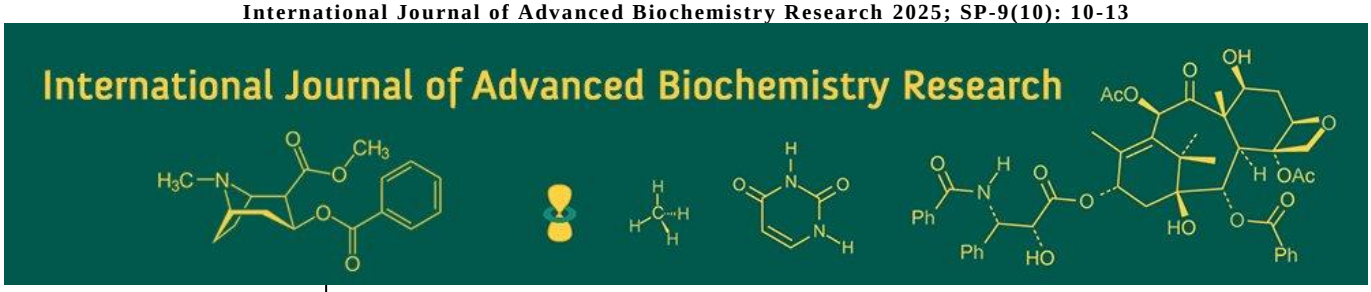

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; SP-9(10): 10-13
www.biochemjournal.com
Received: 05-07-2025
Accepted: 09-08-2025

Sonal D Rekhate
PG Scholar, Department of Molecular Biology and Biotechnology Dr. PDKV Akola Maharashtra, India

AG Deshmukh
Assosiate Professor, Department of Molecular Biology and Biotechnology Dr. PDKV Akola Maharashtra, India

PV Jadhav
Assosiate Professor, Department of Molecular Biology and Biotechnology, Dr. PDKV Akola Maharashtra, India

MP Moharial
Assosiate Professor Department of Molecular Biology and Biotechnology Dr. PDKV Akola Maharashtra, India

NK Patke
Assosiate Professor Department of Agronomy Dr. PDKV Akola Maharashtra, India

Kalyani D More
PhD Scholar, Department of Molecular Biology and Biotechnology Dr. PDKV Akola Maharashtra, India

Gayatri C Dhondekar
PG Scholar, Department of Molecular Biology and Biotechnology Dr. PDKV Akola Maharashtra, India

Prajakta Shelke
PhD Scholar, Department of Plant Pathology Dr. PDKV Akola Maharashtra, India

RR Tahakik
PhD Scholar Department of Molecular Biology and Biotechnology Dr. PDKV Akola Maharashtra, India

Rupali B Patle
PG Scholar Department of Molecular Biology and Biotechnology Dr. PDKV Akola Maharashtra, Indi

Corresponding Author:
Sonal D Rekhate
PG Scholar, Department of Molecular Biology and Biotechnology Dr. PDKV Akola Maharashtra, India

Enhanced antimicrobial activity of palmarosa (*Cymbopogon martinii*) essential oil against gram-positive bacterial pathogens

Sonal D Rekhate, AG Deshmukh, PV Jadhav, MP Moharial, NK Patke, Kalyani D More, Gayatri C Dhondekar, Prajakta Shelke, RR Tahakik and Rupali B Patle

DOI: https://www.doi.org/10.33545/26174693.2025.v9.i10Sa.5812

Abstract
Palmarosa (*Cymbopogon martinii*) essential oil is widely valued for its antimicrobial properties, largely attributed to its high geraniol content. This study evaluated oils extracted from fresh and dried Palmarosa samples for antibacterial activity against *Bacillus cereus* using agar well diffusion and broth microdilution (MIC) assays. Zones of inhibition (ZOI), MIC, and IC_{90} values were determined to compare potency. Dried-sample oil exhibited significantly larger ZOI (24.44 mm) than fresh-sample oil (18.50 mm), indicating stronger antibacterial activity. MIC assays showed concentration-dependent inhibition between 2-4 µL/mL, with IC_{90} values of 3.18 µL/mL for dried oil and 3.60 µL/mL for fresh oil. These findings demonstrate that drying Palmarosa biomass can optimize essential oil bioactivity, offering a promising strategy for developing natural antibacterial agents in therapeutic and preservative applications.

Keywords: Essential oils, Minimum inhibitory concentration (MIC), *Cymbopogon martini Bacillus cereus*, Palmarosa

1. Introduction
The *Cymbopogon* genus, part of the *Poaceae* family, holds significant economic value due to its frequent use in the perfume and pharmaceutical industries. This genus comprises approximately 140 species, each rich in various phytochemicals, with their essential oils extracted through hydro-distillation and steam distillation. The essential oils from *Cymbopogon* are composed of elements like citral, geraniol, citronellol, and citronellal, which possess insecticidal properties and exhibit antibacterial, repellent, and antifungal activities. *Cymbopogon martinii* is a perennial herbaceous species It is a tall, clumping grass that can grow up 3 to 5 meters in height. It has long, slender, green leaves and produces flowering tops that are harvested for oil extraction. The entire plant is aromatic, emitting a sweet, rosy fragrance when crushed, which is attributed to its high concentration of the chemical compound geraniol and geranyl acetate compounds constitute approximately 75-92% of the total essential oil
Pre-drying *Cymbopogon martinii* prior to essential oil extraction has been shown to improve geraniol content while facilitating bulk handling and storage. Advances in drying and harvesting protocols enable producers to maintain or even enhance oil quality compared to fresh biomass, simultaneously reducing logistical and production costs. This approach offers a scalable and economically viable strategy for essential oil production.
Microorganisms have the ability to develop resistance to conventional antimicrobial agents when exposed over time. This growing resistance underscores need for research focused on formulating new-generation therapeutics to combat infectious diseases effectively. Among the various biological activities of essential oils, their antibacterial potential is particularly significant, as they demonstrate strong efficacy against a broad spectrum of bacterial pathogens.
The present study has been planned to determine the zone of inhibition, Minimum Inhibitory Concentration (MIC) and Percentage Growth Inhibition of Essential oils against Gram positive bacterial pathogen.

2. Materials and Methods

2.1 Materials

- Palmarosa essential oil
- Nutrient broth and agar
- **Bacterial Strain:** *Bacillus cereus*,
- **Media:** Nutrient Agar and Nutrient Broth (for bacteria).
- **Inoculum Standardization:** 0.5 McFarland standard (~1.5×10^8 CFU/mL).
- **Solvent:** DMSO and Tween 80 for oil emulsification.
- **Sterile Equipment:** Cork borer (6-8 mm diameter), micropipettes, petri dishes, Sterile swab

2.2 Collection of Essential Oils

Palmarosa (*Cymbopogon martinii*) essential oils were obtained from the Nagarjuna Medicinal Plant Garden, Dr. PDKV, Akola. Oils were extracted from both fresh and dried plant materials and stored under refrigeration until further use.

2.3 Maintenance of Bacterial Cultures

The test organism, *Bacillus cereus*, was maintained on nutrient agar (NA) slants and stored at 4 °C to ensure viability and purity. Sub-culturing was performed periodically to sustain active cultures for experiments.

2.4 Agar Well Diffusion Assay

Antibacterial activity of the essential oils was initially evaluated by the agar well diffusion method. Sterile nutrient agar was poured into Petri plates and allowed to solidify. The bacterial inoculum was uniformly spread across the agar surface using a sterile swab. Wells of 5-6 mm diameter were bored aseptically into the medium. A test solution was prepared by emulsifying 25 µL of essential oil in Tween-80 and dimethyl sulfoxide (DMSO) with vortexing to achieve homogeneity, and added into the wells. Control wells contained only Tween-80 and DMSO. Plates were pre-incubated at room temperature for 1 h to allow diffusion and then incubated at 37 ± 1 °C for 24-48 h. The diameter of the inhibition zones (mm) was measured with a digital Vernier caliper.

2.5 Minimum Inhibitory Concentration (MIC) Assay

MIC values were determined using the broth dilution method. A loopful of *B. cereus* was inoculated into 5 mL of nutrient broth (NB) and incubated at 37 °C for 12 h to obtain actively growing cultures. The turbidity was adjusted to the 0.5 McFarland standard (~1.5×10^8 CFU/mL). Essential oils were serially diluted in NB containing 0.1% (v/v) Tween-80 and 1% (v/v) DMSO to achieve final concentrations of 0.5, 1.0, 2.0, and 4.0 µL/mL. Control tubes contained NB with Tween-80 and DMSO only. Each tube was inoculated with standardized bacterial suspension and incubated at 37 ± 1 °C for 22-24 h. Growth was assessed visually for turbidity and confirmed spectrophotometrically at 600 nm using a UV-Visible spectrophotometer.

3. Results and Discussion

According to the agar well diffusion assay (Table 1), Palmarosa essential oil exhibited notable antibacterial activity against *Bacillus cereus* at a concentration of 25 µL. The essential oil extracted from fresh plant material produced a zone of inhibition (ZOI) measuring 18.50 mm. In comparison, the oil obtained from dried samples showed a significantly larger ZOI of 24.44 mm. These findings suggest that the drying process may have enhanced the concentration or bioavailability of active constituents responsible for the antibacterial effect. The higher inhibitory activity of the dry sample oil highlights its potential as a more effective source of antimicrobial agents against *B. cereus*.

The antibacterial effect of Palmarosa essential oils, both Fresh sample and dry sample was evaluated against the Gram-positive, *Bacillus cereus*. Minimum Inhibitory Concentration (MIC) assays were conducted at four concentrations (0.5, 1.0, 2.0, and 4.0 µl/ml), and the results are presented in Table 2. A concentration-dependent response was observed across all treatments, with inhibition percentages increasing proportionally with essential oil concentration. At 0.5 µl/ml, inhibition ranged from 52.11-55.89%, which significantly increased to 64.11-71.68% at 1.0 µl/ml. At 2.0 µl/ml, inhibition ranged between 75.89-83.89%, while the highest concentration (4.0 µl/ml) demonstrated maximum inhibition values of 92.53-95.58%, particularly in dry sample oils. The IC_{90} value for Fresh sample oil was calculated as 3.60 µl/ml, whereas dry sample oil exhibited an improved IC_{90} value of 3.18 µl/ml. The MIC for *B. cereus* was determined to fall within the range of 2-4 µl/ml, confirming that dry sample extraction significantly enhanced the antimicrobial potency of Palmarosa essential oil compared to fresh sample oil.

Table 1: Zone of Inhibition (ZOI) of Palmarosa Essential Oil Against *Bacillus cereus*

Sample Type	ZOI (mm)
Fresh Sample essential oil	18.50
Dry Sample essential oil	24.44

Table 2: Minimum Inhibitory Concentration (MIC) assay showing the antibacterial effect of Palmarosa essential oil (Fresh and Dry samples) against *Bacillus cereus*.

Concentration (µL/mL)	% Inhibition - Fresh Sample (mean ± SE)	% Inhibition - Dry Sample (mean ± SE)	Significance
0.5	52.11 ± 0.84^{d}	55.89 ± 0.91^{d}	$p < 0.05$
1.0	64.11 ± 1.02^{c}	71.68 ± 1.15^{c}	$p < 0.05$
2.0	75.89 ± 1.34^{b}	83.89 ± 1.27^{b}	$p < 0.05$
4.0	92.53 ± 0.98^{a}	95.58 ± 0.85^{a}	$p < 0.05$
IC_{90} (µL/mL)	3.60	3.18	-
MIC range (µL/mL)	2-4	2-4	-

Different superscript letters within a column indicate significant differences ($p<0.05$).

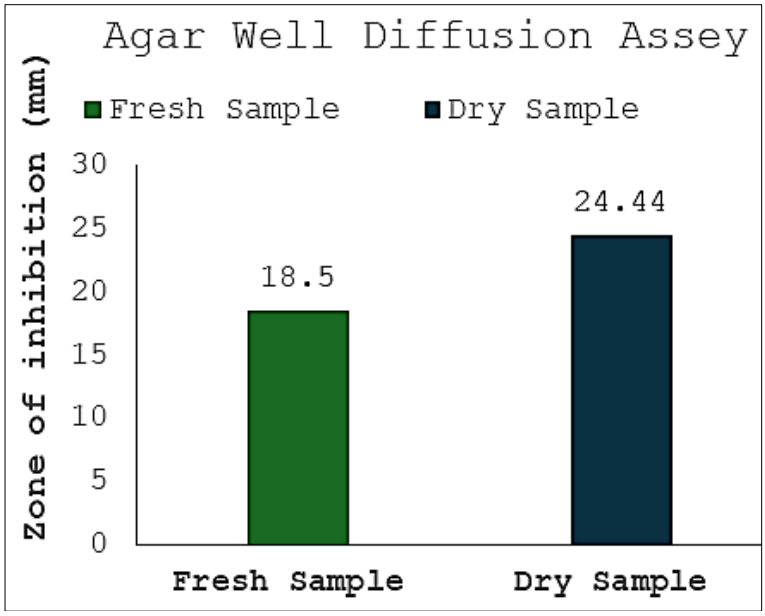

Fig 1: Zone of Inhibition (ZOI) of Palmarosa Essential Oil against *Bacillus cereus*

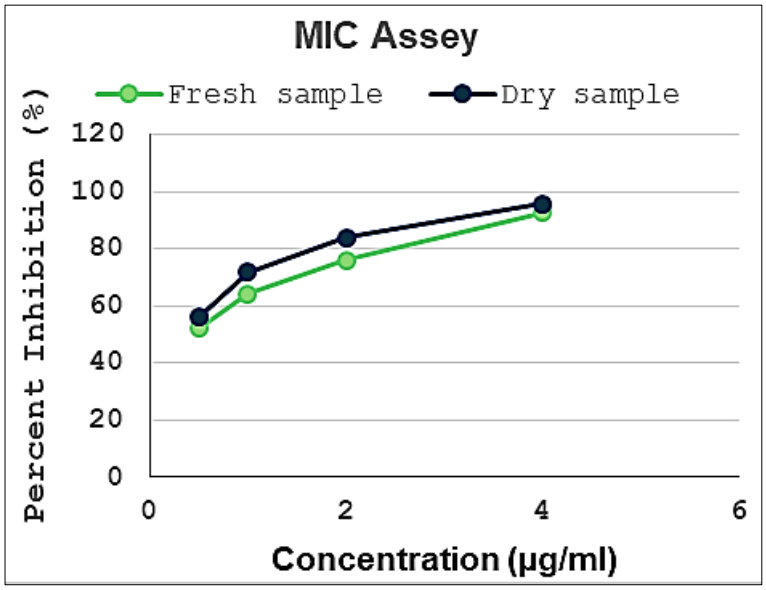

Fig 2: Percentage growth inhibition of *Bacillus cereus* at different concentrations of Palmarosa essential oil.

4. Conclusion

The present investigation demonstrated the significant antibacterial activity of Palmarosa (*Cymbopogon martinii*) essential oil against *Bacillus cereus*. A clear dose-dependent inhibition was observed, with higher concentrations producing stronger antibacterial effects. Dry sample essential oil enhanced both the efficacy and potency of the oil, as reflected in lower IC_{90} values and higher inhibition percentages compared to fresh sample oil. The MIC was determined within the range of 2.0-4.0 µl/ml, with dry sample oil exhibiting superior performance. The enhanced activity is attributed to the concentration of key bioactive compounds, primarily geraniol, during the dehydration process. These findings highlight that dry sample not only improves essential oil yield but also enhances its quality and antimicrobial properties, underscoring the potential application of Palmarosa essential oil as a natural, effective alternative for managing bacterial pathogens, particularly in food preservation and pharmaceutical formulations.

Acknowledgement

The authors would like to thank the Department of Agricultural Biotechnology and Nagarjuna medicinal and Plant Garden laboratory Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, for offering the laboratory facilities and support for conducting this research.

References

1. Gürer ES, Tunc T. Investigation of antimicrobial and cytotoxic activities of palmarosa (*Cymbopogon martinii*) essential oil. Cumhur Sci J. 2022;43(4):594-9.
2. Lodhia MH, Bhatt KR, Thaker VS. Antibacterial activity of essential oils from palmarosa, evening primrose, lavender and tuberose. Indian J Pharm Sci. 2009;71(2):134-8.
3. Nagalakshmi S, Saranraj P, Sivasakthivelan P. Determination of minimum inhibitory concentration (MIC) and percentage bacterial growth inhibition of essential oils against gram positive bacterial pathogens. J Drug Deliv Ther. 2019;9(3):33-5.

4. Van NTB, Vi OT, Yen NTP, Nhung NT, Cuong NV, Kiet BT, *et al.* Minimum inhibitory concentrations of commercial essential oils against common chicken pathogenic bacteria and their relationship with antibiotic resistance. J Appl Microbiol. 2022;132(2):1025-35.
5. Microbe Online. Minimum inhibitory concentration (MIC) broth dilution method: procedure and interpretation [Internet]. 2023 [cited 2025 Sep 30].
6. Lal RK, Gupta P, Chanotiya CS, Mishra A, Kumar A. Eminent high essential oil yielding and photosynthesis efficient genotype selection across multi-environments in the palmarosa (*Cymbopogon martinii* (Roxb.) Wats.). Ecol Genet Genomics. 2023;27:100167.
7. Soni K, Kalbande S. Influence of energy sources and shade drying period on oil yield and chemical composition of the essential oil extracted from palmarosa grass (*Cymbopogon martinii*) through hydrodistillation method. Bioresour Technol Rep. 2023;22:101491.
8. Jamwal S, Thakur M, Walia S, Kumar R. Effect of season on essential oil content and composition of palmarosa [*Cymbopogon martinii* (Roxb.) Will. Watson] cultivated in the mid-hill conditions of the Western Himalayas. Indian J Nat Prod Resour. 2024;15(4):546-54.