Journal of Applied Botany and Food Quality 98, 53 - 65 (2025), DOI:10.5073/JABFQ.2025.098.006

[1]Department of Botany, Lahore College for Women University Lahore, Pakistan
[2]Department of Physics, College of Science and Humanitiesi Al-Kharj, Prince Sattam binAbdulaziz University, Al-Kharj, Saudi Arabia
[3]Department of Plant Pathology, Institute of Agricultural Sciences, University of the Punjab, Lahore Pakistan

Impact of leaf growth stages on essential oil composition and bioactivity in lemongrass (*Cymbopogon citratus*)

Rutaba Saeed[1], Arusa Aftab[1*], Zainab Maqbool[1], Zubaida Yousaf[1*], Awais Khalid[2], Zill-e-Huma Aftab[3]

(Submitted: December 14, 2024; Accepted: March 26, 2025)

Summary

Lemongrass (*Cymbopogon citratus*) essential oil plays a crucial role in managing digestion, blood cholesterol levels, inflammation, and the immune system. It also has a calming effect on the mind and body in aromatherapy. Among its constituents, β-myrcene has been reported as a potent analgesic, antidiabetic, antioxidant, anti-inflammatory, and anticancer agent. This study aimed determine the optimum leaf development stage for extracting essential oil enriched with therapeutic phytochemicals such as citral, β-myrcene, and perillaldehyde. In lemongrass, essential oil is produced by glandular trichomes.

Microscopic analysis revealed the presence of capitate-stalked trichomes on the abaxial epidermis of the leaves. GC-MS analysis identified key phytochemicals such as citral and β myrcene in significant amounts across all leaf growth stages, with essential oil yields of 52.03% (lag phase), 64.26% (log phase), and 69.27% (stationary phase). Antioxidant activity was evaluated at 50 µL/mL, with the highest value (84.42 ± 0.03%) observed in the lag phase. Total phenolic content was also highest in the lag phase (1123.61 ± 16.71 µg GAE/g), whereas total antioxidant capacity reached its maximum in the stationary phase (0.7 ± 0.15). The stationary phase exhibited the highest anti-inflammatory potential (82.12 ± 0.04%). Both the stationary and lag phases demonstrated the highest anti-diabetic potential compared to the commercial drug sitagliptin-metformin. The stationary phase exhibited the highest biofilm inhibitory potential against *S. aureus* (38.78 ± 0.12%) and *R. solanacearum* (91.00 ± 0.07%), while the lag phase showed the strongest inhibition against *X. oryzae* (89.57 ± 0.01%). GC-MS analysis identified citral, β myrcene, and perillaldehyde. These findings suggest that *Cymbopogon citratus* is a valuable source of citral, β-myrcene, and perillaldehyde, particularly in the log and stationary phases of leaf development.

Keywords: β-myrcene, Citral, *Cymbopogon*, nutritional, phytochemical

Introduction

The recent development of functional foods and pharmaceutical products based on medicinal and edible plants, particularly fruits and vegetables, has led to significant improvements in various aspects of life (Raina et al., 2022). Traditional plant-based medicines are widely used in developing countries due to their easy accessibility and fewer side effects compared to modern medicine (Sandhya *et al*., 2011). Approximately 30-50% of drugs originate from plants, highlighting the significance of plant biodiversity in medicine (Anand et al., 2019). Therefore, studying plant-based medicines or pharmacognosy is crucial for modern drug development (Mohanty et al., 2014). With the global population expected to reach 9.6 billion by 2050, there is an increasing need for sustainable solutions, including plant-based medicines and natural products.

Plant diseases caused by phytopathogens affect 10% of global food production, especially in developing countries (Daulagala, 2021). Over two hundred different bacterial phytopathogens have been identified including *Xanthomonas*, *Ralstonia*, *Erwinia*, *Pseudomonas*, and *Pectobacterium* species (de Kraker et al., 2016). Biofilms, complex structures formed by bacteria, are a major contributor to antibiotic resistance (Jamal et al., 2019). It is recognized that plants have significant medicinal properties (Sharma et al., 2021; Velu et al., 2021). Essential oils (EOs) derived from plants can be used as natural pest control agents, replacing harmful chemical insecticides (Duarte et al., 2016). EOs contain diverse chemical compounds (Perczak et al., 2019), which are known for their significant biological properties (Falleh et al., 2020). While thousands of EOs exist, only around 300 are commercially available (Sil et al., 2020).

In recent years, there has been a surge in interest regarding EOs derived from species of the *Cymbopogon* genus (Olayemi et al., 2018). This taxonomically diverse genus comprises over one hundred species primarily distributed across tropical regions (Zigene et al., 2018). The genus *Cymbopogon*, a member of the Poaceae family, has traditionally been used for its potent therapeutic effects (Avoseh et al., 2015). *Cymbopogon citratus* (DC.) Stapf commonly referred to lemongrass, is a potential source of essential oils (Ekpenyong and Akpan, 2017). The lemongrass essential oil exhibits a range of biological properties including antioxidant (Widelska et al., 2018), antibacterial (Shendurse et al., 2021), antifungal, and biofilm inhibition activities (Martinazzo et al., 2019).

The aerial parts of the plant, particularly the leaves, contain two types of trichomes: glandular and non-glandular (Sawant et al., 2023). Trichomes are hair-like structures on the epidermis that exhibit a wide range of morphological and functional variations (Choi and Kim, 2013). The metabolites secreted through glandular trichomes contribute to the characteristic aroma observed in the foliage of many plant species. Notably, these phytochemicals often possess antimicrobial properties, as observed in the foliage of *Cymbopogan* species (Happyana et al., 2013; Schuurink et al., 2020). This study aims to optimize the leaf development stages of *Cymbopogon citratus* (lemongrass) to extract essential oils enriched with therapeutic phytochemicals such as citral, β-myrcene, and perillaldehyde. This research seeks to identify the stages that yield the highest concentrations of these bioactive compounds, providing insight into their potential as natural remedies.

Plant material collection

Fresh leaves of *C. citratus* were collected at different growth stages (February to April, 2024) from Botanical garden (coordinates: latitude: 31.5437°N and longitude: 74.320°E), Lahore college for Women

* Corresponding authors

University, Lahore. Leaves were harvested from each selected stage (lag, log, and stationary) (Fig. 1).

Leaf epidermal anatomy

Leaves were dipped in 60% ammonia solution for 4 h. After removing from ammonia solution, leaves were boiled in a water bath (Model No. HH-S2) for about 10 min and subsequently placed in 88% lactic acid. The leaf epidermal layer was peeled off using a sharp razor blade. Through scrapping, mesophyll and epidermis layer were removed and stained with safranine for 5 min. Excessive stain was removed by using distilled water and ethanol solution and examined under a light microscope (Olson and Clark, 2021). Photographs were recorded, and different anatomical characters such as trichomes length, width, and area were calculated using MOTIC IMAGES PLUS 2.0 software (Karanović et al., 2022). Scanning microscope imaging was done using fresh leaf samples washed with distilled water and then ethanol to remove any debris and air dried for 4 h and put on stubs to observe under SEM (Gul et al., 2019).

Essential oil extraction

The essential oils of selected stages were extracted by hydro-distillation method using a modified Clevenger-type apparatus (Abed, 2007). Fresh plant material was prepared by rinsing and chopping lemongrass into 1 cm pieces, which were partially dried at room temperature for 72 h. About 1000 g of partially dried leaves were placed in hydro-distillation for a minimum of 3 h (Bagheri et al., 2014). To remove water, anhydrous sodium sulfate was used and the extracted oil was aliquot in amber glass vials stored at 4 °C in a refrigerator to reduce susceptibility (Ranitha et al., 2014; Bistgani et al., 2018). After extracting the essential oil from selected leaf growth phases, the oil was serially diluted in 5% DMSO, and varying concentrations were prepared ranging 50, 25, 12.5, 6.25, to 3.125 µl/mL and stored till further use (Sawant et al., 2023).

Estimation of phytochemicals composition at different leaf growth stages

Gas chromatography-mass spectrometry (GC-MS) was applied for the chemical characterization of lemongrass essential oil (Gao et al., 2020). GC TRACE-1300 chromatography coupled with mass spectrometry, single quadruple, and autosampler (AI-1310; Thermo Scientific, Waltham, MA, USA) were applied. A Capillary column of TR-35 MS GC Column 30 mx, 25 mm IDx, and 25 µm was used. The injector temperature was set at 280 °C, column temperature was initially maintained at 50 °C for 5 min and then programmed at 3 °C/min to 240 °C and 5 °C/min to 300 holds for 3 min. The initial sample delay time was 3.5 min. The transfer line temperature was set at 300 °C, while the ion source temperature was 250 °C. Helium (He) (99.99%) was used as the carrier gas with a linear gas flow of 1.5 mL/min through split-less injection. The injected volume was kept at 1 µL, and mass spectra were taken at 70 eV (Shamsheer et al., 2022). The individual volatile constituents of essential oil samples were analyzed by their retention indices (Adams et al., 2017), and a comparison with reference spectra (Wiley and NIST (https://webbook.nist.gov/chemistry/) databases) (Van Den Dool and Kratz, 1963). The percentages of identified compounds exceeding 0.1% were then calculated based on their respective GC peak areas (Valková et al., 2021).

Antioxidant activity

The antioxidant potential of essential oil of selected leaf growth stages was evaluated by following three essays:

DPPH Free radical scavenging assay

Briefly, 0.6 M solution of DPPH in ethanol was prepared. 0.5 ml of DPPH solution was added to test tubes, followed by 0.5mL of essential oil of varying concentrations. The mixture was stirred thoroughly and stored in the dark for 30 min. Then, the absorbance was measured at 517 nm. Alpha-tocopherol was used as positive control, while DPPH acted as the negative control (Kalpoutzakis et al., 2023; Aftab et al., 2020). The scavenging activity was calculated through

$$\%\ \text{Scavenging activity} = \left[\frac{A_{517}\ \text{of control} - A_{517}\ \text{of sample}}{A_{517}\ \text{of control}} \right] \times 100.$$

Determination of total phenolic content

Concisely, 0.5 mL of essential oil was mixed with 1.25 mL of 10% (w/v) Folin–Ciocalteu reagent. After 5 min, 1 mL of Na_2CO_3 (75%) was added to the mixture and incubated at 50 °C for 10 min with occasional stirring. Then the sample was cooled and absorbance was measured at 765 nm by using a UV Spectrophotometer (UV-6000). A standard curve was prepared using gallic acid. The results obtained were expressed as µg/g of gallic acid equivalence (GAE) (Thummajitsakul et al., 2020; Aftab et al., 2019).

Total antioxidant capacity

Briefly, 0.1 ml aliquot of various concentrations of the essential oil (50, 25, 12.5, 6.25, and 3.125 µl/ml) were mixed with 1 ml of reagent solution (600 mM sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The test tubes were covered with aluminum foil and incubated in a water bath (Model No. HH-S2) at 95 °C for 90 min. Then extracts were cooled at room temperature, and absorbance of the reaction mixture was determined at 765 nm compared with Butylated hydroxyl toluene (BHT) (Aftab et al., 2023).

Anti-inflammatory activity

Firstly, 1 ml essential oil of concentrations (50, 25, 12.5, 6.25, and 3.125 µl/ml) was mixed with, 1.4 ml Phosphate buffer saline (PBS) and 0.1 ml egg albumin. The reaction mixture was incubated for 30 min at 37 °C. Then the reaction mixture was heated at 70 °C for 15 min. The absorbance was measured at 680 nm. Standard drug dicloran (diclofenac sodium @Novartis) was used as positive control whereas distilled water served as negative control. The percentage inhibition was calculated through

$$\%\ \text{Percentage inhibition} = \frac{\text{Abs of Control} - \text{Abs of sample}}{\text{Abs of control}}$$

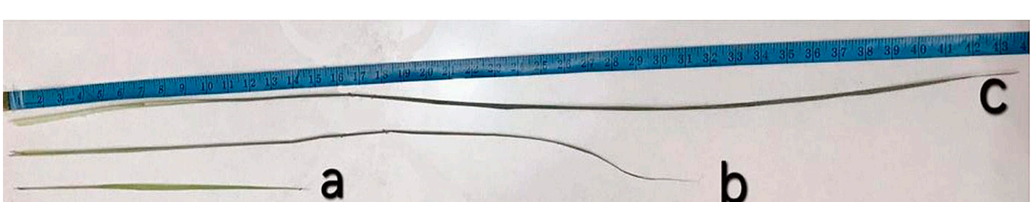


Fig. 1: Length **a)** lag phase **b)** log phase **c)** stationary phase of *C. citratus* leaves

Anti-diabetic activity
The inhibition of carbohydrate hydrolyzing enzymes such as α-amylase can be an important strategy to lower postprandial blood glucose levels. Sitagliptin and metformin help manage blood sugar levels, which in turn may reduce the body's need for alpha amylase, potentially improving blood glucose control (Kim et al., 2017). For this, 50 µl of alpha amylase solution was mixed with 50 µl of essential oil and incubated at 30 °C for 10min. Then 50 µl of 1% starch solution was added and incubated for 3 min. Then reaction was stopped by adding 50 µl of DNSA reagent and were incubated for 10 min in a water bath (Model No. HH-S2) at 85-90 °C. (Miller, 1959). The absorbance was measured at 540 nm in UV-spectrophotometer (U-6000) (Jaber, 2023). Sitaglumet (Hilton Pharma Pvt Ltd), a standard drug, was employed as positive control (Kim et al., 2017). The % amylase inhibition was calculated using the following formula:

$$\% \ \alpha - \text{amylase inhibition} = \frac{100 \times \text{Abs}_{100\%\text{control}} - \text{Abs}_{\text{Sample}}}{\text{Abs}_{100\%\text{control}}}$$

Antibacterial profile of *C. citratus* essential oil
The antibacterial profile of essential oils was evaluated through the agar well diffusion method, minimum inhibitory concentration, minimum bactericidal concentration assay and biofilm inhibition assay. Pure bacterial cultures were obtained from Institute of Agriculture Sciences (IAGS), university of the Punjab, Lahore Pakistan. Bacteria used in the following experiments were *Xanthomonas oryzae* (FCBP-PB-0133), *Staphylococcus aureus* (ATCC 6539) and *Ralstonia solanaceaerum* (FCBP-PB-0407) (Sharma et al., 2024).

Agar-well diffusion assay
Bacterial strain was diluted to attain optical density (OD_{595}) of 0.1. The inoculum was smeared on nutrient agar media plates and wells of 6 mm utilizing aseptic cork borer. Essential oil (50, 25 to 3.125 µl/ml) were transmitted to the wells. The agar plates were left to stand at room temperature for about 15 min and incubated at 37 °C. After 24 hours, the zone of inhibition was measured in mm (Kaur and Sharma, 2015; Sharma et al., 2024).

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assay
In a 96-well microtitre plate, 100 µl of diluted pathogenic bacterial strains and 5 µl of the plant essential oil were added. The plate was incubated for 24 hours at 37 °C, and the turbidity of the plate was checked visually. MIC was calculated as the reciprocal of the lowest concentration at which turbidity was absent. Minimum bactericidal concentration (MBC) was evaluated by adding MIC broth and incubating for 24 h at 37 °C. MBC was considered the minimum concentration at which no bacterial growth was visible (Ibrahim and Al-Mizraqchi, 2024; Parvekar et al., 2020)).

Bacterial biofilm inhibition assay
A 96-well plate was prepared by adding 100 µL essential oil, 100 µL of autoclaved nutrition broth, and 20 µl of overnight grown indicator pathogenic bacterial culture to each well (OD_{595} of 0.1). The reaction mixture was incubated at 37 °C to promote the production of biofilm in the wells. After fixing the adhering cells in 200 µl of methanol for 15 min, the wells were removed and allowed to air dry. 200 µl of 2% crystal violet was used to stain the fixed biofilm for 5 min. The excessive stain was removed by washing with distilled water. The dye from adhering cells was extracted using 100 µl of 33% Glacial acetic acid. The OD_{595} was then measured. Gentamycin was used as the positive control (Sharma et al., 2018). The percentage (%) biofilm inhibition was calculated through

$$\text{Biofilm inhibition (\%)} = 100 - \frac{\text{OD of sample} \times 100}{\text{OD of control}}$$

Hemolytic activity
The fresh human blood was taken and washed at least 3 times by centrifugation in isotonic PBS solution. The 2% Erythrocyte solution with PBS was prepared after removing the buffy coat of the pellet. Then a mixture of 0.1 ml of plant extract, 0.1 ml of 2% erythrocyte solution was prepared in test tubes. The reaction mixture was incubated at 37 °C for 3 h. Then absorbance value was measured at 540 nm in a UV spectrophotometer (UV-6000). The 0.1% solution of Triton X was used as a positive control (Rodríguez-Garza et al., 2023; Atta and El-Shabasy, 2022). The % Hemolysis was calculated through

$$\% \text{ hemolysis} = \frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

Statistical analysis
All experiments were conducted in triplicate, and the data were analyzed using Excel. Results were presented as mean ± standard deviation, by using Analysis of variance (ANOVA) (Atta and El-Shabasy, 2022).

Results

Leaf morphology and distribution of glandular trichomes
The lengths of leaves at the three developmental stages were as follows: lag phase (20-30 cm), log phase (50-60 cm), and stationary phase (100-120 cm) (Fig. 1). Leaves of *C. citratus* exhibited parallel venation, a glabrous surface with a dentate cuticle. The study revealed that the composition and size of trichomes varied across leaf developmental stages, showing notable differences in density and morphology (Tab. 1). In the intercostal zone, long cells were interspersed with shorter ones, along with dumbbell-shaped stomata and a layer of glandular microhairs. In the coastal zone, both long and short epidermal cells were observed, along with prickle hairs (Fig. 2). On the abaxial surface, the density of trichomes was higher during the lag and log phases than in the stationary phase, as observed in microscopic analysis. The trichomes in lemongrass were glandular capitate-stalked. The glandular trichomes consisted of a bulb-shaped trichome head joined to the stalk by a narrow, short segment. The glandular trichomes were located close to the stomata, which could explain the plant's strong aroma (Fig. 3).

Scanning electron microscope (SEM)
Leaf specimens were examined under SEM (Fig. 4) and capitate-stalked trichomes were identified in all leaf stages at the abaxial epidermis.

Tab. 1: Number of trichomes on abaxial surface and abaxial leaf surface of selected leaf growth stages (lag, log and stationary)

Growth stage	No. of trichomes (n)	Length (µm)	Width (µm) (µm)	Area (μm^2)
Lag	11	121.36±3.12	28.1±2.22	3410.216
Log	10	132.16±2.14	33.53±3.1	4431.325
Stationary	9	155.6±4.59	42.43±5.9	6299.308

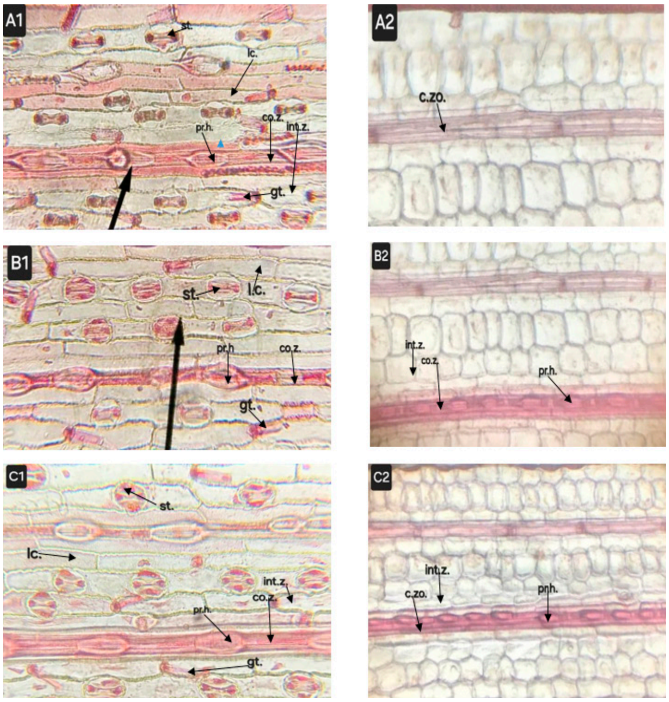


Fig. 2: Adaxial and abaxial epidermises of *C. citratus* leaf. A1, abaxial epidermis of lag phase; A2, adaxial epidermis of lag phase; B1, abaxial epidermis of log phase; B2, adaxial epidermis of log phase; C1, abaxial epidermis of stationary phase; C2, adaxial epidermis of stationary phase, st., stomata; lc., long cell; pr.h., prickle hair; co.z., costal zone; int.z., intercostal zone; gt., glandular trichome

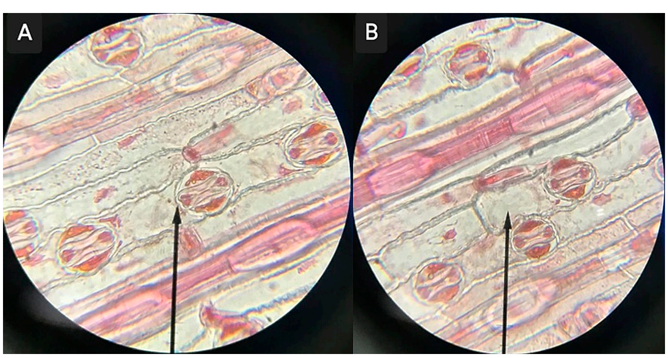


Fig. 3: Glandular trichrome located near stomata

Gas Chromatography-Mass Spectrometry analysis of *C. citratus* essential oil

The essential oil extracted from the leaf growth stages i.e. lag, log and stationary characterized 8, 8 and 12 compounds respectively. The yields of essential oils were 0.15%, 0.14%, and 0.12% (V/W) from the lag, log, and stationary phases, respectively, using 1000 g of leaves. The most common compounds present in the essential oils at all three developmental stages were geranial, neral and β myrcene (Tab. 2). The lag phased showed the presence of 2-Methyl Pentane, 3-methylpentane, Butane, 2,2,3- Trimethyl, Methylcyclopentane, cyclohexane, β-Myrcene, Neral and Geranial, log phase Butryic acid hydrazide, β-Myrcene, Perrillaldehyde, geraniol, Neral, Geranial, N-Pentacontanol, 2-Hexyl-1-Decanol and β-Myrcene, β-ocimene (3Z), 3-carene, Linalool, Verbenol, Citronellol, Neral, Perillaldehyde, geranial, geranyl acetate, ledol and cyclohexanol.

The GC–MS analysis displayed an increase in the percentage of monoterpene, β myrcene from 3.13% (lag phase) (Fig. 5a) to 5.3% (stationary phase) (Fig. 5c). Citral (neral+geranial) present in all leaf growth stages exhibited an increasing trend in peak % area; lag phase 52.03% (22.34+29.69), log phase 64.26% (25.86+38.4), and stationary phase 69.27% (29.35+39.92). Accordingly, the highest citral content was observed in the stationary phase of lemongrass leaves. These data demonstrated significant qualitative and quantitative differences in essential oil composition across the three developmental stages of lemongrass leaves.

Tab. 2: Comparative analysis of *C. citratus* essential oil at three leaf growth stages through GC-MS

Compound	Lag phase (%)	Log phase (%)	Stationary phase (%)
β Myrcene	3.13	3.75	5.3
Neral	22.34	25.86	29.35
Geranial	29.69	38.4	39.92
Perillaldehyde	-	1.61	3.4

Antioxidant activity

DPPH radical scavenging activity

Data showed that as the concentration increased, the absorbance decreased. The lag phase exhibited the highest value (84.42±0.03%), followed by the log phase (60.03±0.05%) and the stationary phase (54.62±0.13%) at 50 µl/ml. The standard showed the highest value of 94.45±0.96% at 50 µl/ml (Fig. 6a).

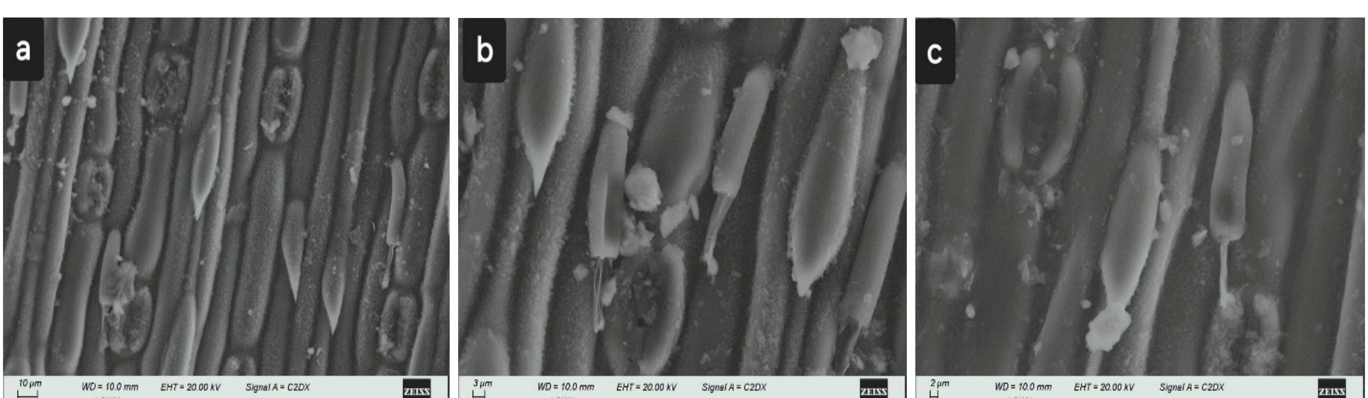


Fig. 4: High magnification SEM micrographs of *Cymbopogan citratus* trichomes of selected leaves growth phases, a-c capitate trichomes (microhair) of lag, log and stationary phase

Total phenolic content estimation

The essential oils were analyzed using a standard curve of gallic acid (50–3.125 µg/mL). The total phenolic content at 50 µl/ml was highest in the lag phase (1123.61±16.71 µg GAE/g), followed by the log phase (847.97±16.71 µg GAE/g) and the stationary phase (526.46±16.71 µg GAE/g). (Fig. 6b).

Total Antioxidant Capacity Assay

The results were calibrated using butylated hydroxyl toluene (BHT) as the standard. Results showed that all leaf growth stages of *C. citratus* at all concentrations exhibited significant total antioxidant capacity ($p < 0.05$). At 50 µL/mL, the total antioxidant capacity was highest in the stationary phase (0.7±0.15%), followed by the lag and log phases (both 0.69±0.15%) (Fig. 6c).

Anti-inflammatory activity

For anti-inflammatory potential, values were compared with the standard drug dicloran (Fig. 7). The values exhibited by lag phase (75.53±0.45%), log phase (79.71±0.04%) and stationary phase were (82.12±0.04%) at 50 µl/ml. Dicloran displayed the highest value (85.23±0.58%) at 50 µl/ml. *C. citratus* at all concentrations displayed significant anti-inflammatory activity (*p< 0.05*).

Anti-diabetic activity

The α-amylase inhibitory assay was assessed to evaluate anti-diabetic potential of selected leaf growth stages (lag, log and stationary) of *C. citratus* essential oil and values were compared with standard drug Sitaglu-Met (Fig. 8). The value exhibited by lag phase (90.06±0.08%), log phase (89.14±0.14%) and stationary phase were (92.56±0.03%) at 50 µl/ml. The standard exhibited highest value (88.87±1.06%) at 50 µl/ml.

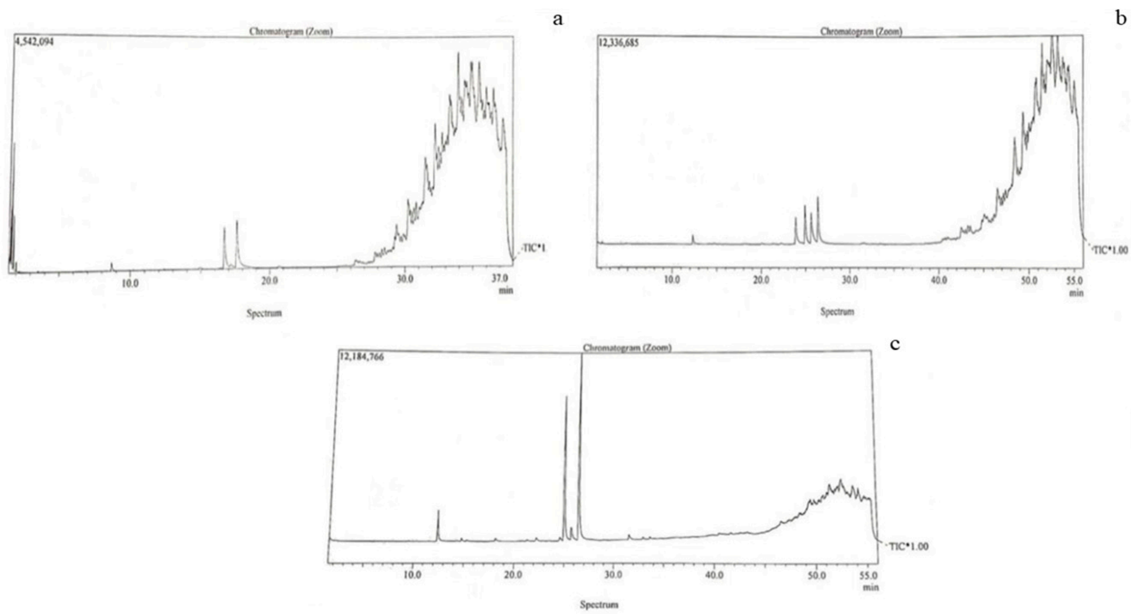


Fig. 5: GC-MS spectrum of *C. citratus* essential oil (a) Lag phase, (b) Log phase (c) Stationary phase

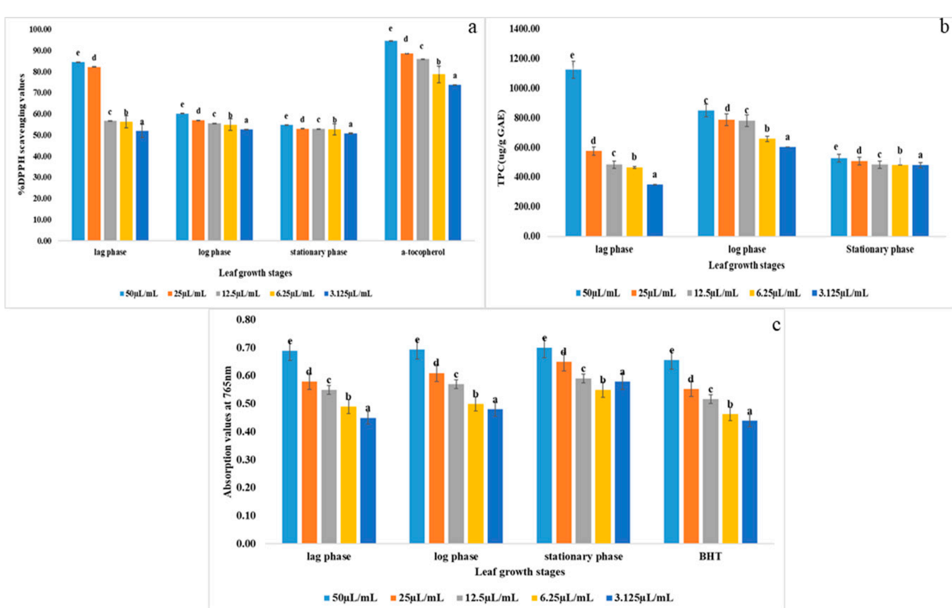
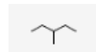
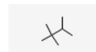
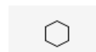
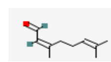
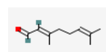
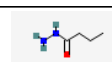
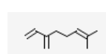
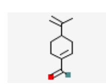
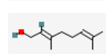
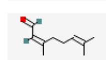
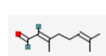
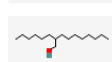


Fig. 6: Antioxidant Evaluation of *C. citratus* essential oil at varying concentrations (a) DPPH Radical scavenging potential, (b) Total phenolic contents, (c) Total antioxidants

Tab. 3: GC-MS analysis of *C. citratus* essential oil at lag phase

Compound	Molecular Weight	Molecular Formula	Structural Formula	Retention Time	Similarity Index	Peak Area %
2-Methyl pentane	86	C_6H_{14}		1.59	93	3.28
3-Methylpentane	86	C_6H_{14}		1.635	94	4.7
Butane, 2,2,3-Trimethyl	100	C_7H_{16}		1.688	91	11.63
Methylcyclopentane	84	C_6H_{12}		1.823	96	5.46
Cyclohexane	84	C_6H_{12}		2.002	87	1.05
β Myrcene	136	$C_{10}H_{16}$		8.662	86	3.13
Neral	152	$C_{10}H_{16}O$		16.65	90	22.34
Geranial	152	$C_{10}H_{16}O$		17.558	96	29.69

Tab. 4: GC-MS analysis of *C. citratus* essential oil at log phase

Compound	Molecular Weight	Molecular Formula	Structural Formula	Retention Time	Similarity Index	Peak Area%
Butyric acid hydrazide	102	$C_4H_{10}N_2O$		2.07	82	0.48
β Myrcene	136	$C_{10}H_{16}$		12.293	91	3.75
Perillaldehyde	150	$C_{10}H_{14}O$		25.669	91	1.6
Geraniol	152	$C_{10}H_{18}O$		24.893	91	1.9
Neral	152	$C_{10}H_{16}O$		25.575	96	25.86
Geranial	152	$C_{10}H_{16}O$		26.363	96	38.4
N-Pentacontanol	718	$C_{50}H_{102}O$		48.428	88	0.23
2-Hexyl-1-Decanol	242	$C_{16}H_{34}O$		51.42	89	0.26

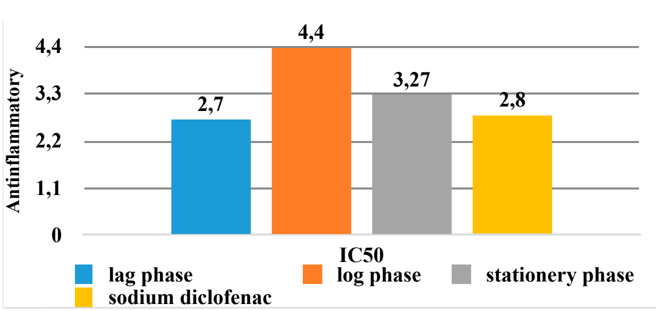


Fig. 7: IC_{50} of *C. citratus* essential oil of three leaf growth stages and diclofenac standard for anti-inflammatory assay

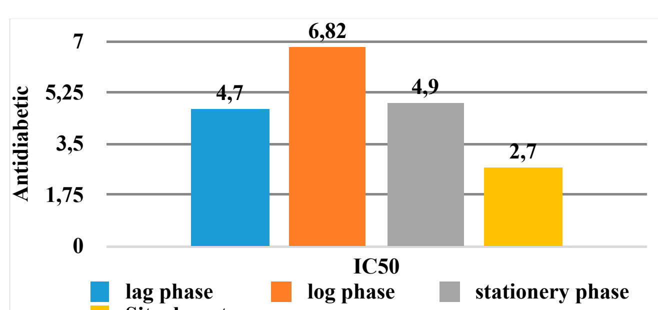
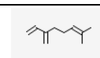
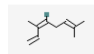
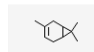
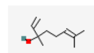
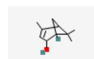
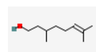
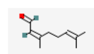
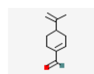
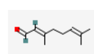
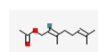
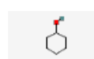


Fig. 8: IC_{50} of *C. citratus* essential oil of three leaf growth stages and Sitaglumet standard for α-amylase inhibition assay

Tab. 5: GC-MS analysis of *C. citratus* essential oil at stationary phase

Compound	Molecular Weight	Molecular Formula	Structural Formula	Retention Time	Similarity Index	Peak Area%
β Myrcene	136	$C_{10}H_{16}$		12.315	95	5.3
Beta-ocimene (3Z)	136	$C_{10}H_{16}$		14.713	87	0.57
3-Carene	136	$C_{10}H_{16}$		15.229	79	0.33
Linalool	154	$C_{10}H_{18}O$		18.102	83	0.84
Verbenol	152	$C_{10}H_{16}O$		22.155	83	1.06
Citronellol	156	$C_{10}H_{20}O$		24.527	89	1.07
Neral	152	$C_{10}H_{16}O$		24.954	92	29.35
Perillaldehyde	150	$C_{10}H_{14}O$		25.669	91	3.4
Geranial	152	$C_{10}H_{16}O$		26.457	97	39.92
Geranyl acetate	196	$C_{12}H_{20}O_2$		31.515	88	1.38
Ledol	222	$C_{15}H_{26}O$		32.946	77	0.4
Cyclohexanol	334	$C_6H_{12}O$		33.652	77	0.48

Antibacterial potential of *C. citratus* essential oil at selected growth stages

Agar-well diffusion method

The antimicrobial activity of essential oil obtained from the selected leaf growth stages (lag, log and stationary) of *C. citratus* essential oil were investigated at different concentrations (50-3.125 µl/ml). The efficacy of oils was assessed against pathogenic bacteria *Xanthomonas oyrzae*, *Staphylococcus aureus* and *Ralstonia solanaceaerum*. The zones of inhibition (mm) were measured on agar well containing different concentration of EOs after 24 hours (Tab. 5a. The results reported that highest zone of inhibition against *Staphylococcus aureus* of lag phase (17±1.31), log phase (20.1±0.36) while stationary phase (37±1.39) at 50 µl/ml was observed (Tab. 5a). The highest value for the zone of inhibition (mm) against *R. solanacearum* of lag phase (29.5±0.71), log phase (21±1.41) whereas stationary phase (27.5±1.19) at 50 µl/ml. (Fig. 9b). Zone of inhibition against *X. oryzae* was analyzed by, lag phase (23.5±1.68), log phase (31±1.49) while stationary phase (28±1.31) at 50 µl/ml Tab. 5a.

Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Assay

Tab. 5b showed MIC of *S. aureus*, *X. syringae* and *R. solanceaerum* treated with *C. citratus* essential oil of three leaf growth stages. Results revealed that lag and stationary phase both exhibited highest MIC. Tab. 6 reported the MBC of three leaf growth stages against pathogenic bacterial strains. These data demonstrated that all lag, log, and stationary phases displayed considerable antibacterial activity.

Effect of *C. citratus* essential oil on biofilm formation

In the current study, (%) biofilm inhibition potential of *C. citratus* essential oil of three leaf growth stages (lag, log and stationary) at sub-MIC values was evaluated against *S. aureus*, *R. solanaceaerum* and *X. oyrzae* pathogenic bacterial strains (Fig. 9-10). Results reported that maximum (%) biofilm inhibition potential value against *S. aureus* was observed by lag phase (34.59±0.07), log phase (38.29±0.12) and stationary phase (38.78±0.12) at 50 µl/ml. Gentamycin displayed highest value (41.3±7.78) at 50 µl/ml (Fig. 9a). Results reported that maximum (%) biofilm inhibition potential value against *R. solanceaerum* was observed by lag phase (64.00±0.07), log phase (56.89±0.11) and stationary phase (91.00±0.00) at 50 µl/ml. Gentamycin displayed (64.32±0.07) at 50 µl/ml) (Fig. 9b). Results reported that maximum (%) biofilm inhibition potential value against *X. oyrzae* was observed by lag phase (89.57±0.03), log phase (89.53±0.01) and stationary phase (89.37±0.01) at 50 µl/ml. Gentamycin displayed (89.32±0.01) at 50 µl/ml (Fig. 9c).

Hemolytic activity

Hemolytic potential of three leaf growth stages (lag, log and stationary) of *C. citratus* essential oil was determined and values were compared with 0.1% Triton X-100. The hemolytic percentage of H_2O_2 (negative control) was calculated as 5% and 100% for Triton X-100 (positive control). Results displayed that maximum value was observed by log phase (6.02±0.02), lag phase (5.36±0.01) and stationary phase (3.97±0.03) at 50 µl/ml. As the concentration of plant decreases, its hemolytic activity also reduces (Fig. 10). The higher

Tab. 5a: Zone of inhibition (mm) produced by various concentration of selected leaf growth stages of *C. citratus* essential oil against *(a) Staphylococcus aureus (b) Ralstonia solanacearum (c) Xanthomonas oryzae*

	Staphylococcus aureus			*Ralstonia solanacearum*			*Xanthomonas oryzae*		
Conc. (µL/mL)	**lag**	**log**	**stationery**	**lag**	**log**	**stationery**	**lag**	**log**	**stationery**
50	17±0.1^{e}	20.1±0.1^{e}	37±0.1^{e}	29.5±0.1^{e}	21±0.1^{e}	27.5±0.1^{e}	23.5±0.1^{e}	28±0.1^{e}	31.5±0.1^{e}
25	15±0.2^{d}	15±0.2^{d}	23±0.1^{d}	28.5±0.1^{d}	17.5±0.1^{d}	26±0.1^{e}	23.5±0.1^{d}	27.5±0.1^{d}	29.5±0.1^{d}
12.5	13.5±0.3^{c}	14.5±0.3^{c}	19±0.1^{c}	24.5±0.2^{c}	14±0.1^{c}	25±0.1^{e}	23±0.1^{c}	26±0.1^{c}	27.5±0.1^{c}
6.25	11±0.3^{b}	14±0.2^{b}	17±0.3^{b}	21±0.1^{b}	10.5±0.3^{b}	23±0.1^{e}	19.5±0.1^{b}	25±0.1^{b}	24.5±0.1^{b}
3.125	10.5±0.1a	12±0.1^{a}	13±0.2^{a}	13±0.2^{a}	67±0.1^{e}	17.5±0.1^{a}	15.5±0.1^{a}	16.5±0.1^{a}	20.5±0.1^{a}

Tab. 5b: Minimum Inhibitory Concentration of *C. citratus* against *S. aureus*, *X. oyrazae* and *R. solanceaerum* isolates at lag, log and stationary phases

MIC (µl/ml)	***Staphylococcus aureus***	***Xanthomonas oyrazae***	***Ralstonia solanceaerum***
lag phase	6.25	25	50
log phase	12.5	50	50
stationary phase	50	50	50
gentamycin	25	50	50

Tab. 6: Minimum Bactericidal Concentration of *C. citratus* against *S. aureus*, *X. oyrazae* and *R. solanceaerum* isolates at lag, log and stationary phases

MBC (µl/ml)	***Staphylococcus aureus***	***Xanthomonas oyrazae***	***Ralstonia solanceaerum***
lag phase	3.125	12.5	12.5
log phase	3.125	6.25	6.25
stationary phase	12.5	12.5	12.5
gentamycin	3.125	50	6.25

EO concentration resulted hemolysis degradation, whereas, lower EO dilutions exhibited higher hemolysis degradation, likely due to increased DMSO concentrations. The DMSO had significant effect on human red blood cells affecting osmotic fragility and increasing hemolysis (Yi *et al*., 2017). IC50 value s for the haemolysis at lag, log and stationary phases were noted as 2.5. 2.2 and 1.6 respectively. Pearson correlation of lag phase: An inverse relation is established between concentration and DPPH (%) radical scavenging activity

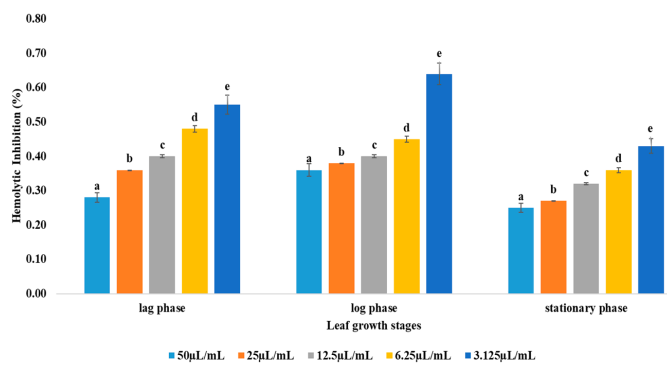


Fig. 10: Hemolytic activity of three leaf growth stages of *C. citratus* at different concentration

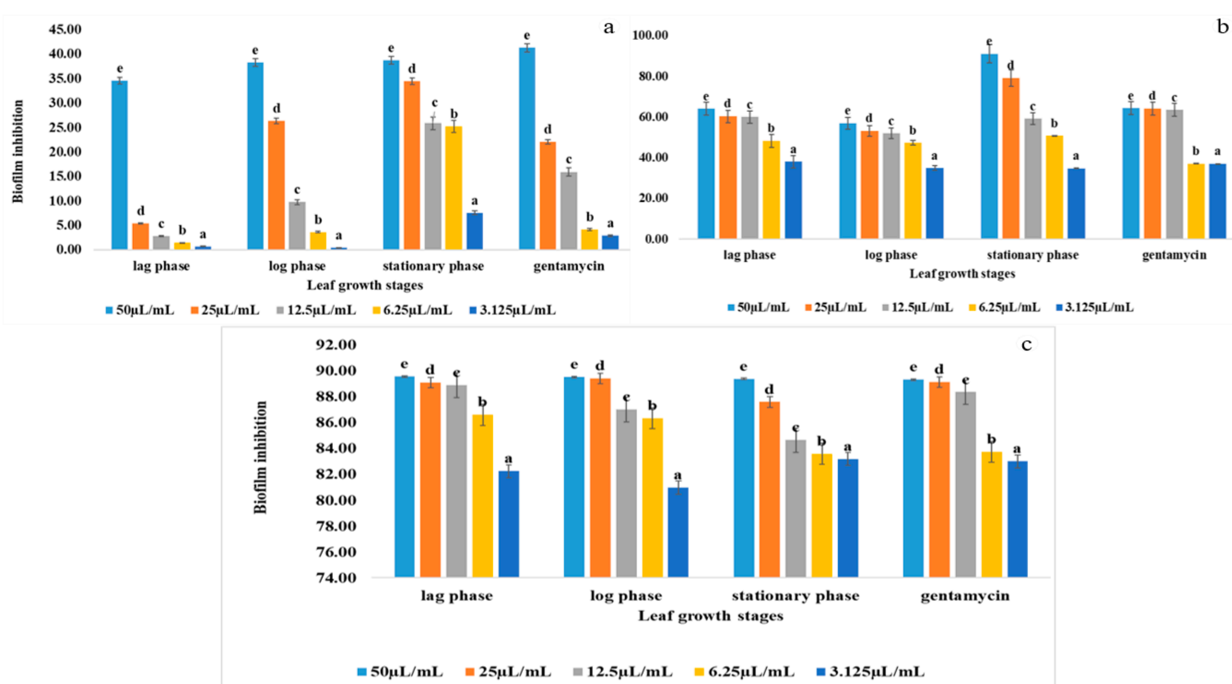


Fig. 9: Biofilm inhibition potential of *C.citratus* essential oil at three selected leaf growth stages against (a) *S. aureus (b) R. solanceaerum* (c) *Xanthomonas oryzae*

(R^2=-0.89009) indicated that increasing concentration caused notable inhibition DPPH (%) radical scavenging activity. On the other hand, a striking positive correlation between biofilm formation of *S. aureus* and biofilm formation of *R. solanceaerum* (R^2=0.97859) implies that biofilm formation in *S. aureus* and *R. solanceaerum* are strongly associated. Pearson correlation of log phase: A strong negative correlation between concentration and total antioxidant capacity (R^2=-0.805) indicates that increasing concentration leads to a marked decrease in total antioxidant capacity. Strong positive correlation between DPPH (%) radical scavenging activity and anti-diabetic activity (R^2=0.924) indicating relation that higher DPPH (%) radical scavenging activity is correlated with higher anti-diabetic activity. Pearson correlation of stationary phase: A striking negative correlation of concentrations with anti-diabetic (R^2=-0.933), inhibition zone of *S. aureus* (R^2=-0.951), and biofilm inhibition of *S. aureus* (R^2=-0.977) indicating that with the increase in concentrations all these activities showed inhibition. However, remarkable positive relation of DPPH (%) radical scavenging activity with anti-diabetic (R^2=0.913) (Fig. 11).

Discussion

Essential oils are complex mixtures of volatile compounds with fragrance and defensive properties derived from plants (Erminawati et al., 2019). To ensure the safety and effectiveness of essential oil preparation, quality uniformity must be maintained (Żukowska et al., 2024). This comprehensive investigation aims to assess the chemical makeup and biological activity of essential oils from *C. citratus* leaves obtained at different leaf growth stages. *Cymbopogon* plants have a diverse range of pharmacological benefits, including anti-inflammatory, antibacterial, antidiabetic, and other advantageous qualities, making them potential sources for both culinary and medicinal purposes (Zhao et al., 2024; Niazi et al., 2024). According to the literature, the plant *Cymbopogon citratus*, known as lemongrass, is abundant in bioactive constituents (Oldaeji et al., 2019). The principal phytochemical compounds from its leaves are flavonoids, alcohols, aldehydes, alkaloids, esters, ketones, tannins, terpenes, and phenolics (Karpagam et al., 2016). These constituents are well-known for their substantial potential in the domains of pharmacology, food science, and agriculture (Hasim et al., 2015).

The leaf surface of *C. citratus* is glabrous (Yeşil et al., 2015). A wide range of glandular trichomes may be the result of several evolutionary processes. These differences include shape, cell quantity, and types of released metabolites (Singh et al., 2019). Many metabolites are stored and released by glandular trichomes at the leaf surface (Shi et al., 2018). The density of plant trichomes can also be impacted by environmental stressors such as excessive salt, low temperatures, drought stress, heavy metal exposure, severe photoperiod, and UV light exposure (Hauser et al., 2014; Tian et al., 2017; Wang et al., 2021). Lewinsohn et al. (1998) conducted a study on localized oil cells in lemongrass leaves. They reported that individual oil cells within the leaf tissues are the site of citral formation. Similarly, Madi et al. (2022) performed an experiment to obtain a comprehensive evaluation of morphological and anatomical characters of lemongrass leaf. They reported the presence of oil cells on the abaxial surface of the leaf. Differences in adaxial and abaxial leaf epidermis were observed by examining the presence of numerous micro-hairs and graminaceous stomata on the abaxial surface only. Our study also affirmed the abundance of micro-hairs and graminaceous stomata in the abaxial epidermis. The capitate-stalked

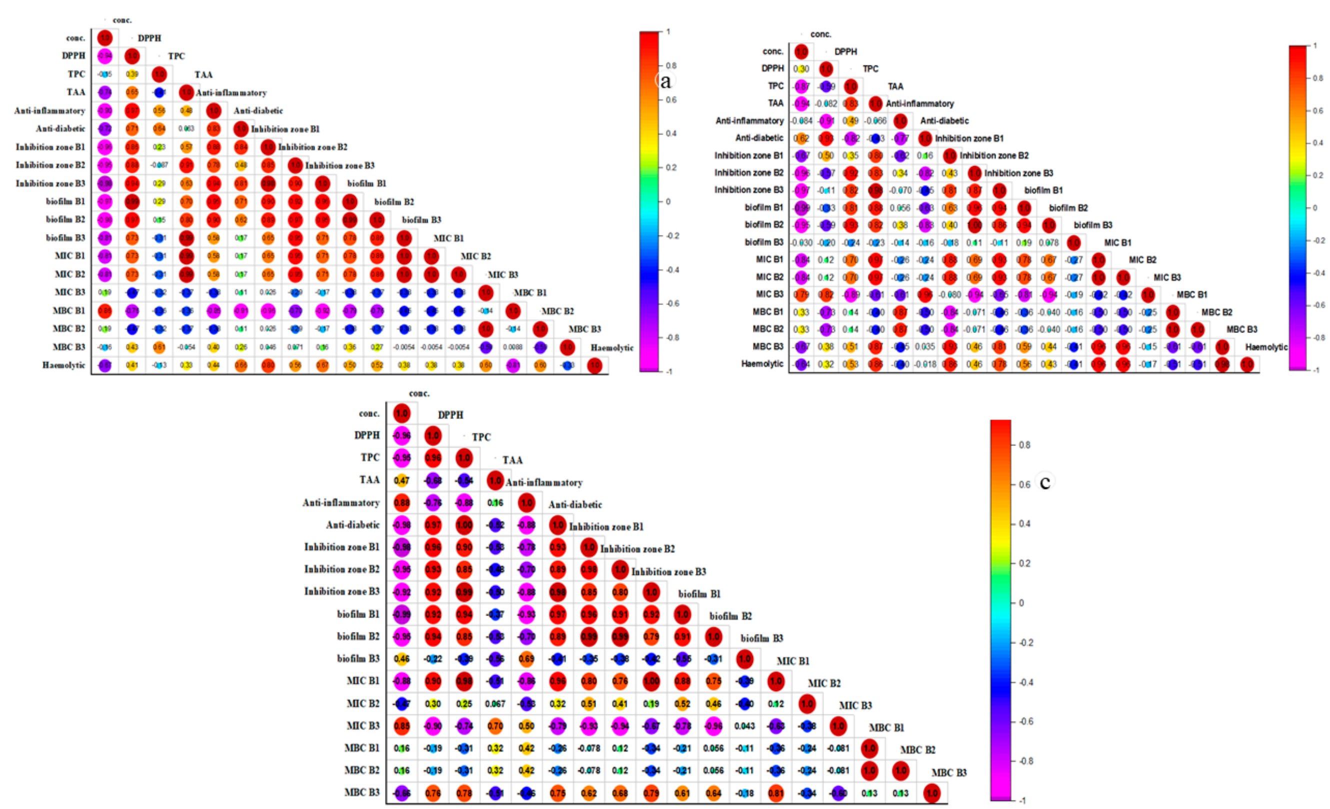


Fig. 11: Pearson Correlation of (a) lag phase, (b) log phase, (c) Stationary phase
Key: conc., concentration; TPC, total phenolic content; TAA, total antioxidant capacity assay; Inhibition zone B1, *S. aureus*; Inhibition zone B2, *R. solanacearum*; Inhibition zone B3, *X. oyrazeae*; Biofilm B1, *S. aureus*; Biofilm B2, *R. solanacearum*; Biofilm B3,1*X. oyrazeae*; MIC B1, minimum inhibitory concentration of *S. aureus*; MIC B2, minimum inhibitory concentration of *R. solanacearum*; MIC B3, minimum inhibitory concentration of *X. oyrzae*; MBC B1, minimum bactericidal concentration of *S. aureus*; MBC B2, minimum bactericidal concentration of *R. solanacearum*; MBC B3, minimum bactericidal concentration of *X. oyrzae*.

trichome structure was described by Guimarães et al. (2008) as having a spherical head made of a layer of secretory cells enclosing a central cell, a stalk cell, and a base cell.

Punja et al. (2023) identified capitate-stalked trichomes in their study. They documented that as the plant reaches maturation, its trichome length increases, which was also noted in our current study. Based on these studies, it was concluded that in the current investigated plant, glandular trichomes reached their maximum length from 121.36±3.12 µm (lag phase), 132.16±2.14 (log phase), and 195.6±4.59 (stationary phase). Only capitate-stalked glandular trichomes have been observed on the abaxial epidermis in this investigation. Analysis of the essential oils produced during the lag, log, and stationary phases identified, oxygenated monoterpenes, monoterpene aldehydes, and alcohols. The details of these compounds are shown in Tab. 1. It was observed that as the plant matures, its oil yield decreased, i.e., lag (0.15%), log (0.14%), and stationary (0.12%). The increase in fiber content of the leaf could be a reason for the decrease in oil yield.

Citral (3,7-dimethyl-2,6-octadien-2-al) is an isomer of two acyclic monoterpene aldehydes, geranial and neral (Ha et al., 2008). Citral was found to be a major component in all leaf growth stages of *C. citratus* essential oils. Citral inhibits oxidative stress and alters enzyme activity involved in drug metabolism (Li et al., 2018). Citral content of essential oil varied when lemongrass was collected at various stages. The finding was in accordance with Tajidinet et al. (2012), who documented the trend of harvesting lemongrass at 5.5, 6.5, and 7.5 months intervals. Variation was observed in citral content, and the highest content was extracted after lemongrass was harvested at 7.5 months. These observations were further affirmed by an experiment conducted by Bekele et al. (2019) that lemongrass produces maximum citral as the plant reaches its maturity stage.

Perillaldehyde, Verbenol, and citronellol were recognized as the main compounds only in the stationary phase of oil. The phytochemical makeup of the essential oils is thought to be influenced by the developmental processes that take place throughout leaf growth, as evidenced by the observed variations in color and scent of the oils produced at different leaf stages as well as the absence of some chemicals in the early developmental stage. Valková et al. (2022) reported that the essential oil extracted from *C. citratus* leaves is high in oxygenated monoterpenes like citral, which is in accordance with the information shown in Tab. 1. Antioxidants are substances that inhibit or neutralize the oxidative reactions caused by atmospheric oxygen. They are fundamental to an organism's defensive mechanism against illnesses brought about by the negative effects of free radicals (Aziz et al., 2022). Since plant-derived antioxidants can counteract oxidative stress and protect the neurological system from age-related deterioration, plants are often researched for their antioxidant qualities (Naseer et al., 2019; Cui et al., 2020; Riaz et al., 2021). In the current study, the antioxidant potential of *C. citratus* was investigated by 2,2-diphenyl-2-picrylhydrazil (DPPH) free radical scavenging activity, total phenolic content (TPC), and total antioxidant capacity (TAC). The DPPH radical is stable and is assessed to evaluate the antioxidant potential of plants. Compounds demonstrating potent DPPH scavenging activity can be assessed as potential therapeutic agents for oxidative stress-related diseases (Gulcin and Alwasel, 2023). *C. citratus* essential oil of the lag phase displayed the highest value (84.42±0.03) DPPH (%) free radical scavenging activity (Bärlocher and Graça, 2020).

The microbial colonies known as biofilms, which are encased in an extracellular matrix that they manufacture on their own, play a major role in persistent bacterial infections (Gautam et al., 2020). Reversible and irreversible attachment, microcolony production, maturation, and ultimately biofilm dispersal are all steps in the process of biofilm formation. Many illnesses linked to tissues, such as kidney infections, meningitis, endocarditis, and persistent wounds that do not heal, have been linked to biofilms (Khatoon et al., 2018; Rather et al., 2021). A potential approach to treating infections is the use of antimicrobial drugs that possess both antibacterial and antibiofilm qualities. This study evaluated the *C. citratus* essential oil's ability to prevent biofilms against *S. aureus*, *R. solanaceaerum*, and *X. oyrzae* at sub-minimum inhibitory concentrations using leaf growth stages (lag, log, and stationary). Numerous latent, acute, and chronic diseases have been linked to these pathogenic bacterial species by their ability to create biofilms (Harrell et al., 2021; Schulze et al., 2021; Rather et al., 2021). *C. citratus* essential oil of leaf growth stages (lag, log, and stationary) displayed significant biofilm inhibitory potential against all pathogenic strains. The results reported that the stationary phase showed the highest (%) biofilm inhibitory potential against *S. aureus* (38.78±0.12), *R. solanacearum* (91.00±0.07), and the lag phase against *X. oryzae* (89.57±0.01).

Toxicological evaluations may determine a range of negative consequences related to the ingestion of therapeutic plants or herbs, particularly in people who are susceptible (Kauser et al., 2018). Hemolysis, also known as erythrocyte rupture, is an indicator of a substance's adverse impact on red blood cells (Velika et al., 2012). These substances or extracts would not be deemed appropriate for ingestion by humans as therapeutic agents (Hussain et al., 2023). The maximum (%) hemolytic value was evaluated from the stationary phase of lemongrass leaf essential oil (0.25±0.03). However, all of the leaf growth stages (lag, log, and stationary) showed hemolytic activity within the range of (0.25-0.64%), suggesting that they might be used safely by humans. The higher EO concentration resulted hemolysis degradation, whereas, lower EO dilutions exhibited higher hemolysis degradation, likely due to increased DMSO concentrations. According to Yi et al. (2017), DMSO has a profound impact on human red blood cells, increasing osmotic fragility and significantly enhancing hemolysis (Yi et al., 2017).

Conclusion

In the present study microscopic analysis of *C. citratus* leaves revealed the presence of capitate-stalked trichomes on the abaxial epidermis. GC-MS analysis of essential oils obtained from leaves at various growth stages identified distinct phytochemical profiles. The lag phase exhibited the highest oil yield and antioxidant activity, while the stationary phase demonstrated strong anti-inflammatory and anti-diabetic properties. Both phases exhibited significant antibacterial and biofilm inhibition potential. Additionally, citral and β-myrcene were consistently present, suggesting their potential roles in the observed pharmacological activities. These compounds, known for their antioxidant, anti-inflammatory, anti-diabetic, antibacterial and anti-biofilm properties**,** highlight the therapeutic potential of *C. citratus* leaf essential oil. Toxicological assessments confirmed the safety of this essential oil for human consumption, supporting its potential application in various health-related fields.

Conflict of interest

No potential conflict of interest was reported by the authors.

References

Abed, K.F., 2007: Antimicrobial activity of essential oils of some medicinal plants from Saudi Arabia. Saudi J. Biol. Sci. 14(1), 53-60.

Adams, R.P., 2017: Identification of essential oil components by gas chromatography/mass spectrometry. 5 online ed. Gruver, TX USA: Texensis Publishing.

Anand, U., Jacobo-Herrera, N., Altemimi, A., Lakhssassi, N., 2019: A comprehensive review on medicinal plants as antimicrobial therapeutics:

potential avenues of biocompatible drug discovery. Metabolites 9(11), 258. DOI: 10.3390/metabo9110258

Atta, E., El-Shabasy, A., 2022: Chemotaxonomic and morphological classification of six Indigofera species in Jazan region, KSA. J. Saudi Chem. Soc. 26(3), 101476. DOI: 10.1016/j.jscs.2022.101476

Avoseh, O., Oyedeji, O., Rungqu, P., Nkeh-Chungag, B., Oyedeji, A., 2015: *Cymbopogon* species; ethnopharmacology, phytochemistry and the pharmacological importance. Molecules 20(5), 7438-7453. DOI: 10.3390/molecules20057438

Aziz, M., Ahmad, S., Iqbal, M.N., Khurshid, U., Saleem, H., Alamri, A., Chohan, T.A., 2022: Phytochemical, pharmacological, and In-silico molecular docking studies of *Strobilanthes glutinosus* Nees: An unexplored source of bioactive compounds. S. African J. Bot. 147, 618-627. DOI: 10.1016/j.sajb.2021.07.013

Bagheri, H., Manap, M.Y.B.A., Solati, Z., 2014: Antioxidant activity of *Piper nigrum* L. essential oil extracted by supercritical CO_2 extraction and hydro-distillation. Talanta 121, 220-228. DOI: 10.1016/j.talanta.2014.01.007

Banerjee, P., Eckert, A.O., Schrey, A.K., Preissner, R., 2018: ProTox-II: a webserver for the prediction of toxicity of chemicals. Nucl. Acids Res. 46(W1), W257-W263. DOI: 10.1093/nar/gky318

Bärlocher, F., Graça, M.A., 2020: Total phenolics. Methods to study litter decomposition: a practical guide, 157-161. DOI: 10.1007/978-3-030-30515-4

Bekele, W., Tesema, M., Mohammed, H., Mammo, K., 2019: Herbage yield and bio-chemical traits as influenced by harvesting age of lemongrass (*Cymbopogon citratus* (DC) STAPF varieties at Wondogenet, South Ethiopia. Int. J. Res. Agric. Sci. 214(2), 149-160.

Choi, J.-S., Kim, E.-S., 2013: Structural features of glandular and non-glandular trichomes in three species of Mentha. Appl. Microscopy 43(2), 47-53. DOI: 10.9729/AM.2013.43.2.47

Cui, X., Lin, Q., Liang, Y., 2020: Plant-derived antioxidants protect the nervous system from aging by inhibiting oxidative stress. Front Aging Neurosci. 12, 209. DOI: 10.3389/fnagi.2020.00209

Daulagala, P., 2021: Chitinolytic endophytic bacteria as biocontrol agents for phytopathogenic fungi and nematode pests: A review. Asian J. Res. Bot. 5(3), 14-24.

de Kraker, M.E., Stewardson, A.J., Harbarth, S., 2016: Will 10 million people die a year due to antimicrobial resistance by 2050?. PLoS medicine 13(11), e1002184. DOI: 10.1371/journal.pmed.1002184

Duarte, A.E., De Menezes, I.R.A., Bezerra Morais Braga, M.F., Leite, N.F., Barros, L.M., Waczuk, E.P., Pessoa da Silva, M.A., Boligon, A., Teixeira Rocha, J.B., Souza, D.O., Kamdem, J.P., Melo Coutinho, H.D., Escobar Burger, M., 2016: Antimicrobial Activity and Modulatory Effect of Essential Oil from the Leaf of *Rhaphiodon echinus* (Nees and amp; Mart) Schauer on Some Antimicrobial Drugs. Molecules 21(6), 743. DOI: 10.3390/molecules21060743

Ekpenyong, C.E., Akpan, E.E., 2017: Use of *Cymbopogon citratus* essential oil in food preservation: Recent advances and future perspectives. Crit. Rev. Food Sci. Nutr. 57(12), 2541-2559. DOI: 10.1080/10408398.2015.1016140

Erminawati, Naufalin, R., Sitoresmi, I., Sidik, W., Bachtiar, A., 2019: Antioxidant activity of microencapsulated lemongrass (*Cymbopogon citratus*) extract. IOP Conference Series: Earth Environmental Science. DOI: 10.1088/1755-1315/250/1/012054

Gao, S., G. Liu, J., Li, J., Chen, L., Li, Z., Li, X., Zhang, S., Zhang, R.F., Thorne, Zhang, S., 2020: Antimicrobial activity of lemongrass essential oil (*Cymbopogon flexuosus*) and its active component citral against dual-species biofilms of Staphylococcus aureus and Candida species. Front Cell Infect Microbiol. 10, 603858. DOI: 10.3389/fcimb.2020.603858

Gautam, N., Salaria, N., Thakur, K., Kukreja, S., Yadav, N., Yadav, R., Goutam, U., 2020: Green silver nanoparticles for phytopathogen control. Proceed National Ac. Sci. India Section B: Biol. Sci. 90, 439-446. DOI: 10.1007/s40011-019-01115-8

Guimarães, E., Di Stasi, L.C., Maimoni-Rodella, R.D.C.S., 2008: Pollination biology of *Jacaranda oxyphylla* with an emphasis on staminode function. Annals Bot. 102(5), 699-711. DOI: 10.1093/aob/mcn152

Gul, S., Ahmad, M., Zafar, M., Bahadur, S., Sultana, S., Ashfaq, S., Ullah, F., Kilic, O., Hassan, F.U., Siddiq, Z., 2019: Foliar epidermal anatomy of Lamiaceae with special emphasis on their trichomes diversity using scanning electron microscopy. Microsc. Res. Techn. 82(3), 206-223. DOI: 10.1002/jemt.23157

Happyana, N., Agnolet, S., Muntendam, R., Van Dam, A., Schneider, B., Kayser, O., 2013: Analysis of cannabinoids in laser-microdissected trichomes of medicinal *Cannabis sativa* using LCMS and cryogenic NMR. Phytochem. 87, 51-59. DOI: 10.1016/j.phytochem.2012.11.001

Harrell, J.E., Hahn, M.M., D'Souza, S.J., Vasicek, E.M., Sandala, J.L., Gunn, J.S., McLachlan, J.B., 2021: Salmonella biofilm formation, chronic infection, and immunity within the intestine and hepatobiliary tract. Front. Cell. Infect. Microbiol. 10, 624622. DOI: 10.3389/fcimb.2020.624622

Hasim, H., Falah, S., Ayunda, R., Faridah, D., 2015: Potential of lemongrass leaves extract (*Cymbopogon citratus*) as prevention for oil oxidation. J. Chem. Pharm. Res. 7(10), 55-60.

Hauser, M.T., 2014: Molecular basis of natural variation and environmental control of trichome patterning. Front. Plant Sci. 5, 320. DOI: 10.3389/fpls.2014.00320

Hussain, S., Javed, W., Tajammal, A., Khalid, M., Rasool, N., Riaz, M., Shah, S.A.A., 2023: Synergistic antibacterial screening of *Cymbopogon citratus* and *Azadirachta indica*: phytochemical profiling and antioxidant and hemolytic activities. ACS omega 8(19), 16600-16611. DOI: 10.1021/acsomega.2c06785

Ibrahim, S.M., Al-Mizraqchi, A.S., 2024: Comparison of the antibacterial activity of *Panax Ginseng* and *Symphytum Officinale* with metronidazole against *P. gingivalis*: An MIC and MBC Analysis. Open Dent. J. 18(1). DOI: 10.3390/antibiotics12081288

Jamal, M., Bukhari, S.M., Andleeb, S., Ali, M., Raza, S., Nawaz, M.A., Hussain, T., Rahman, S.U., Shah, S.S., 2019: Bacteriophages: An overview of the control strategies against multiple bacterial infections in different fields. J. Basic Microbiol. 59(2), 123-133. DOI: 10.1002/jobm.201800412

Karanović, D., Zorić, L., Zlatković, B., Luković, J., 2022: Leaf and stem anatomy and micromorphology of four Inuleae (Compositae) genera, with notes on their taxonomic significance. Nordic J. Bot. 7, e03566. DOI: 10.1111/njb.03566

Kaur, S., Sharma, P., 2015: Protease-sensitive inhibitory activity of cell-free supernatant of Lactobacillus crispatus 156 synergizes with ciprofloxacin, moxifloxacin and streptomycin against *Pseudomonas aeruginosa*: an in vitro study. Probiot. Antimicrob. Prot. 7, 172-180. DOI: 10.1007/s12602-015-9188-4

Kauser, A., Shah, S.M.A., Iqbal, N., Murtaza, M.A., Hussain, I., Irshad, A., Riaz, M., 2018: In vitro antioxidant and cytotoxic potential of methanolic extracts of selected indigenous medicinal plants. Progess Nutr. 20(4), 706-712. DOI: 10.23751/pn.v20i4.7523

Khatoon, Z., McTiernan, C.D., Suuronen, E.J., Mah, T.F., Alarcon, E.I., 2018: Bacterial biofilm formation on implantable devices and approaches to its treatment and prevention. Heliyon 4(12). DOI: 10.1016/j.heliyon.2018.e01067

Kim, S.S., Kim, I.J., Lee, K.J., Park, J.H., Kim, Y.I., Lee, Y.S., Lee, S.J., 2017: Efficacy and safety of sitagliptin/metformin fixed-dose combination compared with glimepiride in patients with type 2 diabetes: A multicenter randomized double-blind study. J. Diabetes 9(4), 412-422. DOI: 10.1111/1753-0407.12432

Lewinsohn, E., Dudai, N., Tadmor, Y., Katzir, I., Ravid, U.Z.I., Putievsky, E.L.I., Joel, D.M., 1998: Histochemical localization of citral accumulation in lemongrass leaves (*Cymbopogon citratus* (DC.) Stapf., Poaceae). Annals Bot. 81(1), 35-39. DOI: 10.1006/anbo.1997.0525

Li, C.-C., Yu, H.-F., Chang, C.-H., Liu, Y.-T., Yao, H.-T., 2018: Effects of lemongrass oil and citral on hepatic drug-metabolizing enzymes, oxidative stress, and acetaminophen toxicity in rats. J. Food Drug Anal. 26(1), 432-438. DOI: 10.1016/j.jfda.2017.01.008

Madi, Y.F., Meselhy, M.R., El-Kashoury, E.S.A., Choucry, M.A., 2022: Morphological and Anatomical Characterization of *Cymbopogon citratus* (DC.) Stapf Cultivated in Egypt. Bulletin Fac. Pharmacy Cairo Uni, 60(1), 6. DOI: 10.54634/2090-9101.1032

Martinazzo, A.P., de Oliveira, F.d.S., de Souza Teodoro, C.E., 2019: Antifungal activity of *Cymbopogon citratus* essential oil against *Aspergillus flavus*. Ciência e Natura, e20-e20. DOI: 10.5902/2179460X36055

Mohanty, S.K., Mallappa, K.S., Godavarthi, A., Subbanarasiman, B., Maniyam, A., 2014: Evaluation of antioxidant, in vitro cytotoxicity of micropropagated and naturally grown plants of *Leptadenia reticulata* (Retz.) Wight and Arn.-an endangered medicinal plant. Asian Pac. J. Tropical Med. 7, 267-271. DOI: 10.1016/S1995-7645(14)60244-3

Naseer, S., Afzal, M.S., Nisa, A., Hussain, S., Ahmad, M.A., Parveen, S., Riaz, M., 2019: Extraction of brown dye from Eucalyptus bark and its applications in food storage. Qual. Ass. Safety Crops Foods 11(8), 769-780. DOI: 10.3920/QAS2019.1569

Niazi, P., Monib, A.W., 2024: The role of plants in traditional and modern medicine. J. Pharmacog. Phytochem. 13(2), 643-647. DOI: 10.22271/phyto.2024.v13.i2d.14905

Olayemi, R., Jawonisi, I., Samuel, J., 2018: Characterization and physicochemical analysis of essential oil of *Cymbopogon citratus* leaves. Bayero J. Pure Applied Sci. 11(1), 74-81. DOI: 10.4314/bajopas.v11i1.14

Parvekar, P., Palaskar, J., Metgud, S., Maria, R., Dutta, S., 2020: The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of silver nanoparticles against *Staphylococcus aureus*. Biomat. Invest. Dent. 7(1), 105-109. DOI: 10.1080/26415275.2020.1796674

Perczak, A., Gwiazdowska, D., Marchwińska, K., Juś, K., Gwiazdowski, R., Waśkiewicz, A., 2019: Antifungal activity of selected essential oils against *Fusarium culmorum* and *F. graminearum* and their secondary metabolites in wheat seeds. Arch. Microbial. 201, 1085-1097. DOI: 10.1007/s00203-019-01673-5

Punja, Z.K., Sutton, D.B., Kim, T., 2023: Glandular trichome development, morphology, and maturation are influenced by plant age and genotype in high THC-containing cannabis (*Cannabis sativa* L.) inflorescences. J. Cannabis Res. 5(1), 12. DOI: 10.1186/s42238-023-00178-9

Raina, A., Laskar, R.A., Wani, M.R., Khan, S., 2022: Plant Breeding Strategies for Abiotic Stress Tolerance in Cereals. In: Omics Approach to Manage Abiotic Stress in Cereals, 151-177. Springer. DOI: 10.1007/978-981-19-0140-9_8

Ranitha, M., Nour, A.H., Sulaiman, Z.A., Nour, A.H., 2014: A comparative study of lemongrass (*Cymbopogon Citratus*) essential oil extracted by microwave-assisted hydrodistillation (MAHD) and conventional hydrodistillation (HD) Method. International J. Chemical Eng. Appl. 5(2), 104. DOI: 10.7763/IJCEA.2014.V5.360

Rather, M.A., Gupta, K., Bardhan, P., Borah, M., Sarkar, A., Eldiehy, K.S., Mandal, M., 2021: Microbial biofilm: A matter of grave concern for human health and food industry. J. Basic Microbiol. 61(5), 380-395. DOI: 10.1002/jobm.202000678

Riaz, M., Fatima, H., Rehman, M.M.U., Qadir, R., Hussain, S., Hafeez, A., Siddique, A.B., 2021: Appraisal of antioxidant potential and biological studies of bogan bail (*Bougainvillea glabra*) leaf extracts using different solvents. Czech. J. Food Sci. 39(3), 176-180. DOI: 10.17221/273/2020-CJFS

Rodríguez-Garza, N.E., Quintanilla-Licea, R., Romo-Sáenz, C.I., Elizondo-Luevano, J.H., Tamez-Guerra, P., Rodríguez-Padilla, C., Gomez-Flores, R., 2023: In vitro biological activity and lymphoma cell growth inhibition by selected mexican medicinal plants. Life 13(4), 958. DOI: 10.3390/life13040958

Sandhya, S., Sai Kumar, P., Vinod, K., Banji, D., Kumar, K., 2011: Plant as potent anti-diabetic and wound healing agents a review. Hygeia. J. Drugs Medicines 3, 11-19.

Sawant, S., Baldwin, T.C., Khan, H., Rahman, A., 2023: Evaluation of the effect of leaf development in *Plectranthus amboinicus* L. on antimicrobial activity and virulence factors of *Pseudomonas aeruginosa* PAO1 and *Staphylococcus aureus* NCTC8325. Current Microbiol. 80(1), 24. DOI: 10.1007/s00284-022-03126-7

Schulze, A., Mitterer, F., Pombo, JP., Schild S., 2021: Biofilms by bacterial human pathogens: clinical relevance-development, composition and regulation therapeutical strategies. Microbe Cell 8(2), 28-56. DOI: 10.15698/mic2021.02.741

Schuurink, R., Tissier, A., 2020. Glandular trichomes: micro-organs with model status? New Phytol. 225(6), 2251-2266. DOI: 10.1111/nph.16283

Shamsheer, B., Riaz, N., Yousaf, Z., Hyder, S., Aftab, A., Iqbal, R., ur Rahman, M.H., Al-Ashkar, I., Almutairi, K.F., El Sabagh, A., 2022: Genetic diversity analysis for wild and cultivated accessions of *Cymbopogon citratus* (DC) Stapf using phytochemical and molecular markers. Peer J. 10, e13505. DOI: 10.7717/peerj.13505

Sharma, A., Rashid, M., Chauhan, P., Kaur, S., Kaur, A., 2024: In vitro antibacterial and anti-biofilm potential of an endophytic *Schizophyllum commune*. AMB Express 14(1), 10. DOI: 10.1186/s13568-024-01663-x

Sharma, P.K., Fuloria, S., Alam, S., Sri, M.V., Singh, A., Sharma, V.K., Kumar, N., Subramaniyan, V., Fuloria, N.K., 2021: Chemical composition and antimicrobial activity of oleoresin of *Capsicum annuum* fruits. Mindanao J. Sci. Technol. 19(1). DOI: 10.61310/mndjstecbe.1030.21

Shendurse, A., Sangwan, R., Kumar, A., Ramesh, V., Patel, A., Gopikrishna, G., Roy, S., 2021: Phytochemical screening and antibacterial activity of lemongrass (*Cymbopogon citratus*) leaves essential oil. J. Pharmacogn. Phytochem. 10(2), 445-449.

Shi, P., Fu, X., Shen, Q., Liu, M., Pan, Q., Tang, Y., Jiang, W., Lv, Z., Yan, T., Ma, Y., 2018: The roles of Aa MIXTA 1 in regulating the initiation of glandular trichomes and cuticle biosynthesis in *Artemisia annua*. New Phytol. 217(1), 261-276. DOI: 10.1111/nph.14789

Sil, A., Pramanik, K., Samantaray, P., Firoz, M., Yadav, V., 2020: Essential oils: A boon towards eco-friendly management of phytopathogenic fungi. J. Entomol. Zool. Stud. 8, 1884-1891.

Singh, S.P., Upadhyay, S.K., Pandey, A., Kumar, S., 2019: Molecular Approaches in Plant Biology and Environmental Challenges, 1-5. Springer.

Thummajitsakul, S., Samaikam, S., Tacha, S., Silprasit, K., 2020: Study on FTIR spectroscopy, total phenolic content, antioxidant activity and anti-amylase activity of extracts and different tea forms of *Garcinia schomburgkiana* leaves. LWT 134, 110005. DOI: 10.1016/j.lwt.2020.110005

Tian, N., Liu, F., Wang, P., Zhang, X., Li, X., Wu, G., 2017: The molecular basis of glandular trichome development and secondary metabolism in plants. Plant Gene 12, 1-12. DOI: 10.1016/j.plgene.2017.05.010

Valková, V., Ďúranová, H., Galovičová, L., Borotová, P., Vukovic, N.L., Vukic, M., Kačániová, M., 2022: *Cymbopogon citratus* essential oil: Its application as an antimicrobial agent in food preservation. Agronomy 12(1), 155. DOI: 10.3390/agronomy12010155

Valková, V., Ďúranová, H., Galovičová, L., Vukovic, N.L., Vukic, M., Kačániová, M., 2021: In Vitro antimicrobial activity of lavender, mint, and rosemary essential oils and the effect of their vapours on growth of Penicillium spp. in a bread model system. Molecules 26(13), 3859. DOI: 10.3390/molecules26133859

Van Den Dool, H., Kratz, P.D., 1963: A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J. Chromatograph. 11, 463-71. DOI: 10.1016/S0021-9673(01)80947-X

Velika, B., Kron, I., 2012: Antioxidant properties of benzoic acid derivatives against superoxide radical. Free Radicals Antiox 2(4), 62-67. DOI: 10.5530/ax.2012.4.11

Velu, V., Banerjee, S., Radhakrishnan, V., Gupta, G., Chellappan, D.K., Fuloria, N.K., Fuloria, S., Mehta, M., Dua, K., Malipeddi, H., 2021: Identification of phytoconstituents of *Tragia Involucrata* leaf extracts and evaluate their correlation with anti-inflammatory and antioxi-

dant properties. Anti-inflammatory and anti-allergy agents in medicinal chemistry (*Formerly Current Medicinal Chemistry-Anti-Inflammatory and Anti-Allergy Agents*) 20(3), 308-315.
DOI: 10.2174/1871523020666210126144506

Wang, X., Shen, C., Meng, P., Tan, G., Lv, L., 2021: Analysis and review of trichomes in plants. BMC Plant Biology 21, 1-11.
DOI: 10.1186/s12870-021-02840-x

Widelska, G., Stelmasiewicz, M., Skalicka-Woźniak, K., Oniszczuk, A., Ludwiczuk, A., 2018: Antioxidant activity of lemongrass essential oil and its constituents. Facta Universitatis, Series: Physics Chem. Technol. 16(1), 132. DOI: 10.3390/antiox11010020

Zhao, J., Fan, Y., Cheng, Z., Kennelly, E.J., Long, C., 2024. Ethnobotanical uses, phytochemistry and bioactivities of Cymbopogon plants: A review. J. Ethnopharmacol. 118181. DOI: 10.1016/j.jep.2024.118181

Zigene, Z.D., Kassahun, B.M., Degu, B., 2018: Agronomic Characteristics and Essential Oil Yield of Java Citronella (*Cymbopogon Winterianus* Jowitt) as Affected by Harvesting Age and Plant Population Density. Acad. Res. J. Agric. Sci. Res. 6, 70-76.

Żukowska, G., Durczyńska, Z., 2024: Properties and applications of essential oils: A review. J. Ecological. Eng. 25(2).

ORCID
Arusa Aftab https://orcid.org/0000-0002-1525-3671
Zainab Maqbool https://orcid.org/0009-0005-5606-9095
Zubaida Yousaf https://orcid.org/0000-0001-8710-3915
Awais Khalid https://orcid.org/0000-0002-6084-8703
Zill-e- Huma Aftab https://orcid.org/0000-0003-3955-1312

Address of the corresponding author:
Department of Botany, Lahore College for Women University Lahore, Pakistan
E-mail: arusa.aftab@lcwu.edu.pk; zubaida.yousaf@lcwu.edu.pk

© The Author(s) 2025.
This is an Open Access article distributed under the terms of the Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by/4.0/deed.en).