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Research Article

Chemical Composition and GC-MS Analysis of Essential Oil from *Cymbopogon martinii var sonfia*

Md. Rageeb Md. Usman *, Gautam P. Vadnere, Shreya C. Jain, Jaydip Wahle, Ganesh Dhondkar, Suraj Ingle

Smt. Sharadchandrika Suresh Patil College of Pharmacy, Chopda, Maharashtra, India

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Abstract

Cymbopogon martinii, commonly referred to as palmarosa, is a perennial aromatic grass from the Poaceae family, known for its valuable essential oil rich in geraniol. This oil is in high demand due to its fragrance and potential therapeutic applications. Its versatility enables use in perfumery, cosmetics, and natural remedies. The growing interest in natural products has led to a surge in studies aimed at improving extraction methods and understanding the phytochemical makeup of such oils. The present work aims to extract essential oil from *C. martinii* var. *sofia* using steam distillation and to evaluate its chemical composition through GC-MS analysis, thereby determining its quality and commercial viability.

Keywords: *Cymbopogon martinii* var. *sofia*, essential oil, steam distillation, geraniol, GC-MS analysis, phytochemical constituents.

*For Correspondence: Md. Rageeb Md. Usman, Smt. Sharadchandrika Suresh Patil College of Pharmacy, Chopda, Maharashtra, India

INTRODUCTION

Cymbopogon martinii var. *Sofia (*Family Poaceae) commonly known as palmarosa yields an essential oil rich in geraniol. Palmarosa oil is of commercial importance being extensively used in perfumes soaps cosmetics toiletry and tobacco products.[1] It is used mainly as asource of high-quality geraniol which is used in high grade perfumes and cosmetics[2]. The characteristic odour of palmarosa oil is due to its high content of total alcohol mainly geraniol and a small but varying amount of esters associated with geraniol[3]. The trace constituents present in the oil are responsible for the characteristic olfactory note of the palmarosa oil. There are several reports regarding the chemical composition of palmarosa oil.[4]

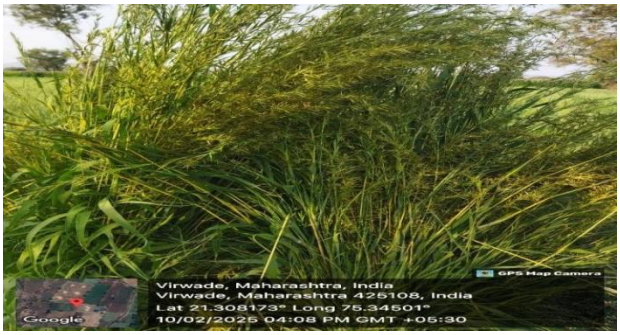

Figure 1: Diagram Palmarosa Plant

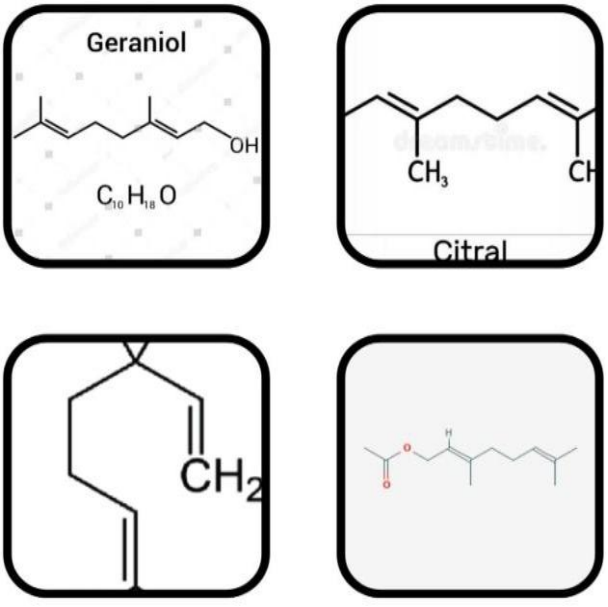

Figure 2: Structure of Chemical Constituents

Table 1: Pharmacognosy of *Cymbopogon martinii* var. *sofia*

Kingdom	Plantae
Clade	Tracheophytes
Clade	Angiosperms
Clade	Monocots
Clade	Commelinids
Odour	Poales
Family	Poaceae
Subfamily	Panicoideae
Genus	Cymbopogon
Family	C. martinii

MATERIALS AND METHOD

Materials needed

1) Fresh or dried Palmarosa plant
2) Distillation apparatus (steam distillation unit)
3) Water (preferably distilled water)
4) Condenser
5) Receiving flask
6) Separation funnel (optional)
7) Ice (optional for cooling)
8) Collection bottles (for storing the essential oil)

Collection of plant material: Plant of *Cymbopogon martinii* var. *sofia* Palmarosa were collected from Swami Herbs Virwade washed with sterile water and dried in shades. Then the samples were powered in mechanical grinder[10]

Extraction of Palmarosa plant

The extraction of Palmarosa plant (essential oil from *Cymbopogon martinii* var. *sofia*, commonly known as holy basil or Palmarosa) is typically done using steam distillation, as it is the most effective method for obtaining essential oils from aromatic plants. This steam distillation method ensures a pure and potent palmarosa essential oil that retains most of the plant's beneficial properties[10,11]. Below is a step-by-step guide for extracting palmarosa oil through this method:

Steps for Steam Distillation

1. Preparation of Plant Material

Harvesting: Choose fresh, young palmarosa plant for the best oil yield and quality. Dried leaves can also be used, though fresh leaves tend to yield more essential oil.

Cleaning: Wash the leaves thoroughly to remove any dirt or contaminants.

Cutting: Gently chop or bruise the leaves to release their essential oils more easily during distillation. This step can help increase the surface area of the leaves, improving extraction efficiency[11]

2. Setting up the Distillation Apparatus

Place the distillation unit (typically consisting of a boiling flask, condenser, and receiving flask) on a stable surface, preferably with heat control. Add water to the boiling flask, just enough to generate steam but not so much that it overflows. Place the chopped palmarosa plant into the distillation chamber. Ensure that the plant material is not submerged in the water, as the steam will pass through the leaves to extract the essential oil[12].

3. Initiating the Steam Distillation

Heat the water in the distillation unit. As the water heats up, it will begin to produce steam. The steam passes through the palmarosa plant, breaking the oil glands and releasing the volatile essential oils into the steam. The steam, along with the essential oils, travels through the condenser where it cools and condenses into liquid form[13].

4. Collection of Distilled Oil

The condensed mixture of water and oil flows into the receiving flask. Since essential oils are less dense than water, they will float on top of the water. The water collected in the flask is called hydrosol, and the essential oil floats on top. The oil can be separated from the water using a separating funnel or by decanting the oil carefully[14].

5. Post-Distillation Steps

After the distillation process, allow the oil to cool to room temperature. Filter the oil to remove any remaining plant debris if necessary.

Storage: Store the palmarosa essential oil in an airtight, dark glass bottle to protect it from light and oxidation. Keep it in a cool, dark place to maintain its potency[14,15].

Figure No. 3: Steam Distillation of Palmarosa plant

Characterization of Palmarosa oil

1. Qualitative phytochemical analysis

The extract was tested following standard biochemical methods as described below.

Test for proteins

Biuret's test: 2ml of Biuret reagent was added to 2ml of extract. The mixture was shaken well and warm for 5 min. Appearance of red or violet colour indicated presence of proteins. **Million's test:** Crude extract was mixed with 2ml of Millon's reagent, if precipitate appeared which turned red on gentle heating confirmed the presence of protein.

Ninhydrin test: Crude extract was mixed with 2 ml of 0.2% solution of Ninhydrin and boiled for some time, if violet colour appeared indicating the presence of amino

acids and proteins[17].

Test for carbohydrates

Fehling's test: Equal amount of Fehling A and Fehling B reagents were mixed and 2ml of it was added to the plant extract and then gently heated the sample. Appearance of brick red precipitate indicated the presence of reducing sugars.

Benedict's test: Crude extract when mixed with 2ml of Benedict's reagent and boiled, a reddish brown precipitate formed which indicated the presence of the carbohydrates.

Molisch's test: 2ml of Molisch's reagent was added to 0.5 ml of crude extract and the mixture was shaken properly. After that, 2ml of concentrated H2SO4 was poured carefully along the side of the test tube. Appearance of a violet ring at the interface indicated the presence of carbohydrate**.**

Iodine test: 2ml of iodine solution was mixed with 0.5 to 1 ml of crude extract. A dark blue or purple coloration indicated the presence of the carbohydrate[18].

Test for phenol: 2 ml of alcohol and 2-3 drops of ferric chloride solution was added to 1 ml of crude extract, blue-green or black coloration indicated the presence of phenols.

Test for tannin: 1 ml of distilled water and 2-3 drops of ferric chloride solution was added to 0.5 ml of crude extract. A black coluration indicated the presence of tannin[17,18].

Test for flavonoids

Shinoda test: Crude extract was mixed with small amount of magnesium and concentrated HCl was added drop wise. Appearance of pink scarlet colour after few minutes indicated the presence of flavonoids.

Alkaline reagent test: 0.5 ml of crude extract was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

Test for saponins: 1ml of crude extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins[17].

Test for glycosides

Liebermann's test: Crude extract was mixed with each of 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully concentrated H2SO4 was added. If colour change from violet to blue to green which indicated the presence of steroidal nucleus, i.e., glycone portion of glycoside.

Salkowski's test: 2ml of chloroform was mixed with crude extract. Then 2ml of concentrated H2SO4 was added carefully and shaken gently. A reddish brown colour indicated the presence of glycoside.

Keller-kilani test: 0.5 ml of crude extract was mixed with 2ml of glacial acetic acid containing 2-3 drops of 2% solution of FeCl3. Then 2ml of concentrated H2SO4 was poured into the mixture. A brown ring at the interface indicated the presence of cardiac glycosides[18].

Test for steroid

(i) 2ml of chloroform was added to the crude extract of Tulsi. Then 2ml of each of concentrated H2SO4and acetic acid were added into the mixture. The presence of steroids was indicated by appearance of a greenish coloration in the reaction mixture.

(ii) Crude extract was mixed with 2ml of chloroform and gently added concentrated H2SO4. A red colour was seen in the lower layer this indicated the presence of steroids.

Test for terpenoids: Crude extract was mixed in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H2SO4 was added and heated for about 2 minutes. Presence of terpenoids was indicated by a greyish colour at the interface[17].

Test for alkaloids: 2ml of 1% HCl was mixed with crude extract and heated gently. After heating, Mayer's And Wagner's reagents were added to the mixture. If precipitate was observed in the reaction mixture which indicated the presence of alkaloids.

Test for anthraquinone: 5ml of chloroform and 5 ml of ammonia solution was added to 0.2 gm of plant extract. Appearance of pink, red or violet colour indicated the presence of anthraquinone.

Oils & Fats: A small quantity of crude extract was pressed between two filter papers separately. An oily appearance on filter paper indicated the presence of fixed oil and fats.

Test for lactones

Baljet's test: Crude extract was treated with sodium picrate solution. Presence of lactone was observed by appearance of yellow to orange colour in the mixture[18].

2. Physical Parameters Analysis

a) Organoleptic:

The physical properties of PEO were observed without changing the identity. Physical properties include smell, taste, and color. The determination of color was carried out by taking a sample of 10mL into a test tube, leaning it on a whiteboard, and observing directly[17].

b) Determination of Relative Density

The pycnometer is filled with distilled water at a temperature of 25±0.2ºC, closed, and weighed. The pycnometer is emptied, washed, and dried. The pycnometer was filled with 1mL of PEO at a temperature of 25±0.2ºC, closed, and weighed, calculating the specific density of PEO[16].

c) Determination of Refractive Index

Dripped oil on the prism at a temperature of 20°C, then adjusted the slides, a clear dark and bright outline was obtained[16].

d) Determination of Solubility in Alcohol

A total of 1mL of PEO, 70% ethanol is added drop by drop at 20°C, and each addition is shaken until the solution is as clear as possible, if the solution is not clear, compare it with the turbidity in 70% ethanol[17].

e) Determination of Acid Value

PEO (0.5mg) dissolved in 10ml of ethanol and 2-3 dropsof PP, then titrated with a standard 0.1N potassium hydroxide solution[19].

f) Determination of Esters Value

25ml of 0.5 N KOH in alcohol, then reflux for 1h. After that, add 10ml of distilled water. Add a few drops of PP and titrate it against 0.5 N HCl[18].

g) Determination of Optical Rotation

2 dm-long polarimeter tube was read at 20°C using D-line polarized sodium light. Different concentrations of oil solutions were prepared in ethanol[18].

h) Determination of Iron

The determination of iron (Fe) was carried out using dry destruction analysis. A sample of 0.5 grams was weighed in a porcelain dish and heated to 800°C for 2 hours in a furnace. The sample was then stored in the furnace for an additional hour. After the cold sample was added 5ml of concentrated HNO3 and heated until dissolved and cooled. After that, 10ml of distilled water was added,bfiltered with Whatman paper, and then analyzed by an AAS (Atomic Absorption Spectrophotometer)[19].

Quantitative analysis of phytochemical in the plant extract Determination of total phenolic contents

The amount of total phenol for aqueous, methanol and ethanol extract were determined by Folin Ciocalteu reagent method. 2.5 ml of 10% Folin- Ciocalteu reagentand 2 ml of 2% Na2Co3 were added to 0.5 ml of plant extract. The mixture was then incubated at room temperature for 30 minutes. Gallic acid was used as standard (1mg/ml). The absorbance of the sample was measured at 765nm. All the tests were done in triplicates and the results were determined from standard curve and were expressed as gallic acid equivalent (mg/g of extracted compound)[20].

Determination of alkaloid

5 g of the sample was taken and 200 ml of 10% acetic acid in ethanol was added to the sample and allowed to stand for 4 hours. Then the solution was filtered and the extract was concentrated on water bath Conc. $NH_4(OH)$ was added drop wise and the whole solution was allowed to settle and the precipitate was then washed with dilute ammonium hydroxide and filtered. The residue was dried and weighed and this was the amount of alkaloid present in the plant material. 10 g of plant sample was taken and extracted repeatedly with 100ml 80% methanol. Then the solution was filtered and the filtrate was transferred into an empty crucible and evaporated into dryness over water bath and weighed. The final weight dry weight was amount of flavonoids in the plant sample[21].

Preparation of stock solution

The extracts were reconstituted in methanol. Methanolic extracts (1 µl) were injected for GC- MS analysis.

Gas Chromatography-Mass Spectrometry analysis

Table 2: Chromatographic Conditions

Parameter	Gas Chromatography	Gas Chromatography–Mass Spectrometry
Instrument	Varian Star 3400CX GC with FID	Hewlett–Packard 5890 GC with HP-5970 MSD
Detector Type	Flame Ionization Detector (FID)	Mass Selective Detector (MSD), EI Mode
Column	Supelcowax-10 (Carbowax 20M)	HP-1 (Methyl silicone)
Column Dimensions	30 m × 0.25 mm × 0.25 µm film thickness	25 m × 0.25 mm × 0.25 µm film thickness
Carrier Gas	Nitrogen (1 mL/min)	Helium (1 mL/min)
Temperature Program	80°C (2 min) to 220°C (5 min) at 7°C/min	60°C to 220°C at 5°C/min
Injector Temperature	200°C	250°C
Detector / Interface Temperature	240°C	280°C
Sample Volume	0.1 µL	0.1 µL
Split Ratio	1:50	1:50
Retention Indices	Standard n-alkanes (C8–C23)	Not specified
Mass Spectra Range	Not applicable	40–400 amu
Scan Speed	Not applicable	1 scan/sec
Ionization Energy	Not applicable	70 eV
Ion Source Temperature	Not applicable	250°C
MS Detector Temperature	Not applicable	150°C
Quantification	GC peak area (no FID response factor correction)	Not specified

RESULT AND DISCUSSION

The gas chromatographic analysis of the volatile oils extracted from Cymbopogon martini revealed a complex mixture of monoterpenes and sesquiterpenes. The major constituents identified based on retention times and comparison with reference standards included geraniol, linalool, citronellol, nerol, and geranyl acetate.

Geraniol was found to be the predominant component, accounting for approximately 40–45% of the total volatile oil composition. This is consistent with literature reports, which consider geraniol a key marker compound in Cymbopogon martini (palmarosa) essential oil. The high concentration of geraniol contributes to the characteristic rose-like fragrance and is largely responsible for the oil's antimicrobial and cosmetic value.

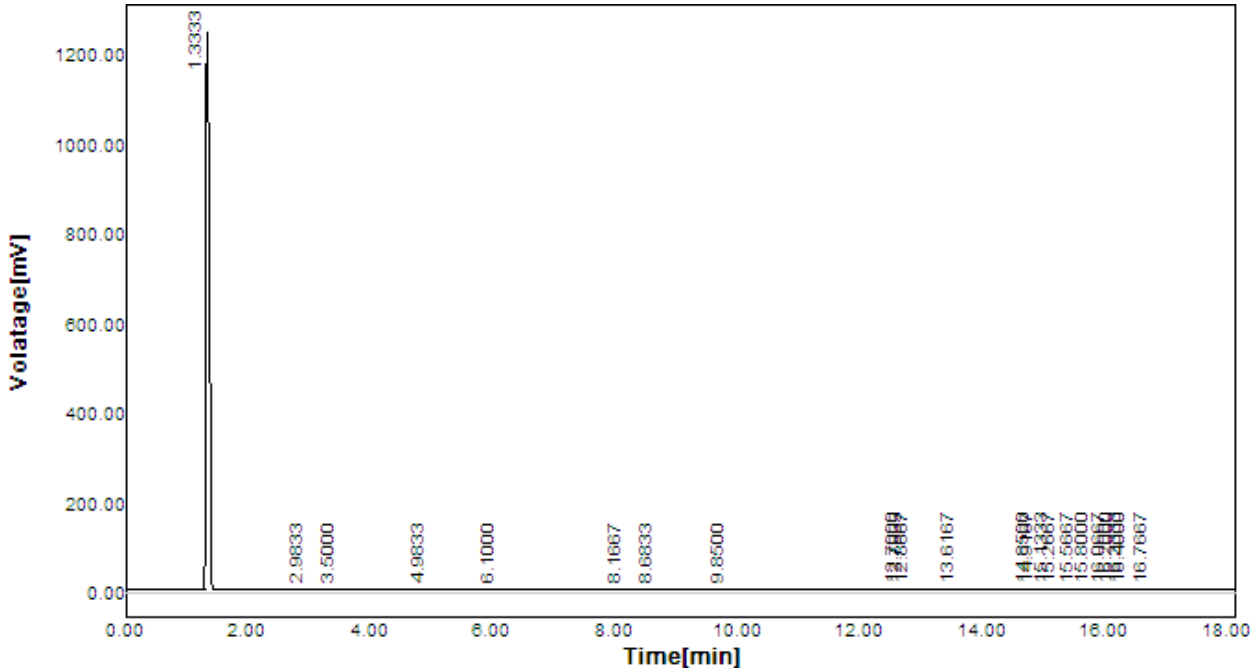

Figure 4: Chromatogram of Palmarosa plant

Table 3: Other Constituents in Palmarosa plant

No.	Name	RT[min]	Area[mV*s]	Area%
1	Geraniol	1.3333	4655.9463	99.73
2	α-Pinene	3.5000	1.2219	0.03
3	α-Pinene	2.9833	1.2803	0.03
4	Limonene	4.9833	2.1029	0.05
5	Myrcene	6.1000	3.3045	0.07
6	Linalool	8.1667	0.5198	0.01
7	Linalool	8.6833	0.8416	0.02
8	Citronellol	9.8500	0.4613	0.01
9	Neral	12.7000	0.1931	0.00
10	Geranial	12.7833	0.3195	0.01
11	α- Humulene	12.8667	0.1419	0.00
12	Geranyl acetate	13.6167	0.1109	0.00
13	β-Caryophyllene	14.8500	0.1383	0.00
Sum			4668.5010	

The GC-MS chromatogram of the essential oil extracted from *Cymbopogon martinii* var. *sofia* revealed the presence of multiple phytochemical constituents, indicating a complex composition. A total of 17 distinct peaks were observed within the retention time range of 1.333 to 16.750 minutes, each corresponding to a different compound. The highest peak was recorded at 1.333 minutes, suggesting the presence of a highly abundant volatile compound, likely geraniol, which is known to be the major constituent of palmarosa oil. The subsequent smaller peaks correspond to various minor components such as linalool, citronellol, geranyl acetate, and other monoterpenes and sesquiterpenes, which contribute to the oil's characteristic fragrance and therapeutic properties. The diversity of the peaks reflects the chemical richness of the essential oil,

supporting its commercial utility in perfumery and pharmaceuticals. These findings align well with previously reported literature indicating that palmarosa oil is rich in alcohols, especially geraniol, along with a range of esters and trace compounds responsible for its pleasant aroma and bioactivity.

CONCLUSION

The steam distillation of *Cymbopogon martinii* var. *sofia* successfully yielded essential oil with a rich phytochemical profile. GC-MS analysis confirmed geraniol as the principal constituent, along with several other minor compounds contributing to its therapeutic and aromatic properties. The results validate the high quality and commercial viability of palmarosa essential oil, making it suitable for applications in cosmetics, perfumery, and natural remedies. The findings also support further exploration of its biological activities and industrial potential.

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Author Contributions:

Md. Rageeb Md. Usman – Conceptualization, Methodology, Supervision, Review & Editing
Gautam P. Vadnere – Validation, Data Interpretation
Shreya C. Jain – Investigation, Data Curation, Original Draft Preparation
Jaydip Wahle – Laboratory Work, Literature Review
Ganesh Dhondkar – Sample Collection, Experimental Work
Suraj Ingle – Data Compilation, Visualization

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Ethical Approval: This study did not involve any human or animal subjects; hence, ethical approval is not applicable.

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