

Chapter 2

Bioactive composition and nutrient profile of leaves of *Cymbopogon citratus*: implications for therapeutic use

Rajesh Kumar Verma¹, Subhalakshmi Rout² and Sugimani Marndi^{2*}

¹Department of Dravyaguna, Naiminath Ayurvedic Medical College and Research Centre, Agra, Uttar Pradesh, India

²Biodiversity and Conservation Lab., Ambika Prasad Research Foundation, Odisha, India

*Email-Id: sugimani2marndi@gmail.com



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Abstract: *Cymbopogon citratus* (lemongrass) is a widely used medicinal and culinary herb valued for its aromatic essential oils and diverse bioactive compounds. Its strong fragrance is attributed to major constituents such as citral, geraniol, citronellal and limonene. Present study aimed to evaluate primary metabolites through proximate analysis and assess major secondary metabolites using qualitative and quantitative phytochemical screening. Secondary metabolites, including alkaloids, flavonoids, tannins, saponins, phenolics and terpenoids, were analysed using standard procedures. The results indicated that *C. citratus* contains a substantial amount of carbohydrates, fibre and essential phytoconstituents, particularly phenolics and flavonoids, which contribute to its antioxidant and therapeutic potential. This integrated biochemical profiling provides scientific support for the nutritional, medicinal and industrial applications of *C. citratus*, further validating its importance in both traditional and modern healthcare systems.

Keywords: Lemongrass, nutritional evaluation, proximate analysis

Introduction

Medicinal plants play a vital role in traditional and modern healthcare systems due to their diverse phytochemical constituents and nutritional properties (Radha et al., 2021). The genus *Cymbopogon*, derived from the Greek words kymbe (boat) and pogon (beard), comprises aromatic perennial grasses belonging to the family Poaceae (Shah et al., 2011). These grasses are widely distributed across tropical and subtropical regions of Asia, Africa, America and Australia (Avoseh et al., 2015). They are known for their essential oil content, strong lemon-like aroma and extensive medicinal, culinary and industrial applications (Maybodi et al., 2025). More than 140 species of *Cymbopogon* have been identified globally, with *C. citratus*, *C. flexuosus*, *C. nardus* and *C. winterianus* being the most economically

significant (Latha et al., 2021). Among these species, *Cymbopogon citratus* (DC.) Stapf is particularly valued for its high aromatic content and multifunctional benefits (Telangi et al., 2022). Commonly known as lemongrass, it is rich in citral cyclic monoterpene responsible for its characteristic lemon scent (Oladeji et al., 2019). The grass contains various phytochemicals including flavonoids, essential oils, phenolic compounds and other metabolites associated with pharmacological activities such as anti-obesity, antibacterial, antifungal, antinociceptive and antidiarrheal effects (Hassan et al., 2024). Traditionally, lemongrass has been used in Ayurveda, folk medicine and culinary practices for its antimicrobial, antioxidant, anti-inflammatory and digestive properties (Ishaya et al., 2025). Scientific evaluation of medicinal plants typically includes the study of both primary metabolites, such as carbohydrates, proteins, lipids and fiber and secondary metabolites, such as alkaloids, flavonoids, phenolics, tannins, terpenoids and saponins, which are responsible for therapeutic activities (Rithu et al., 2020). Although *C. citratus* is widely used, systematic biochemical profiling that integrates proximate analysis and secondary metabolite evaluation is essential for validating its therapeutic potential. Therefore, present study was undertaken to analyze the nutritional composition and phytochemical constituents of *C. citratus*.

Methodology

Collection of plant sample

The leaves of *Cymbopogon citratus* were collected from the nearby Mahanadi River areas of Cuttack district of Odisha. The plant species was identified by the authors (Figures 1 & 2; Nayak and Kumar, 2023). Soxhlet extraction method was adopted using four solvents for phytochemical analysis (Kumar et al., 2013; Marndi et al., 2025). Detection of eight secondary metabolites was conducted using standard methods (Devi et al., 2022; Jena et al., 2024; Marndi et al., 2024).

Qualitative phytochemical analysis

Primary metabolites

Test for protein: One ml of the filtrate of leaf extract was mixed with 2-5 drops of Millon's reagent and kept in the water bath for 30 minutes. The pink coloration indicates the presence of protein.

Test for carbohydrate: One ml of the filtrate of leaf extract was mixed with 2-5 drops of Fehling's solution A and B and kept in the water bath for 30 minutes. A reducing sugar reacts with Fehling's reagent in an alkaline



Figure 1: Plant sample (*C. citratus*) for quantitative and qualitative analysis

medium to form orange-to-red precipitate. The appearance of an orange or red precipitate indicates the presence of carbohydrates.

Test for starch: To 1 ml of the filtrate of leaf extract, 2-3 drops of concentrated HCL were added and heated for 1-2 minutes. NaOH, following Benedict's reagent, was added to this solution and kept in the water bath. The change in coloration from bluish-green to red-orange indicates the presence of starch.

Test for sucrose: About 1 ml of the filtrate of the leaf extract was mixed with 2-3 drops of iodine. The appearance of a blue-black color indicates the presence of sucrose.

Test for lipid: About 1 ml of the filtrate of leaf extract was mixed with 2-5 drops of Sudan-III. The appearance of pink droplets indicates the presence of lipids.

Test for amino acid: One ml of the filtrate of leaf extract was mixed with 5 drops of Ninhydrin reagent and kept in the water bath for 30 minutes. Ninhydrin is a powerful oxidizing agent that gives a purple-colored product termed Rhuemann's purple.



Figure 2: Habit and habitat of *Cymbopogon citratus*

Secondary metabolites

Test for tannin: About 1 ml of the leaf extract was taken. Three to five drops of 10 % lead acetate solution were added. The gelatinous precipitate formation confirms the presence of tannin.

Test for saponin: About 1 ml of the leaf extract was taken and 1 ml of distilled water was added and shaken well. The formation of persistent froth confirms the presence of saponin.

Test for flavonoids: About 1 ml of the leaf extract was taken. Two ml of 2 % NaOH solution and 3 to 4 drops of diluted HCl were added. The color initially turns to an intense yellow color with NaOH solution and later becomes colorless. This color change in appearance confirms the presence of flavonoids.

Test for terpenoids: About 1 ml of the leaf extract was added with 5-6 drops of chloroform and placed in the water bath for a few minutes. Then 5-6 drops of concentrated sulphuric acid were added. The reddish-brown interface confirms the presence of terpenoids.

Test for phenolic groups: About 1 ml of the leaf extract was taken. A few drops of 5 % ferric chloride solution were added. The dark bluish-black appearance confirms the presence of phenolic compounds.

Test for reducing sugars: About 1 ml of the leaf extract was taken and 2 drops of Fehling's solution A followed by Fehling's solution B were added and kept in the water bath for some time. The presence of red-orange precipitate confirms the presence of reducing sugar.

Test for steroids: About 1 ml of the leaf extract was taken. 1 ml of chloroform and 1 ml of concentrated sulphuric acid were added to it. The appearance of upper red and lower yellow colors with green fluorescence indicated the presence of steroids.

Test for alkaloids: About 1 ml of the leaf extract was taken and 3 to 4 drops of Dragendorff's reagent were added. The formation of a reddish-brown precipitate confirms the presence of alkaloids.

Quantitative estimation of secondary metabolites

Estimation of tannin: 200 mg of tannic acid was mixed with 20 ml of distilled water. From this solution, 5, 15, 25, 35 and 50 μ l were taken in different test tubes. The volume was made up to 1 ml. Then 0.5 ml of Folin reagent and 2.5 ml of 20 % sodium carbonate were added to each test tube. The mixed solutions were shaken for 5 minutes in the dark. The solutions in each test tube

were left for 40 minutes. After 40 minutes, readings were taken at 720 nm to make a standard graph.

Extraction of tannins: 200 mg of the sample was transferred into a 100 ml conical flask to which 75 ml of distilled water was added and boiled for 30 minutes. The whole solution was centrifuged at 2000 rpm for 20 minutes. Supernatant was then taken in a 100 ml volumetric flask and made up to the volume. 1 ml of the sample was taken from a 100 ml volumetric flask and 75 ml of distilled water, 5 ml of Folin reagent and 10 ml of 20 % sodium carbonate were added. The volume was made up to 100 ml. After shaking for 5 minutes, the readings were taken at 720 nm. The amount of tannin was calculated from the standard graph ([Sadasivam and Manickam, 2019](#)).

Estimation of saponin: The determination of total saponin was done by the method of Obadoni and Ochuko, (2001) with minor modifications. 2 g of sample was added to 30 ml of 20 % aqueous-ethanol and kept in a flask on a stirrer for half an hour. It is heated for 4 hours at 45 °C. The mixture was filtered by using Whatman filter paper-1 and the residue was again extracted with another 20 ml of 25 % aqueous ethanol. The combined extracts were concentrated. The concentrate was transferred into a separator funnel and extracted twice with 10 ml diethyl ether. The ether layer was discarded while the aqueous layer was kept and re-extracted with 15 ml n-butanol. The n-butanol extract was washed twice with 5 ml of 5 % aqueous sodium chloride. The remaining solution was evaporated. After evaporation, the sample was dried in the hot air oven at 40 °C to a constant weight. The saponin content was calculated.

$\% \text{ of saponins} = (\text{Final weight of sample} / \text{Initial weight of extracts}) \times 100$

Extraction of phenol: Three replicas of 0.5 g of the sample were taken and crushed with 60 % methanol in a mortar and pestle. Samples were centrifuged 5 times at 5000 rpm for 20 minutes.

Estimation of phenols: Seven test tubes were taken, including a blank and 2 test tubes for each replica. 0.1 and 0.2 ml of the sample were taken in each test tube except the blank. 60 % methanol was added to each test tube to bring the volume up to 1 ml. 1 ml of 0.1 N HCl was added and allowed to stand for a few minutes. 1 ml of sodium nitrite molybdate mixture was added, shaken well and allowed to stand for a few minutes, diluted with 5 ml of distilled water. After dilution, 2 ml of 1N NaOH was added and allowed to stand for 20 minutes. Readings were taken at 515 nm. The amount of phenol present in the sample was calculated from the standard graph ([Swain and Hillis, 1959](#)).

Estimation of flavonoids: A sample of 100 µl of the extract was mixed with 100 µl of 5 % sodium nitrite and allowed to stand for 5 minutes. Next, 100 µl of aluminium trichloride was added and incubated for 6 minutes, followed by the addition of 1 ml of 1 M sodium hydroxide. The total volume of the mixture was made up to 5 ml with distilled water and incubated for 15 minutes at room temperature in the dark for taking absorbance at 510 nm. The amount of flavonoid present in the sample was calculated from the standard graph. The standard graph was prepared for quercetin ([Chang et al., 2002](#); [Raina and Misra, 2023](#)).

Results

The qualitative screening of primary metabolites in different leaf extracts of *Cymbopogon citratus* revealed considerable variation depending on solvent polarity ([Table 1](#)). Non-polar extracts such as n-hexane showed no detectable primary metabolites. In contrast, polar solvents exhibited the presence of key nutritional components. Both ethanol and methanol extracts tested positive for carbohydrates, indicating their good solubility in medium to high-polarity solvents. The aqueous extract showed the presence of carbohydrates as well as proteins, suggesting that water is an effective solvent for extracting hydrophilic primary metabolites from *C. citratus*.

Table 1: Qualitative phytochemical analysis for the detection of primary metabolites

Polarity	Extract	Detected primary metabolites
Non-Polar  Polar	n-Hexane	No tested metabolites were detected
	Ethanol	Carbohydrates only
	Methanol	
	Aqueous	Protein and carbohydrates

The evaluation of secondary metabolites demonstrated that metabolite detection depended strongly on solvent polarity ([Table 2](#)). The non-polar n-hexane extract did not exhibit the presence of any of the tested secondary metabolites, confirming the limited solubility of phytochemicals such as tannins and phenolics in non-polar solvents. Among the polar extracts, ethanol showed the presence of tannins and reducing sugars. Methanol and aqueous extracts demonstrated a broader phytochemical profile, showing tannins, phenols and reducing sugars. Methanol and water, due to their high

polarity, were the most efficient solvents for extracting phenolic constituents from *C. citratus*.

Table 2: Qualitative phytochemical analysis for the detection of secondary metabolites

Polarity	Extract	Detected secondary metabolites
Non-Polar  Polar	n-Hexane	No tested metabolites were detected
	Ethanol	Tannin and reducing sugars
	Methanol	Tannin, phenol and reducing sugars
	Aqueous	

The phytochemical patterns observed in this study highlighted the significant influence of solvent polarity on the extraction efficiency of both primary and secondary metabolites of *Cymbopogon citratus* leaves. Non-polar extraction using n-hexane yielded no detectable metabolites, which is consistent with the previous findings that primary metabolites (such as carbohydrates and proteins) and most secondary metabolites (such as phenolics and tannins) are predominantly polar in nature. Polar solvents, particularly ethanol, methanol and water, successfully extracted key nutritional and bioactive compounds. The detection of carbohydrates in ethanol and methanol extracts indicated the moderate solubility of these primary metabolites in organic solvents (Table 3).

Table 3: Quantitative estimation of total Phenol, Tannin, Flavonoid contents and Saponin percentage

<i>Cymbopogon citratus</i> (Leaves)	Bioactive compound	Content
	Total Phenol (mg/100g)	0.14
	Tannin (mg/100g)	1.1
	Flavonoid (mg/100g)	0.14
	Saponin (%)	1.1

The presence of carbohydrates and proteins in the aqueous extract aligns with the hydrophilic properties of these compounds. The detection of

tannins, phenols and reducing sugars in methanol and aqueous extracts is noteworthy, as these secondary metabolites are associated with significant pharmacological activities, including antioxidant, antimicrobial and anti-inflammatory properties (Hossen et al., 2025). Methanol, being a highly polar solvent, often demonstrate superior extraction ability for phenolic compounds (Shi et al., 2022), and this was evident in the present results. Similarly, the aqueous extract's richness in phenolics further supports the traditional use of water-based lemongrass preparations (such as infusions and decoctions) in medicinal applications. It contains various uses such as culinary, aroma therapy, medicinal and insect repellent properties (Ishaya et al., 2025). The results indicated that *C. citratus* contain a diverse range of bioactive and nutritional components, particularly in polar extracts. These findings support the plant's established therapeutic effects and justify its widespread use in traditional medicine systems (Figure 3). The presence of phenolics and tannins, especially in methanol and aqueous extracts emphasizes the therapeutic potential of lemongrass as a natural source of antioxidants and health-promoting compounds.

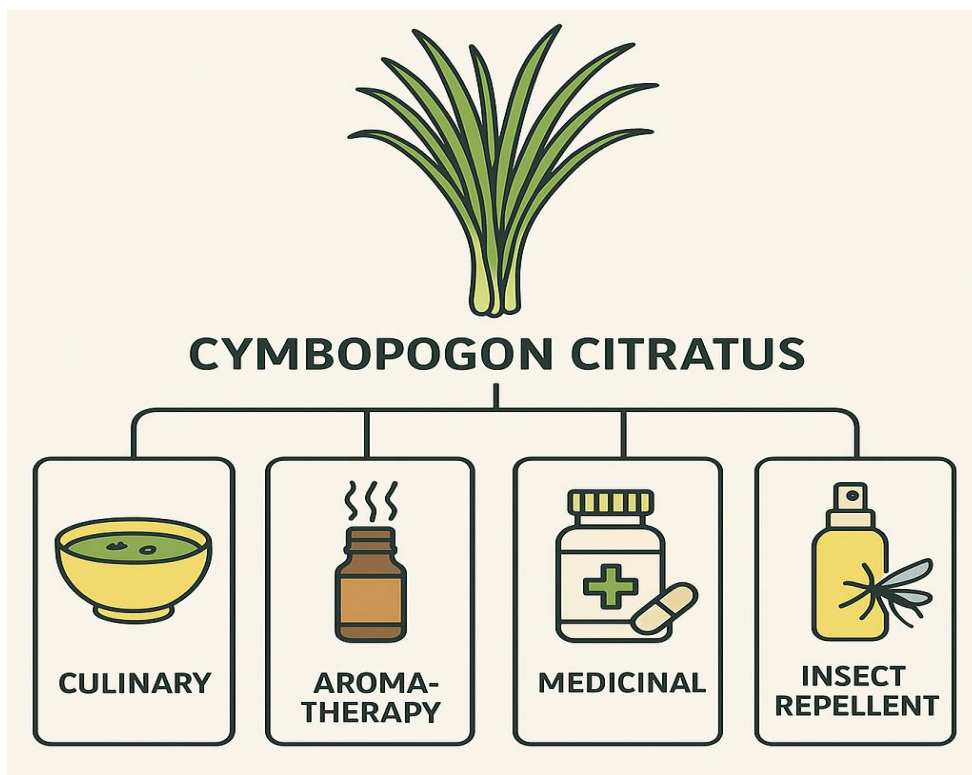


Figure 3: Illustration of various uses of *Cymbopogon citratus*

Conclusion

Present study provides a comprehensive assessment of the nutritional and phytochemical composition of *C. citratus* leaves using qualitative and quantitative methods. Solvent polarity played a significant role in metabolite extraction, with polar solvents, particularly water and methanol, proving most efficient. The aqueous extract contained essential primary metabolites such as carbohydrates and proteins, while methanol and water extracts were rich in tannins, phenols and reducing sugars. Quantitative analysis further confirmed the presence of phenolics, tannins, flavonoids and saponins, which contribute to the antioxidant, antimicrobial and anti-inflammatory properties of lemongrass. These findings reinforce the therapeutic potential of *C. citratus* and validate its longstanding use in traditional medicine. Further advanced studies, including bioactivity assays and compound isolation, are recommended to fully elucidate its pharmacological applications.

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