

Chapter 8

Phytochemical screening and flavonoid quantification of *Cymbopogon citratus* (inflorescence)

Subhalakshmi Rout¹, Sumitra Jethy¹, Sugimani Marndi¹ and Anjali Jaiswal^{2*}

¹Biodiversity and Conservation Lab., Ambika Prasad Research Foundation, Odisha, India

²Science Educationist, JCPRO Academy, Bangalore, Karnataka, India

*Email-Id: 94anjali@gmail.com



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Abstract: *Cymbopogon citratus* (lemon grass) is an economically and medicinally important aromatic grass widely used in traditional medicine and nutraceutical formulations. Present study aimed to evaluate the qualitative and quantitative phytochemical composition of *C. citratus* inflorescence using different solvent extracts. Primary and secondary metabolites were screened through standard phytochemical assays, while total flavonoid content was quantified using the aluminium chloride colorimetric method with quercetin as the standard. The qualitative analysis revealed that non-polar extracts showed no detectable metabolites, whereas polar extracts exhibited a broad spectrum of bioactive compounds. Aqueous extracts showed the richest profile, containing proteins, carbohydrates, starch, tannins, flavonoids and reducing sugars, indicating that most metabolites in *C. citratus* are water-soluble. Quantitative estimation revealed a total flavonoid content of 31.31 mg/100 g, suggesting moderate antioxidant potential of the inflorescence. The current work highlights the phytochemical richness and potential therapeutic value of *C. citratus*, especially in its polar extracts.

Keywords: Antioxidant activity, *Cymbopogon citratus*, ethnopharmacology, flavonoid content, phytochemical screening

Introduction

Medicinal plants play a significant role in both traditional and modern healthcare schemes because of their diverse phytochemical constituents and associated therapeutic properties. Over a period of centuries, medicinal plants have made a promising over modern medicine nowadays. Within this group, the genus *Cymbopogon* (family Poaceae) comprises more than 140 aromatic grass species distributed across the tropical and subtropical regions of Asia, Africa and Australia (Tibenda et al., 2022). Species of this genus are well-known for their essential oils, rich secondary metabolites and extensive applications in culinary, cosmetic and

pharmaceutical industries (Das and Tiwari, 2025). *Cymbopogon citratus*, commonly known as lemon grass, is one of the most extensively cultivated species in the genus. It is highly valued for its characteristic lemon-like fragrance, primarily attributed to its citral-rich essential oil. Traditionally, the plant has been used as an herbal tea and as a natural remedy with antibacterial, antimicrobial, anti-inflammatory, digestive and antioxidant properties (Oladeji et al., 2019; Hussain et al., 2023). Although several studies have focused on its essential oil composition, comparatively fewer investigations have explored the phytochemical constituents present in its inflorescence extracts. Phytochemical screening is an essential preliminary procedure for identifying primary metabolites (such as carbohydrates, proteins, starch and lipids) and secondary metabolites (including tannins, flavonoids, phenolics, saponins and alkaloids), which collectively contribute to the nutritional and medicinal significance of plants (Maheshwaran et al., 2024). The efficiency of metabolite extraction is strongly influenced by the polarity of the solvent used, as it determines the solubility and extraction capacity for different classes of compounds. This study aims to assess the qualitative and quantitative phytochemical profile of *C. citratus* inflorescence using both non-polar and polar solvent extracts. Standard biochemical assays were performed to detect various metabolites and the total flavonoid content was quantified through the aluminium chloride colorimetric method. The findings provide a comprehensive understanding of the distribution of bioactive constituents in *C. citratus*, highlighting its potential as a valuable natural source of nutritionally and therapeutically important phytochemicals.

Methodology

Collection of plant sample

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



Figure 1: Inflorescence of *C. citratus* in wild habitat

Test for carbohydrate: One ml of the filtrate of the inflorescence 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 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 the inflorescence 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 inflorescence 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 the inflorescence 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 the inflorescence 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 as Rhuemann's purple.



Figure 2: Plant sample for experimental work

Secondary metabolites

Test for tannin: About 1 ml of the inflorescence 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 inflorescence 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 inflorescence extract was taken. Two ml of 2 % NaOH solution and 3 to 4 drops of diluted HCl were added. The color initially turned to an intense yellow color with NaOH solution and later

became colorless. This color change in appearance confirms the presence of flavonoids.

Test for terpenoids: About 1 ml of the inflorescence 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 inflorescence 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 inflorescence 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 inflorescence 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 provides the presence of steroids.

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

Quantitative estimation of secondary metabolites

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 and discussion

The qualitative screening of primary metabolites in different solvent extracts of *Cymbopogon citratus* revealed notable variations depending on solvent polarity ([Table 1](#); [Figure 3](#)). Non-polar (n-hexane) extract did not show the presence of any of the tested primary metabolites, indicating that major nutritional compounds of the plant are polar and require medium to high polarity solvents for extraction. Polar solvents such as ethanol, methanol and

aqueous extracts tested positive for carbohydrates, while the aqueous extract also showed the presence of proteins and starch. The detection of these essential metabolites suggested that *C. citratus* inflorescence possesses important nutritional components. The aqueous extract showed the broadest profile of primary metabolites, which may be attributed to the ability of water to dissolve a wide range of hydrophilic nutrients.

Table 1: Qualitative phytochemical analysis for detection of primary metabolites in *C. citratus* (inflorescence)

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

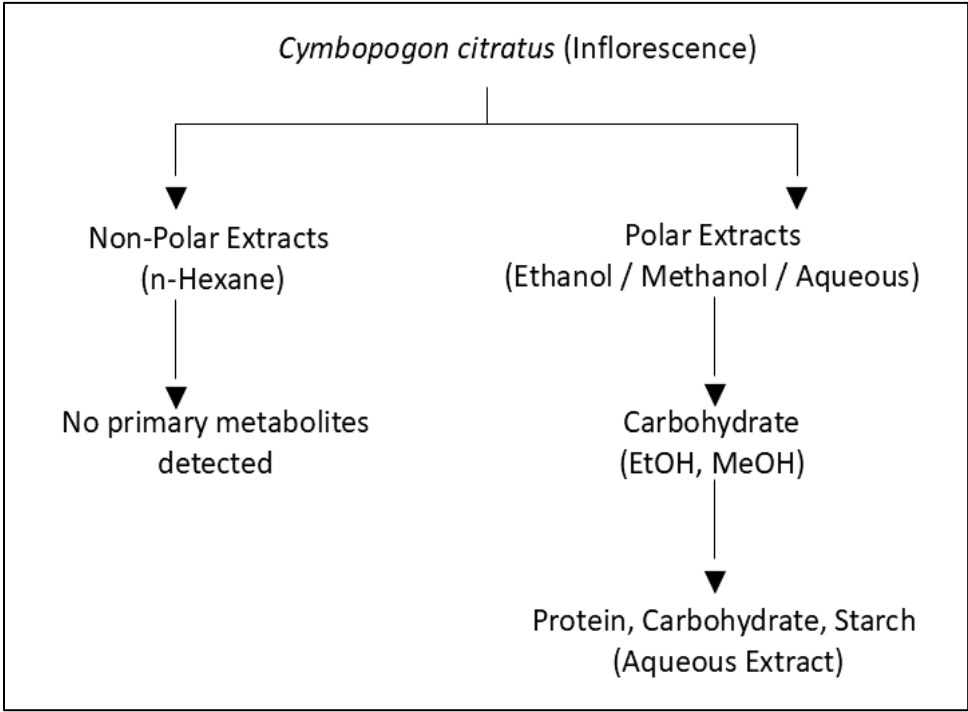


Figure 3: Extraction of primary metabolites from *C. citratus*

The non-polar n-hexane extract again showed no detectable secondary metabolites, confirming that key phytoconstituents in *C. citratus* are polar (Table 2; Figure 4). Among polar extracts, ethanol demonstrated the presence of tannins and reducing sugars. Methanol extracts showed a broader spectrum including tannins, phenols and reducing sugars. Aqueous extracts revealed tannins, flavonoids and reducing sugars. Flavonoids were not detected in alcohol extracts but were prominent in the aqueous fraction, suggesting that flavonoid compounds in *C. citratus* are predominantly water-soluble.

Table 2: Qualitative phytochemical analysis for detection of secondary metabolites in *C. citratus* (inflorescence)

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

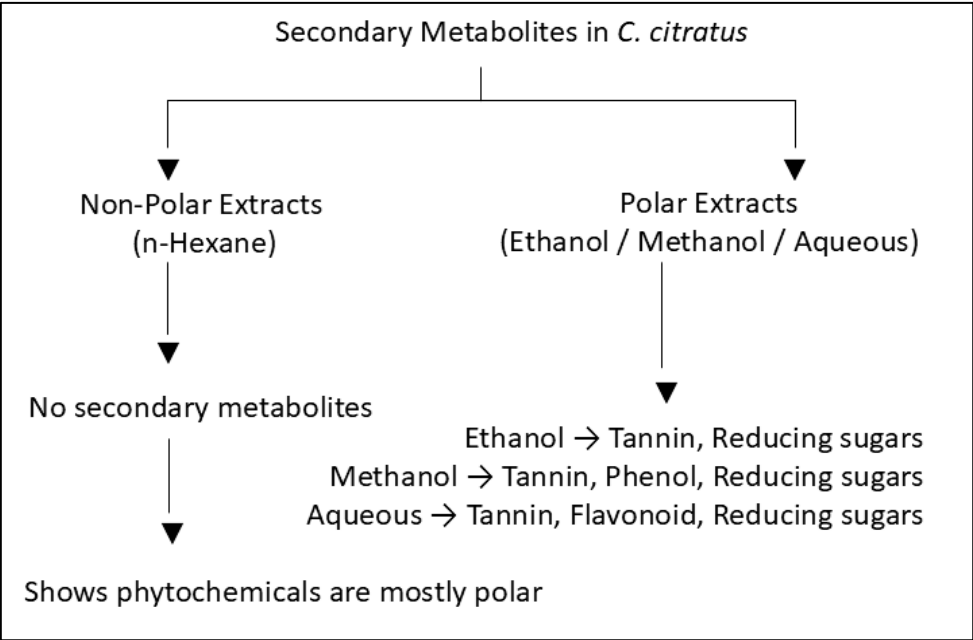


Figure 4: Distribution of secondary metabolites in *C. citratus*

Quantitative analysis revealed that the *Cymbopogon citratus* inflorescence contains 31.31 mg/100 g of flavonoids (Table 3; Figure 5). This indicates a moderate level of flavonoid bioactivity compared to typical medicinal plant standards. Flavonoids are known for antioxidant, anti-inflammatory and antimicrobial properties, suggesting that the inflorescence may serve as a potent source for nutraceutical and therapeutic applications.

Table 3: Quantification of total flavonoid content

<i>Cymbopogon citratus</i> (Inflorescence)	Bioactive compound	Content (mg/100g)
	Flavonoid	31.31

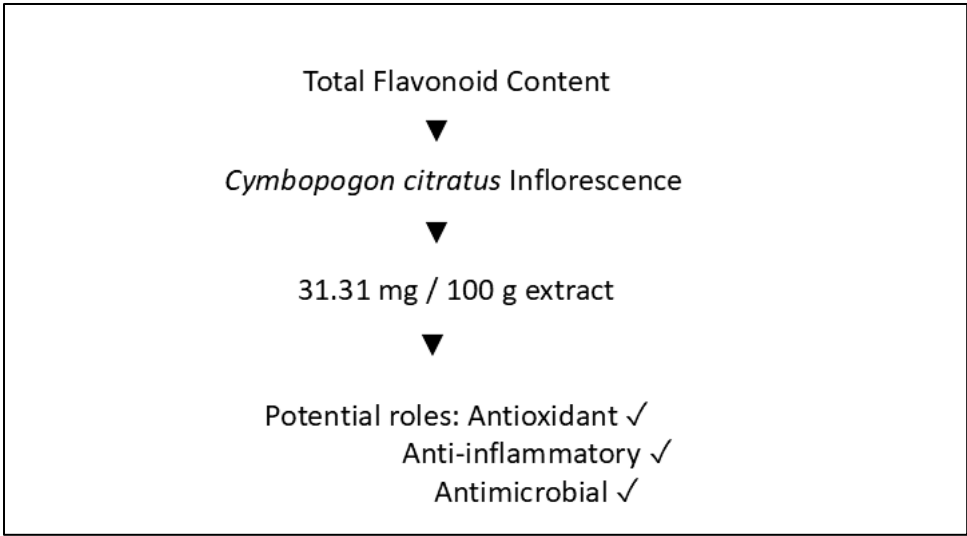


Figure 5: Illustration of flavonoid quantification in *C. citratus*

Conclusion

The current study showed that *Cymbopogon citratus* inflorescence contain several important primary and secondary metabolites, especially in polar solvent extracts. Aqueous extracts exhibited the highest phytochemical richness. The total flavonoid content (31.31 mg/100 g) indicates notable antioxidant potential. Therefore, *C. citratus* is a valuable natural source of bioactive compounds with nutritional and therapeutic significance.

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