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To evaluate the in vitro antibacterial activity of ethanolic extract of Cymbopogon citratus against selected bacterial strains by using the agar well diffusion method

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ABSTRACT

Cymbopogon citratus is a widely used medicinal and aromatic plant known for its high essential oil content and strong lemon-like aroma. The plant is rich in bioactive compounds, particularly citral, which is a mixture of geranial and neral isomers and is mainly responsible for its characteristic fragrance and significant therapeutic properties. Citral is an important phytochemical that exhibits a wide range of pharmacological activities, making lemongrass an important plant in traditional and modern medicine.[1,2]

In this project, citral is extracted from lemongrass leaves using the Soxhlet extraction method, which is an efficient technique for obtaining essential oil components using suitable organic solvents. The extracted crude oil is then subjected to purification and analysis to identify the presence of citral and evaluate its yield. Further identification and characterization of the compound can be carried out using analytical techniques such as thin-layer chromatography (TLC) and gas chromatography (GC), which help in confirming the purity and composition of the extract.[3]

Citral has been widely reported for its antimicrobial, antioxidant, anti-inflammatory, antifungal, antiulcer, and potential anticancer activities. These properties make it a valuable natural alternative to synthetic chemical agents in pharmaceutical and medicinal applications. In addition, citral finds extensive use in cosmetic formulations, perfumery, food flavoring, preservatives, and aromatherapy due to its pleasant aroma and relatively low toxicity.[4,5]

Keywords: Cymbopogon citratus, Citral, Lemongrass, Antimicrobial activity

1. Introduction -

Medicinal and aromatic plants have contributed significantly to human civilization since centuries due to their therapeutic, nutritional and industrial applications. One of the most widely used aromatic grasses of family Poaceae among these plants is Cymbopogon citratus, commonly known as lemongrass. It is widely grown in the tropical and sub-tropical parts of the world including India, Sri Lanka, Brazil and Southeast Asian countries. Lemongrass is valued for its unique lemony fragrance, which is largely due to the presence of an important component of the essential oil called citral[21].

Citral is an aromatic compound that occurs naturally in the essential oil of Cymbopogon citratus. It is a mixture of two geometric isomers, geranial (citral A) and neral (citral B). Citral is widely used in the pharmaceutical, cosmetic, perfumery and food industries due to its pleasant citrus fragrance and diverse biological properties. Recently, the increasing interest of citral for its antimicrobial, antioxidant, anti-inflammatory, antifungal and anticancer activities has been observed. The growing demand for natural products and plant-derived bioactive compounds has further increased the importance of citral from lemongrass.

Extraction and characterization of citral from Cymbopogon citratus has become one of the important research areas in natural products chemistry and

phytochemistry. Different extraction methods such as steam distillation, hydrodistillation, solvent extraction and supercritical fluid extraction are used to obtain citral rich essential oil from lemongrass leaves. Steam distillation and hydrodistillation are the most commonly used of these methods because of their cost-effectiveness and efficiency in large-scale production.

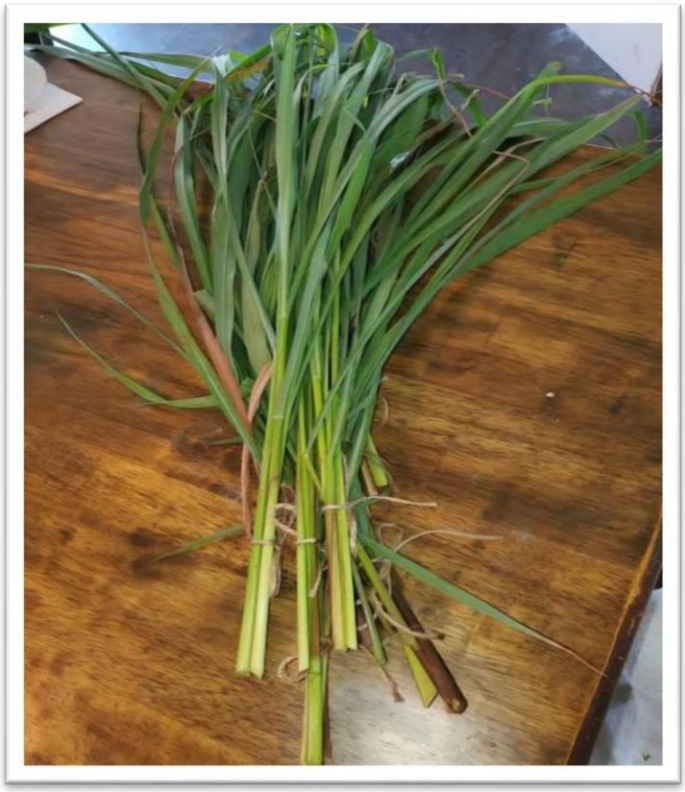
Lemongrass essential oil content generally varies from 0.2% to 0.5% depending on climatic conditions, geographical location, harvesting time and extraction method. Lemongrass is one of the richest natural sources of citral, which constitutes approximately 70–85% of the total essential oil composition. Besides citral, lemongrass oil contains other components in smaller amounts such as myrcene, limonene, citronellal, and geraniol. [21].

Material and Method;

Collection and Authentication of plant;

Collection of plant;

The plant material of cymbopogon citratus L was collected from Sangli, Maharashtra, India. The collected plant was healthy, disase-free and full grown stage. After collection, it was properly washed and shade dried at room temperature. [21]



(Fig.no.2.Collection of plant)

Authentication of plant:

The plant was authenticated by [Mr.S.M.Sabale], Department of Botany, [P.V.P. College, Kavthe Mahankal] Sangli, Maharashtra, India. The authenticated specimen was confirmed as cymbopogon citratus [14]

Plant Profile; Lemongrass

1. Botanical Name Cymbopogon citratus L.
2. Family Poaceae (Gramineae)
3. Common Names

- Lemongrass
- West Indian Lemongrass
- Citronella Grass
- Fever Grass
- Barbed Wire Grass

4. Plant Description: Cymbopogon citratus is a perennial aromatic grass that grows in dense clumps. It is widely recognized for its strong lemon-like fragrance due to the presence of essential oils.[21,23]

1. Morphological Features :

- Height: 1–2 meters
- Leaves: Long, narrow, linear leaves with rough margins and aromatic oil glands
- Color: Green to pale green leaves
- Roots: Fibrous root system

6. Distribution: Cymbopogon citratus is native to tropical regions of Asia, particularly India and Sri Lanka, and is now cultivated widely .It grows well in warm climates with adequate rainfall and well-drained soil.

7. Traditional Uses: Traditionally, lemongrass has been used in Ayurvedic, folk, and traditional medicine systems for centuries.

8. Common Traditional Uses

- Treatment of fever and colds
- Relief from digestive disorders
- Management of stomachache and diarrhea

- Reduction of anxiety and stress
- Treatment of cough and flu
- Used as a mild sedative
- Applied for headaches and muscle pain
- Insect repellent and antimicrobial agent

9. Phytochemical Constituents

The plant contains volatile oils, flavonoids, phenolics, and terpenoids responsible for its medicinal properties.[29]

10. Major Phytochemicals

Citral (major constituent; mixture of geranial and neral) , Myrcene , Geraniol , Limonene , Citronellal , β-Caryophyllene , Flavonoids , Tannins , Saponins , Alkaloids , Phenolic compounds

11. Essential Oil Content

The essential oil obtained from leaves is highly valued in pharmaceutical, cosmetic, and food industries.

12. Pharmacological Activities

Research studies have shown several biological activities of Cymbopogon citratus.[29]

- Antibacterial activity
- Effective against bacteria and fungi.
- Anti-inflammatory activity
- Helps reduce inflammation and pain.
- Antioxidant activity
- Neutralizes free radicals and oxidative stress.
- Analgesic activity
- Provides pain-relieving effects.
- Antidiabetic activity
- May help regulate blood glucose levels.
- Anxiolytic and sedative activity
- Produces calming and relaxing effects.

13. Conclusion: Cymbopogon citratus is an important medicinal and aromatic plant with extensive traditional and modern therapeutic applications. Its rich phytochemical composition, especially citral-containing essential oil, contributes to numerous pharmacological activities including antimicrobial, antioxidant, anti-inflammatory, and anxiolytic effects. The plant continues to be valuable in pharmaceutical, nutraceutical, cosmetic, and food industries.

Drying of plant Material:

Drying of plant material is an essential step in pharmacognostic and phytochemical studies, as it helps in the removal of moisture and prevents microbial growth, enzymatic degradation, and decomposition of active constituents. Proper drying preserves the bioactive compounds present in the plant material and maintains its quality for further analysis [13].

The freshly collected plant material of Cymbopogon citratus is first washed thoroughly with running water to remove dirt, dust, and other impurities. After washing, excess surface water is removed, and the plant material is subjected to shade drying at room temperature. Shade drying is preferred over direct sunlight because exposure to intense heat and sunlight may lead to the degradation of volatile oils and heat-sensitive phytochemicals, particularly citral, which is the major active constituent of lemongrass [16].

The leaves are spread in a thin layer on clean trays or paper sheets to ensure uniform drying and proper air circulation. The material is turned periodically to avoid fungal contamination and uneven drying. The drying process is continued for about 7–10 days or until the leaves become completely dry and brittle [15].

After complete drying, the plant material is checked for residual moisture content. Properly dried leaves should break easily when pressed. The dried material is then powdered using a mechanical grinder and stored in airtight containers or sealed polythene bags to protect it from moisture, contamination, and loss of volatile constituents until further pharmacognostic and phytochemical studies are carried out [13,15].

vb

(Fig.no.3 Dried Plant)

(fig. no. 4. Plant Powder)

Extraction of Plant Material:

- **Method of extraction:** Extraction of Leaf Powder of Cymbopogon Citratus By Soxhlet Method (Using Ethanol 70%)

Soxhlet extraction is a continuous hot extraction technique commonly used to isolate bioactive compounds from plant materials using suitable solvents. This method ensures efficient and repeated extraction of phytochemicals from the plant material [5].

1. Preparation of Leaf Powder

Fresh leaves of Cymbopogon citratus are collected and washed thoroughly with running water to remove dust, dirt, and other impurities. The cleaned leaves are shade dried at room temperature to avoid degradation of heat-sensitive and volatile compounds such as citral. After complete drying, the leaves are ground into a fine powder using a mechanical grinder and sieved to obtain uniform particle size. The powdered material is stored in an airtight container for further use [16].

2. Loading of Sample

Approximately 50 g of dried leaf powder is packed into a filter paper / thimble and placed inside the Soxhlet extractor for extraction [16]

3. Selection of Solvent

Ethanol (70%) is used as the extraction solvent because it effectively extracts a broad range of phytochemicals including Citral (major constituent; mixture of geranial and neral), Myrcene , Geraniol, Limonene ,Citronellal, β-Caryophyllene ,Flavonoids ,Tannins, Saponin and essential oil constituents present in Cymbopogon citratus. These compounds are associated with antimicrobial, antioxidant, anti-inflammatory, and other pharmacological activities [13].

4. Soxhlet Extraction Procedure

The Soxhlet apparatus is assembled using a round-bottom flask containing ethanol and connected to a condenser. Upon heating, ethanol vapors rise through the distillation arm, condense in the condenser, and drip into the thimble containing the leaf powder. The extraction chamber gradually fills with solvent and siphons back into the flask repeatedly, enabling continuous extraction of phytoconstituents. The extraction process is continued for about 6–8 hours or until the solvent in the siphon tube becomes colorless, indicating complete extraction [5,13].

5. Concentration of Extract

After completion of extraction, the ethanol extract is concentrated by evaporating the solvent using a water bath at controlled temperature. This process yields a semi-solid or dry crude extract containing the bioactive constituents of the plant [16].

6. Storage

The concentrated extract is transferred into airtight containers and stored at 4°C until further pharmacognostic, phytochemical, and pharmacological studies are carried out [5].

7. Importance

Soxhlet extraction provides maximum yield of active phytoconstituents from Cymbopogon citratus and is widely used for the evaluation of its medicinal and biological activities such as antimicrobial, antioxidant, and anti-inflammatory effects [13].

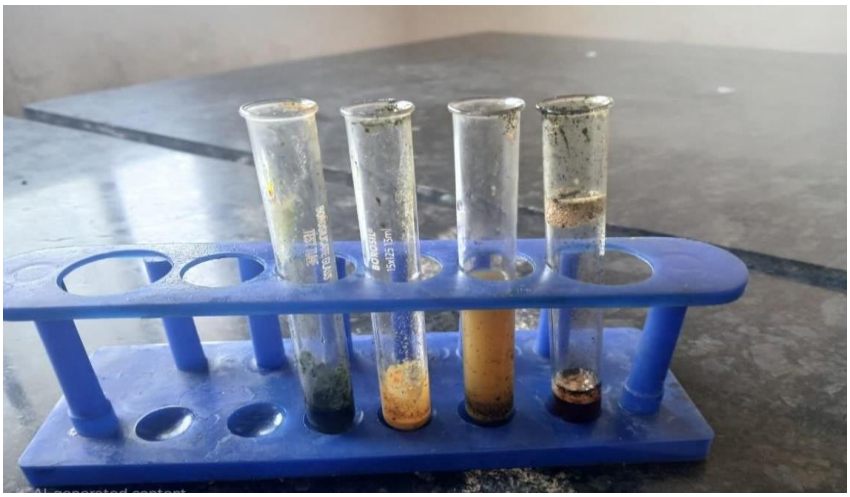
Percentage (%) Yield = Weight of Extract / Total weight of sample X 100

(Fig.no.5.Extraction process)

Result And Discussion : Phytochemical testing :
Chemical Tests for Terpenoids
Terpenoids are important secondary metabolites present in medicinal plants such asCymbopogon citratus. They are identified by different qualitative chemical tests based on characteristic color reactions.

Test	Procedure	Observation	Result
Salkowski Test	Add 2 mL chloroform and concentrated H_2SO_4 to the extract.	Reddish-brown ring at interface.	Presence of terpenoids.
Liebermann–Burchard Test	Add acetic anhydride and concentrated H_2SO_4 to the extract.	Green or bluish-green color appears.	Indicates terpenoids.
Copper Acetate Test	Add copper acetate solution to the extract.	Emerald green color develops.	Presence of terpenoids confirmed.
Sulfuric Acid Test	Add concentrated H_2SO_4 to the extract.	Yellow or reddish-brown color forms.	Positive for terpenoids.
Vanillin–Sulfuric Acid Test	Add vanillin reagent and sulfuric acid; heat gently.	Pink or purple coloration appears.	Presence of terpenoids.
Acetic Anhydride Test	Mix extract with acetic anhydride and H_2SO_4.	Blue-green coloration observed.	Indicates terpenoids.
Tin and Thionyl Chloride Test	Add tin and thionyl chloride to extract.	Pink coloration develops.	Presence of terpenoids.
Trichloroacetic Acid Test	Add trichloroacetic acid solution to extract.	Yellow to red coloration appears.	Positive terpenoid test.
Carr-Price Test	Add antimony trichloride reagent to extract.	Blue color develops.	Indicates terpenoid compounds.
Noller's Test	Heat extract with tin and thionyl chloride.	Pink to red color appears.	Presence of terpenoids confirmed.

These tests are commonly used in phytochemical screening to identify terpenoid compounds present in medicinal plant extracts.



(Fig.no.8.Phytochemical testing)

Authentication letter:

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kvt95.cl@unishivaji.ac.in

Date:

Authentication Certificate

This is to certify that, the plant specimen submitted by **Tanmay Tanaji Kamble** of Nootan Pharmacy College, Landagewadi has been authenticated and identified as follows:

Specimen 1:

- Scientific/ Botanical Name: ***Cymbopogon citratus*** L.
- Family: Poaceae
- Common Name: Lemon grass, Gavati Chaha
- Collection Location: Kavathe Mahankal

The authentication has been carried out based on morphological characteristics of specimen and comparison with existing literature and Botanical Flora's.

Authenticated by,
Mr. S. M. Sabale

Acting Principal
Padmabhushan Vasantraodada Patil Mahavidyalaya,K.Mahankal,Dist-Sangli.

Activity Checked By in Vitro Study:

Introduction: The antimicrobial activity is estimated by comparing the inhibition of growth of sensitive micro-organisms produced by known concentration of the isolated substance or extract or synthetic compound to be examined against a reference substance

Method of Analysis: Two general method usually employed; One is the cup-plate method [Agar well diffusion method)-The agar cup plate method depends upon diffusion of the antibiotic from a vertical agar [well] Cylinder through a solidified agar layer on a Petri dish. Sterile Agar is inoculated by suspension of the microbial inoculum. Then well with diameter of 6 to 8 mm is punched aseptically, and then of the antimicrobial solution at desired concentration is introduced into the well. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain entirely in a zone around the cylinder containing a solution of the substance to be tested.

Test	Antimicrobial Screening
Method	The Agar Well plate method
Location	Biocyte Microbiological Testing Center, Sangli

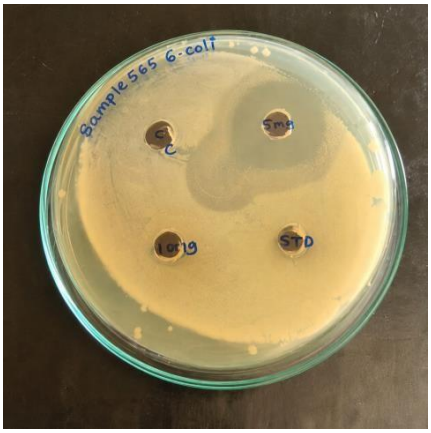
Description of Equipment/Instruments:

Analytical Balance	Vernier caliper
Water Bath	Incubator- 20 to 25 °

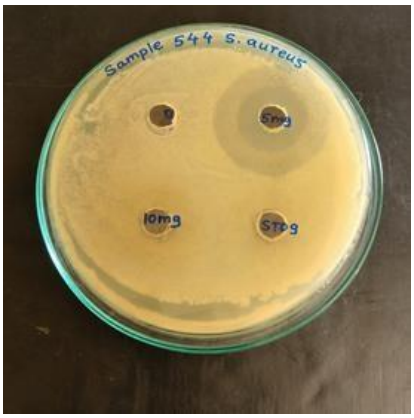
Laminar Air flow	Incubator- 30 to 35 "C
Colorimeter	Refrigerator-2 to 8 ℃
Zone Reder	Cyclomixer
sonicator	Micro-pipettes

Media and reagents preparation: Antibiotic Assay Medium No. 19 (Ph Is 6.1 ± 0.2):

Ingredient	**Weight**
Peptone	9.4g
Yeast Extract	4.7 g
Beef Extract	2.4 g
Sodium Chloride	10.0 g
Dextrose	10.0 g
Agar	23.5 g
Water	1000 mL



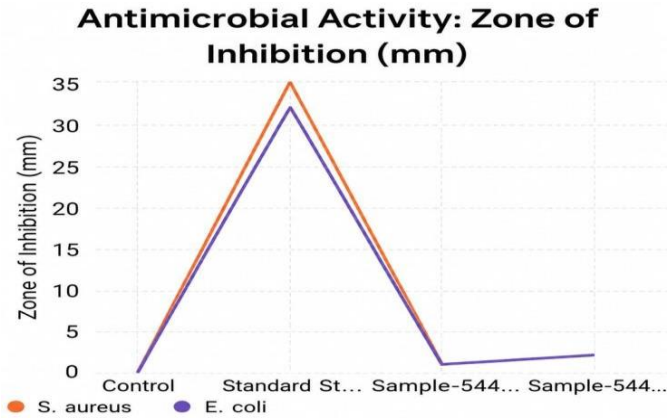
Escherichia Coli ATCC no-8739



Staphylococcus aureus ATCC no-6538

Observation Table:

Sr. No.	**Sample**	**Concentration**	**Zone of Inhibition (mm)** ***S.aureus***	**Zone of Inhibition(mm)** ***E.coli***
1	Control		-	-
2	Standard Streptomycin	1 mg/ml	35	32
3	Sample-544	5mg/ml 10mg/ml	01 02	01 02



Conclusion:
The antibacterial activity study demonstrated that the standard drug Streptomycin exhibited significant inhibitory activity against both Staphylococcus aureus and Escherichia coli, producing zones of inhibition of 35 mm and 32 mm respectively at 1 mg/mL concentration. In comparison, Sample-544 showed very weak antibacterial activity against both bacterial strains. At 5 mg/mL concentration, the sample produced only 1 mm zone of inhibition, while at 10 mg/mL concentration, a slight increase to 2 mm was observed against both organisms. The results indicate that Sample-544 possesses negligible to very low antibacterial potential against both Gram-positive and Gram-negative bacteria under the tested conditions.[31,32,33]

Collection;
The whole plant Cymbopogon citratus L was collected in month of march 2026 , from Sangli, Maharashtra, India.
Authentication;
After collection the plant was identified, conformed and authenticated by Mr. S. M. Sabale. Head of department of botany P.V.P. college of Kavathe Mahankl, Sangli.

Extraction of Plant :

The dried leaf powder of Cymbopogon citratus was extracted using ethanol by the Soxhlet extraction method. Ethanol was selected as the extraction solvent because of its ability to extract a wide range of phytoconstituents including citral (major constituent; mixture of geranial and neral), myrcene, geraniol, limonene, citronellal, β-caryophyllene, flavonoids, tannins, and saponins. These compounds are responsible for various pharmacological activities of the plant.
The extraction process yielded a yellowish semi-solid crude extract with a characteristic lemon-like odor due to the presence of volatile essential oils. The percentage yield of the ethanolic extract indicated efficient extraction of bioactive constituents from the plant material. Soxhlet extraction was found to be an effective method for continuous extraction and maximum recovery of phytochemicals present in Cymbopogon citratus.

Discussion:
The present study confirmed that ethanolic leaf extract of cymbopogon citratus contains several important phytoconstituents responsible for biological activites. The phytochemical analysis revealed the presence of compounds with known anti bacterial properties

Conclusion:
The present study was carried out to evaluate the phytochemical constituents and in vitro antibacterial activity of the ethanolic leaf extract of Cymbopogon citratus (DC.) Stapf. Preliminary phytochemical screening revealed the presence of several important bioactive secondary metabolites, including flavonoids, phenolic compounds, tannins, saponins, terpenoids, and essential oil constituents such as citral (a mixture of geranial and neral), limonene, and geraniol, which are well known for their antimicrobial properties.
The ethanolic leaf extract exhibited significant in vitro antibacterial activity against selected bacterial strains using the agar well diffusion method. The antibacterial effect was found to increase with increasing concentration of the extract, indicating a clear dose-dependent response. This observed activity may be attributed to the synergistic action of the identified phytoconstituents, particularly the essential oil components and phenolic compounds, which are known to disrupt bacterial cell membranes and inhibit microbial growth.
The findings of the present investigation support the traditional medicinal use of Cymbopogon citratus and highlight its potential as a promising natural source for the development of plant-based antibacterial agents. However, further studies involving the isolation, purification, and detailed characterization of active compounds, along with comprehensive pharmacological and toxicological evaluations, are necessary to confirm its therapeutic efficacy and ensure safety for potential clinical applications.

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