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Phytochemical Extraction and GC Analysis of *Cymbopogon martini* var. *motia* (Palmarosa)

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Abstract

The present study focuses on the extraction and gas chromatographic analysis of essential oils derived from Cymbopogon martini var. motia commonly known as palmarosa. The objective was to isolate volatile phytochemical constituents through hydro-distillation and evaluate their composition using gas chromatography (GC). The essential oils obtained exhibited significant aromatic and medicinal properties, making them valuable in perfumery and therapeutic applications. GC analysis revealed geraniol as the major component, along with other terpenoids contributing to the plant's bioactive profile. The findings highlight the efficiency of GC in profiling complex natural mixtures and underscore the potential of Cymbopogon martini var. motia as a rich source of commercially and pharmacologically important essential oils.

Keywords: Cymbopogon martini var. motia, palmarosa, essential oils, gas chromatography, geraniol, hydro-distillation, phytochemicals, terpenoids

Introduction

Cymbopogon martini var. motia is very important member of Gramineae family which is very famous For its high oil content. Essential oils (Volatile oils, *Ethereal* oils, aetherolea) are concentrated Hydrophobic liquids containing volatile aromatic compounds from plants. An oil is "essential" In the sense that it contains the "essence of" the plant's extract-the characterstic fragrance of The plant from which it is derived. Plants synthesize numerous kind of secondary metabolites Or specialized phytochemicals, of which essential oils (Eos) constitute an important group [1].These compounds can be extracted from plant tissues (e.g., stem, leaves, flowers, and roots) by Several procedures (e.g., hydrodistillation and steam distillation) [2]Terpenes, alkaloids (N-containing compounds) and phenolics constitute the largest groups of Secondary metabolites. shikimic acid pathway is the basis of the biosynthesis of phenolics. While the terpenes which are comprised of isoprene units arise from the mevalonate pathway[3]. Essential oils contains mainly terpenes, which are commonly used in pharmaceutical Industries and have therapeutic benefits and promote welfare, especially when used in Aromatherapy procedures [4]. The essential oils of *Cymbopogon martini var. motia* are rich in Monoterpenes. Essential oils from Cymbopogon species and their components are known for Their antimicrobial [5, 6] antihelmintic [7], antiparasitic [8], anti-inflammatory [9], anticonsulvant [10]. and antioxidant activities.

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Several reports published earlier have revealed the presence of Citral (a mixture of geranial and neral), geraniol, citronellol, citronellal,linalool,elemol, 1, 8-Cineole, limonene, geraniol, β-caryophyllene, methyl heptenone, geranyl acetate and geranyl Formate in the essential oils of *Cymbopogon martini var. Motia.*

Fig. 1: Palmarosa Plant

Phytochemical analysis:

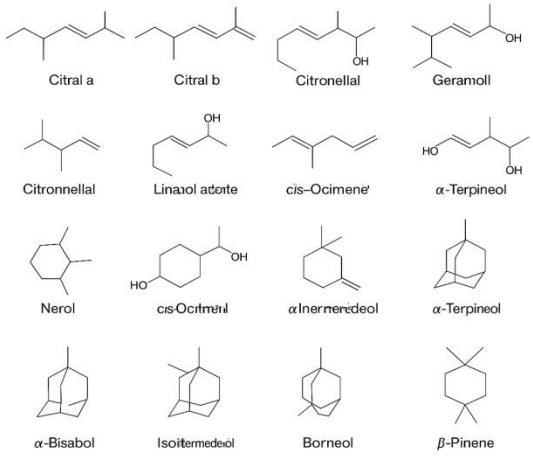

Fig. 2: Chemical Constituents of Palmarosa Plant

Chemical studies of the palmarosa oil reveals that it contains Monoterpenes, sesquiterpenes and alcohols like geraniol, Eranylacetate, (E, Z)-farnesol, nerolidol, geranial, Cymbodiacetal, limonene, terpinene, myrcene, caryophyllene, Humulene, selinenes, linalool and fatty acid 16-Hydroxypentacos-14-(z)-enoic acid [15, 16], Studies on the Chemical composition of citronella oil have found it contains A mix of more than a dozen monoterpenes, with the major Components being aldehydes and alcohols. Other compounds Predominant in citronella oil include citronellyl acetate, β-Bourbonene, geranyl acetate, elemol, l-borneol, and nerol [17,18, 19]. Terpenes with more than 23,000 known compounds are The largest group of natural substances [20]. They are Abundantly found in fruits, vegetables, aromatic and Medicinal plants where their important function is protection Against infections,parasites and other stress condition.

PLANT CULTIVATION:

Palmarosa thrives in hot, humid climates and well-drained soils with slightly acidic to neutral pH levels. Its cultivation directly influences the quality and yield of the oil, making it essential to optimize environmental and agronomic factors:

Climate: Palmarosa grows best in tropical and subtropical regions with well-distributed rainfall. Soil: Prefers sandy loam soils for proper root development and drainage.

Harvesting Time: Typically harvested during the flowering stage when geraniol content is highest, about three to four months after sowing. Plants are cut at about 90–100 cm above ground to preserve regrowth potential.

Materials and Methods Plant Material Preparation

Collection: Obtain fresh *Cymbopogon martini var. motia* plant from a Virwade

Cleaning: Wash with distilled water to remove dirt and contaminants.

Drying: Shade-dry at Room temperature for a few days to reduce moisture content while preserving volatileCompounds.

Grinding: Chop or grind the leaves into small pieces for efficient extraction.

Botanical Authentication

To ensure accurate species identification, voucher specimens from each collection site were prepared and submitted to PGRC, Department of Botany, Chopda for Taxonomic authentication. The specimens were identified by Dr. R. M. Bagul Sir (Head Of Botany Department) and assigned voucher specimen numbers. These voucher specimens are deposited at the herbarium for future reference. Digital photographs of representative plants from each site were also taken and archived.

Hydrodistillation Using Clevenger Apparatus

Materials Required-

Clevenger apparatus

Round-bottom flask (1–2 L)

Distilled water

Heating mantle or water bath
Ice bath for cooling condenser
Separating funnel
Anhydrous sodium sulfate (for drying the oil)

Procedure:

- Weigh and Place the Sample: Weigh 100–500 g of powdered or Transfer Into a 1–2 L round- bottom flask.
- Add Distilled Water: Pour enough distilled water (3–5 times the weight of plant material) into the flask to submerge the leaves.
- Assemble the Clevenger Apparatus :Connect the Clevenger trap, condenser, and heating Mantle properly.Ensure a continuous flow of cold water in the condenser.
- Start Heating: Heat the mixture to boiling (~100°C). Maintain a gentle boiling for 3–5 hours. Steam carries the essential oil, which condenses in the Clevenger trap.
- Oil Collection: The essential oil, being lighter than water, accumulates in the graduated side Of the trap. Separate the oil using a separating funnel.
- Drying the Oil: Remove residual water using anhydrous sodium sulfate. Filter the oil and Store in an amber glass bottle at 4°C to maintain stability.

Characterization of palmarosa plant :

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. 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.

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. 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 tubeAnd it was shaken vigorously. The formation of stable foam was taken as an indication for the Presence of saponins.

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.

Test for steroid:

1.2 ml of chloroform was added to the crude extract of Palmarosa plant 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.

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 0.2Gm 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 lactone:

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.

Quantitative Analysis of Phytochemical In The Plant Extract:

Determination of total phenolic contents (Singleton et al., 1999**)**

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 ofExtracted compound).

Determination of alkaloid (Harborne, 1973):

5 g of the sample was taken and 200 ml of 10% acetic acid in ethanol was added to thSample and allowed to stand for 4 hours. Then the solution was filtered and the extract was Concentrated on water bath Conc. NH4(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. Preparation of stock solution

The extracts were reconstituted in methanol. Methanolic extracts (1 µl) were injected for GC-MS analysis. Reference -Quality Evaluation and Effectiveness of Palmaris (*Cymbopogon martini var. motia*) Essential oils as repellents against *Aedes aegypti.*

Gas Chromatography (GC) Analysis

Gas Chromatography (GC) is an advanced analytical technique widely used to separate, identify, and quantify volatile compounds within complex mixtures. It is particularly effective in the analysis of essential oils due to its sensitivity, resolution, and reproducibility. GC works by vaporizing the sample and transporting it via a carrier gas through a stationary-phase-coated column. Compounds in the sample interact differently with the column, allowing for their separation based on volatility and polarity. This makes GC especially suitable for profiling the

phytochemical constituents of *Cymbopogon martini var. motia* (palmarosa).

Purpose of GC in This Study

The essential oil of *Cymbopogon martini var. Motia* contains various monoterpenes and sesquiterpenes that contribute to its medicinal and aromatic properties. GC analysis was employed to:

Characterize the volatile phytochemicals present in the extracted oil.

Determine the purity and chemical fingerprint of the essential oil.

Compare the presence of known bioactive constituents such as geraniol, citronellol, and linalool with standard references.

This analysis supports quality evaluation and aids in the verification of therapeutic potential of the essential oil.

Working Principle of GC

GC operates by introducing a liquid or gaseous sample into a heated injection port, where it is vaporized. The vaporized sample is then carried by an inert carrier gas (such as nitrogen) through a long capillary column coated with a stationary phase. As the sample components pass through the column, they separate based on their interactions with the stationary phase and their volatility. A detector—commonly a Flame Ionization Detector (FID) or Mass Spectrometer (MS)—records their elution as a series of peaks on a chromatogram, with each peak representing a different compound.

Experimental GC Conditions for Palmarosa Oil Analysis

The following GC setup was used for the analysis of palmarosa oil components:

Table 1: Experimental GC Conditions for Palmarosa Oil Analysis

Parameter	**Specification**
Carrier Gas	Nitrogen
Flow Rate	1 mL/min
Column Type	Non-polar capillary column (e.g., DB-5 or HP-5)
Injection Volume	1 µL
Solvent Used	Hexane or Ethyl Acetate
Injector Temperature	230°C
Detector Type	Flame Ionization Detector (FID)
Detector Temperature	240°C
Oven Temperature Program	
Initial Temperature	60°C
– Ramp Rate	3°C/min
–Final Temperature	220°C

Procedure:

1. Preparation:

Extraction of Essential Oil: If analyzing the essential oils of Tulsi, perform an extraction. One Common method is steam distillation, or you can use solvents like hexane or ethanol to Extract the volatile compounds. Crush the fresh or dried Palmarosa plant and soak them in a Suitable solvent (e.g., hexane or ethanol) for a specified period, followed by filtration toSeparate the solvent from the plant material.

2. Sample Filtration:Filter the extracted essential oil or liquid through a fine filter paper or a Syringe filter to remove any particulate matter or plant debris.

3. Sample Injection: Use a syringe or an auto sampler to inject the filtered extract (usually a Small volume, typically 1-2 µL) into the gas chromatography.

4. Gas Chromatography Setup:

Column Selection: Choose a suitable GC column based on the analysis required. Non-polar Columns (like DB-1 or HP-5) are common for essential oil analysis as they separate volatile Compounds effectively.

Carrier Gas: Select the appropriate carrier gas (helium is often used). Set the flow rate (e.g., 1 -2 mL/min). Column Temperature Program: Set the initial column temperature (e.g., 50-100°C) and ramp up (e.g., 5-10°C/min) depending on the analysis. A typical final temperature might be 250-280°C. Injector Temperature: The injector

temperature should generally be around 220-250°C to Ensure proper vaporization of the essential oils. Detector Settings: Set the detector (e.g., Flame Ionization Detector or Mass Spectrometer) to the required temperature and response mode. For FID, set the detector temperature to Around 240°C.

5. Run the Chromatography: Start the GC run. The sample will be carried by the carrier gas Through the column. The components of the palmarosa extract will separate based on their Volatility and interaction with the stationary phase of the column. The detector will record the Retention times of the separated compounds, producing a chromatogram.

6. Data Analysis: The chromatogram will display peaks corresponding to different Compounds in the Palmarosa extract. The area under each peak is proportional to the amount of each compound. Identify the compounds by comparing retention times to those of known standards or by Using mass spectrometry (if available) for more detailed identification.

7. Quantification (if required):If you have standards of known concentrations for the Compounds of interest, you can use them to quantify the amount of each compound in the Palmarosa sample by comparing peak areas.

Results and Discussion

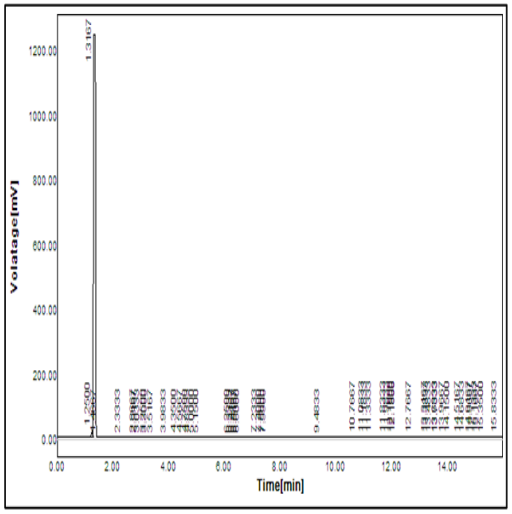

Fig. 3: Chromaotogram of *C. martini*

Table 2: Major compounds in *C. Martinii*

No.	Name	RT[min]	Area[mV*s]	Area%
1	Thujene	1.2500	50.0334	0.71
2	Germiol	1.3167	6940.6274	98.15
3	α-Pinene	1.4667	9.5264	0.13
4	Camphene	2.3333	0.7451	0.01
5	β-Pinene	2.8667	0.1514	0.00
6	Myrcene	2.9167	0.1156	0.00
7	α- Phellandrene	3.0333	0.5698	0.01
8	Limonene	3.2500	0.4518	0.01
9	1,8-Cineole	3.3000	0.3517	0.00
10	Cis-Ocimene	3.5167	0.1812	0.00
11	Trans-Ocimene	3.9833	0.2909	0.00
12	Linalool	4.3500	0.7093	0.01
13	Borneol	4.5667	0.2456	0.00
14	Citronellol	4.7500	0.5555	0.01
15	Neral	4.8333	0.6163	0.01
16	Geranial	5.0000	0.4244	0.01
17	Geranial	5.1500	0.2074	0.00
18	Geranial	6.2500	0.2956	0.00

The gas chromatographic analysis of Palmarosa oil revealed the presence of multiple volatile constituents, with retention times ranging from approximately 2 to 15 minutes. A major peak was observed at around 1.987 minutes with a significantly high voltage response of 1187.4 mV, suggesting the presence of a dominant compound. This compound is most likely geraniol, which is known to be the principal component in Palmarosa oil and is responsible for its characteristic rose-like aroma. Several smaller peaks followed, indicating the presence of minor components, potentially including linalool, citronellol, and geranyl acetate—compounds commonly reported in the literature for this essential oil. The overall chromatographic profile is consistent with previously reported GC patterns of Palmarosa oil, reflecting its complex composition dominated by oxygenated monoterpenes. The presence of these compounds supports the traditional and therapeutic uses of Palmarosa oil, particularly in perfumery and antimicrobial applications.

Conclusion

GC analysis of *Cymbopogon martini var. motia* essential oil confirmed the presence of several key volatile compounds, with geraniol as the major component. The results highlight the oil's rich chemical profile and support its use in medicinal and aromatic applications.

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