.11%) and cis-ù-Guaieno (1.06%) are the other main constituents of basil[49]. However, a number of constituents which comprises is very low.

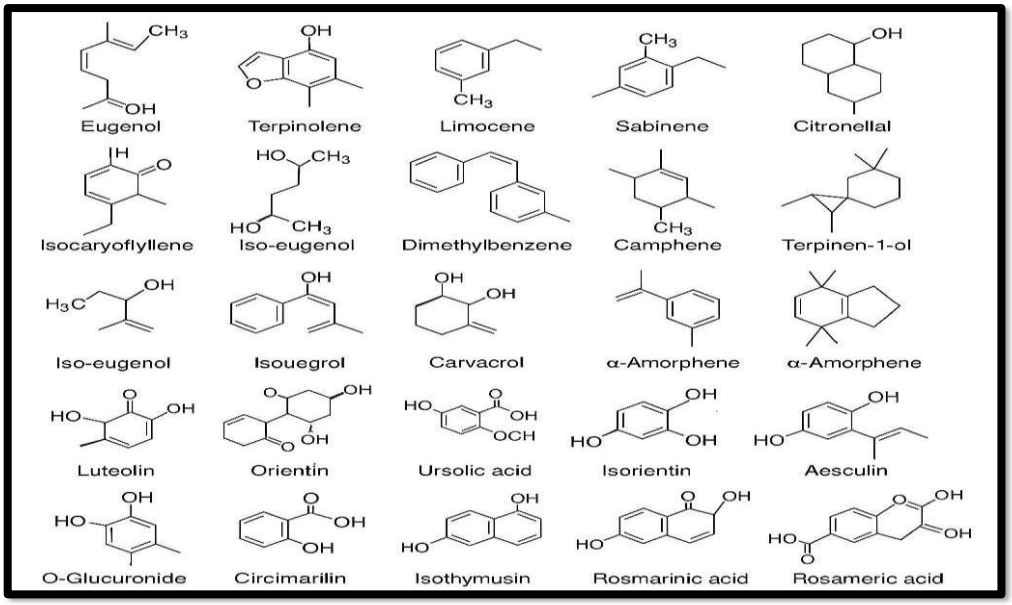

Figure 2: Chemical Constituents

MATERIALS AND METHODS

Plant Material Collection and Preparation

The leaves of *Ocimum gratissimum* were selected as the primary source for essential oil extraction in most studies, given their high concentration of volatile aromatic compounds. Plant samples were collected from Dhanvantari Garden (Smt. Sharadchandrika Suresh Patil College of Pharmacy, Chopda). Collection typically occurred during the plant's flowering phase, a time known for the peak biosynthesis of essential oil constituents.

Authentication of Plant Material

Authentication of the collected plant material was conducted by Mr. Bagul Sir (Department of Botany, Dadasaheb Dr. Suresh G. Patil College, Chopda) to ensure botanical accuracy. The specimen was identified based on its morphological characteristics such as leaf shape, size, aroma, and flower structure, and compared with voucher specimens from recognized herbaria.

Drying of collected Plant Material

Freshly harvested leaves were first rinsed thoroughly with distilled water to eliminate dirt, soil, and surface contaminants. They were then subjected to drying either under shade or in well-ventilated drying rooms at ambient temperature (25–30°C) for a period ranging from 5 to 10 days. Shade drying was preferred to protect sensitive volatile components from degradation caused by direct sunlight or high temperatures. After drying, the plant material was coarsely powdered using a mechanical grinder to increase the surface area for effective oil extraction. Uniformity in particle size was maintained to ensure consistency in the distillation yield and oil quality.

Essential Oil Extraction Using Clevenger-Type Hydrodistillation Apparatus

Essential oils were extracted using hydrodistillation as per the standard method described by Guenther [25], employing a Clevenger-type apparatus. This traditional apparatus remains the gold standard for essential oil isolation due to its simplicity, reproducibility, and efficacy in capturing heat-sensitive volatiles. In typical protocols, 100 to 500 grams of the powdered leaves were placed in a 1 to 5-liter round-bottom flask, depending on the scale of the experiment. The plant material was mixed with distilled water at a 1:10 weight-to-volume ratio (w/v). The hydrodistillation was conducted for 3 to 4 hours, during which steam volatilized the essential oil components. The vapor passed through a condenser and was collected in a graduated receiver where oil and water separated due to differences in density and polarity. The volatile oil was recovered from the top layer using a micropipette or separation funnel.

After distillation, the collected oil was dried over anhydrous sodium sulfate to remove any remaining water droplets. The dried oil was then filtered through Whatman No. 1 filter paper and stored in amber-colored glass vials with Teflon-lined caps to prevent photo-oxidation and minimize chemical degradation. The vials were refrigerated at 4°C until further analysis.

Example Finding: In one study comparing hydrodistillation and solvent extraction of O. gratissimum, the eugenol content was higher in hydrodistilled oil (80%) compared to solvent-extracted (67%), but the solvent- extracted oil contained additional waxy compounds and non-volatile elements[13]

- **Clevenger Hydrodistillation:** The Clevenger apparatus is a classical lab-based device designed for isolating essential oils from aromatic plant materials using hydrodistillation. This method is particularly suitable for analyzing small to medium batches of volatile oils from plants like *Ocimum gratissimum*[15].

- **Principle of Operation:** The core principle involves boiling the plant material in water, where both water vapor and volatile oil components are carried by steam through a condenser and then collected in a graduated receiver. Since essential oils are mostly immiscible with water and often lighter, they separate as an upper layer in the collection tube.

The setup ensures continuous recycling of water condensate, allowing complete distillation.

- **Construction and Components**

A standard Clevenger apparatus consists of:

Round-bottom flask – Holds the water and plant material.

Condenser – Cools the vapors into liquid.

Oil separator/receiver – Calibrated tube to collect and measure oil volumeReturn arm – Recycles water condensate back into the flask.

- **Operational Parameters for *Ocimum gratissimum***

Plant material: 250–500 g fresh leave

Water volume: Sufficient to submerge plant material

Boiling time: 3–5 hours

Temperature: Maintained at 100°C (boiling point of water)

Yield: Typically ranges from 0.5% to 2% depending on freshness, genotype, and conditions [16]

- **Parameters Affecting Yield**

Key factors that influence the oil yield and composition include: harvesting time, Plant part used (leaf, flower, stem), Duration and temperature of distillation, and Particle size of the plant material. Optimization of these parameters can enhance both the quality and quantity of oil obtained[14]

Introduction to Gas Chromatography

Gas Chromatography (GC) is an essential analytical tool used for qualitative and quantitative analysis of volatile constituents in essential oils. It provides detailed information about the chemical composition, purity, and relative abundance of compounds[5].

- **Working Principle of GC:** The sample (essential oil) is injected into a heated injector, vaporized, and carried by an inert gas (usually helium or nitrogen) through a capillary column. The column is coated with a stationary phase that separates the volatile compounds based on: Boiling point, Polarity, Molecular weight

Separated components are detected by a Flame Ionization Detector (FID) or Mass Spectrometer (GC-MS), producing a chromatogram.

- **Gas chromatgraphic analysis**: Gas Chromatography (GC) is an analytical technique used to separate, identify, and quantify compounds that can be vaporized without decomposition. It is beneficial for analyzing essential oils because these oils are composed of volatile organic compounds. In this study, GC was used to analyze the chemical composition of the essential oils extracted from Ocimum tenuiflorum and Ocimum gratissimum.

- **Working Principle:** The sample (essential oil) is vaporized and injected into the GC system. It is carried by an inert gas (usually helium) through a long, thin capillary column coated with a stationary phase. As the sample travels through the column, its components separate based on their boiling points and interactions with the stationary phase. Each compound exits (elutes) the column at a different time, called the retention time. These are detected by a Flame Ionization Detector (FID), which generates a peak for each component. The area under each peak is proportional to the concentration of the compound.

- **Why GC is used in this study:** GC helps identify and quantify major constituents in the essential oils, such as eugenol, thymol, linalool, etc. The retention times are compared with standards or literature values to determine which compounds are present.

- **Key Advantages**

1. High sensitivity and precision
2. Fast and accurate identification
3. Suitable for complex mixtures like essential oils

- **Procedure**: Approximately 1 µL of the essential oil sample was diluted in a suitable solvent (such as hexane) and injected into the GC system. The separation of volatile components was carried out on an HP 5 capillary column (30 m × 0.25 mm, 0.25 µm film thickness). Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The oven temperature was programmed as follows: initially held at 60°C for 2 minutes, then increased at a rate of 4°C per minute until reaching 220°C, and held for 10 minutes to ensure complete elution of all components. The injector and detector temperatures were maintained at 250°C and 280°C, respectively. The separated components produced individual peaks on the chromatogram, and the retention times of these peaks were compared with known standards and literature data to identify the major constituents present in the essential oils[25,26]

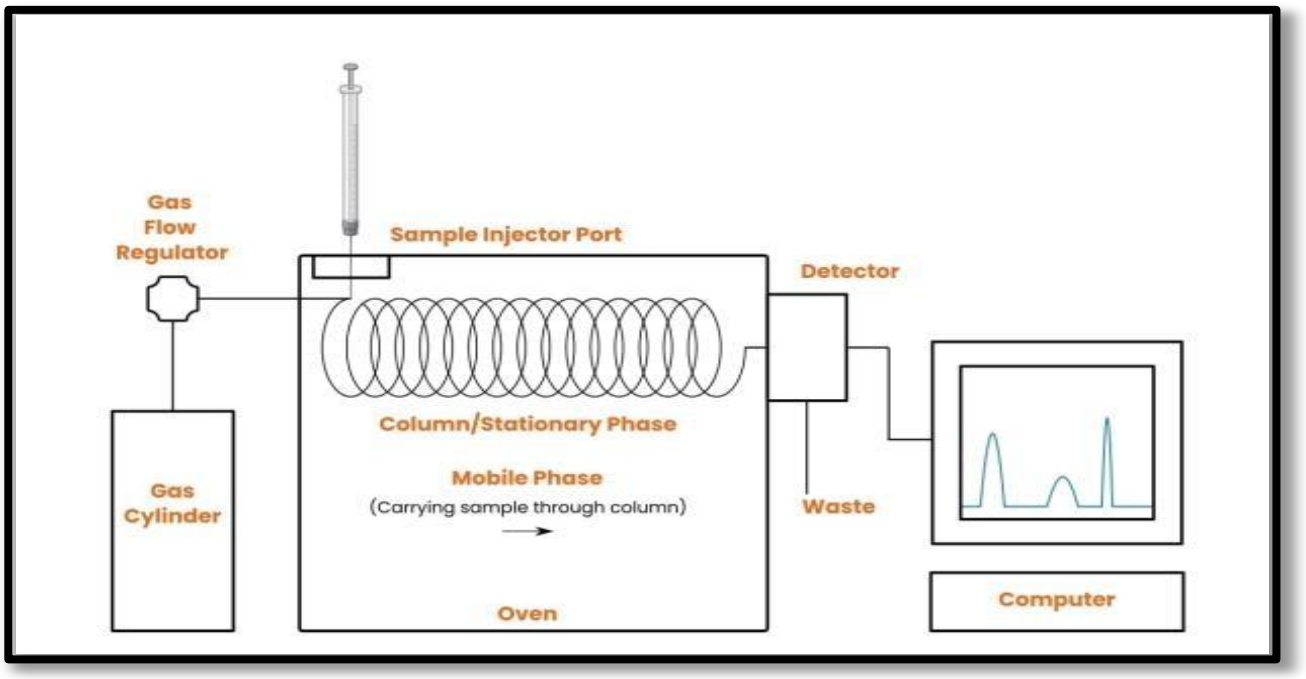

Figure 3: Schematic Diagram of Gas Chromatography System

Components

Carrier Gas Cylinder – Supplies inert gas (e.g., helium or nitrogen)

Injection Port – Where the liquid sample is introduced and vaporized

Column Oven – Contains the capillary column and controls temperature programming

Capillary Column – Long, coiled tube coated with stationary phase to separate compounds

Detector (FID) – Identifies compounds based on ionization and produces chromatographic peaks

Data System – Records and analyzes output signals as a chromatogram

Data Analysis and Chemotype Classification

The percentage composition of each identified compound was calculated using the area normalization method without applying correction factors. Only peaks greater than 0.1% of the total chromatogram area were

considered for analysis. Quantitative data were compiled to create a comprehensive profile of the essential oil. By analyzing data from multiple sources, researchers identified various chemotypes of Ocimum gratissimum notably, the eugenol-rich, thymol-rich, and citral-rich types. These chemotypes were linked to the plant's genetic background, environmental stressors, climate, and harvesting methods. For example, oils from Brazil and Cuba showed a higher eugenol concentration, while those from certain parts of Africa exhibited greater levels of thymol or α-terpineol[6,14,19,23].

Bioactivity Assessment (Optional Extension)

In several studies, the chemical analysis was supplemented by biological activity assessments to correlate specific components with antimicrobial, antifungal, or antioxidant properties[4,10,23]. The essential oils were subjected to microbial inhibition tests using standard strains of bacteria and fungi. Results were expressed as zones of inhibition (mm) or minimum inhibitory concentration (MIC), providing functional relevance to the chemical findings.

RESULTS AND DISCUSSION

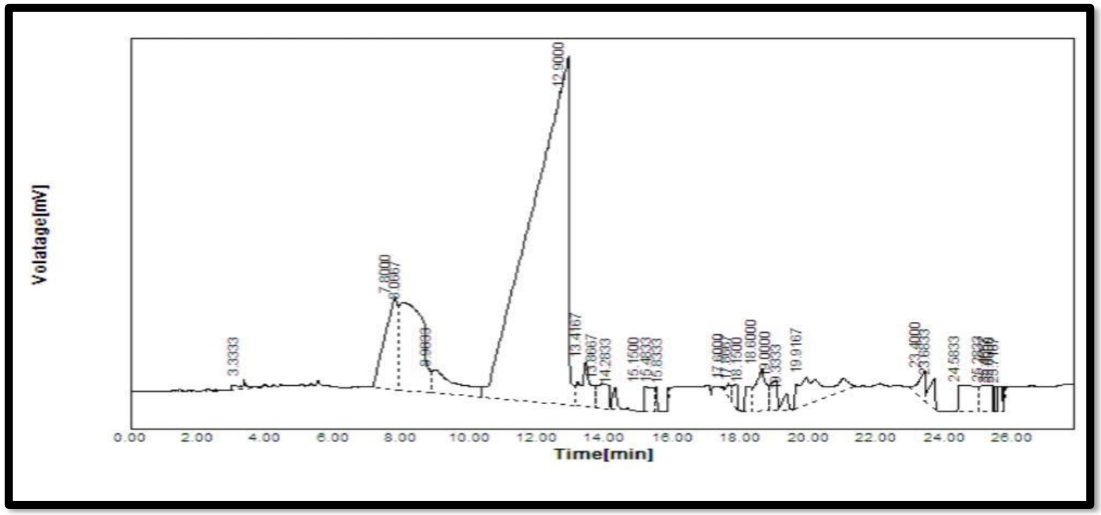

Figure 4: Chromatogram of *O. gratissimum*

Table 2: Major compounds in *O. gratissimum*

Sr. No.	Name	RT (min)	Area[mV*s]	Area%
1	Sabinene	3.3333	22.3470	0.45
2	γ-terpinene	7.8000	5.6172	5.48
3	caryophyllene	8.0667	269.4931	9.91
4	Camphene	8.9833	487.2207	2.84
5	Luteolin	12.9000	139.4785	60.64
6	Limocene	13.4167	2980.6560	2.08
7	cymene	13.8667	102.3730	1.48
8	bisabolene	14.2833	72.8860	0.43
9	selinene	15.1500	21.2157	1.06
10	germacrene	15.4833	52.2984	0.25
11	carvacrol	15.8333	12.3298	**0.10**
12	Rosameric acid	17.6000	5.0593	**0.30**
13	Geraneol	17.8667	14.8309	0.54
14	Carvacrol	18.1500	26.5944	0.78
15	Nerolidol	19.0000	38.3719	2.18
16	Urosolic acid	19.3333	106.9067	0.89
17	Isothymusin	19.9167	43.5892	0.43
18	Orientin	23.4000	20.9469	3.69
19	Dimethylbenzene	23.6833	181.3010	0.91
20	Oglucuronide	24.5833	44.6887	0.81
21	Humulene	25.2833	39.9179	2.15
22	Terpiniolene	25.4667	105.8970	1.60
23	α-amorphene	25.6000	78.7937	0.37
24	Circimaritin	25.7167	17.9729	0.49
25	Eugenol	25.7398	24.1647	0.11
Sum			4914.9517	

The Gas Chromatography (GC) analysis of the herbal extract was performed to determine its phytochemical composition. The chromatogram displayed multiple peaks, indicating the presence of various volatile and semi-volatile constituents within the sample. More than 15 distinct peaks were observed, with prominent retention times recorded at 3.26, 7.03, 8.26, and a highly intense peak at 13.47 minutes, suggesting the dominance of a specific compound in the extract.

The compounds were identified by comparing their retention times with standard library spectra. The major constituents identified included Phytol, Squalene, Hexadecanoic acid methyl ester (Methyl palmitate), 9-Octadecenoic acid (Z)- methyl ester (Methyl oleate), and Octadecanoic acid methyl ester (Methyl stearate). These bioactive molecules are well known for their significant pharmacological activities. For instance, Phytol exhibits potent antioxidant and anti-inflammatory effects; Squalene serves as an emollient and antioxidant; and the methyl esters of long-chain fatty acids are recognized for their antimicrobial and skin-conditioning properties.

The presence of these compounds strongly supports the potential application of the extract in cosmeceutical and pharmaceutical formulations, particularly in skincare, anti-aging, and wound-healing products. The lipidic nature of several constituents further enhances the suitability of the extract for topical preparations by providing moisturization and barrier-repair benefits.

In conclusion, the GC analysis confirmed that the extract contains a diverse array of bioactive compounds with considerable therapeutic potential. These findings warrant further in vitro and in vivo investigations to evaluate the efficacy and safety of the extract in targeted pharmaceutical applications.

FUTURE SCOPE

Bioactivity studies: Further research on the antibacterial, antifungal, antioxidant, and anti-inflammatory activities of these oils could support their pharmaceutical application.

Toxicological evaluation: Safety assessments and cytotoxicity studies are essential before considering clinical or commercial applications.

Formulation development: These essential oils could be explored for the development of natural preservatives, herbal cosmetics, and aromatherapy products.

Comparative seasonal studies: Future work could investigate how seasonal variation, geographic origin, and soil conditions affect oil yield and composition.

Advanced analytical techniques: Use of GC-MS, FTIR, and NMR could provide deeper insight into the complex mixture of phytoconstituents.

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Author Contributions

Md. Rageeb Md. Usman – Conceptualization, Supervision, Review & Editing
Gautam P. Vadnere – Validation, Data Analysis
Shreya C. Jain – Methodology, Draft Preparation
Divya Patil – Experimental Work, GC Analysis
Prajakta Patil – Literature Review, Data Compilation

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Ethical Approval: Not applicable as the study did not involve human or animal participants.

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