

Chapter 5

Bioactive compounds of Lemon grass (stems) and its flavonoid estimation

Nibedita Jena, Sugimani Marndi and Sweta Mishra*

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

*Email-Id: swetamishra.aprf@gmail.com



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Abstract: Grasses play a vital role in our biodiversity conservation with respect to food, medicinal, ecological and economic value. *Cymbopogon citratus* (lemon grass) is one of the famous aromatic medicinal grasses widely valued for its therapeutic properties and bioactive constituents. However, its stems remain comparatively underexplored. Present study aimed to investigate the qualitative phytochemical profile and quantitative flavonoid content of *C. citratus* stem using solvent extraction of varying polarity. Soxhlet extraction was carried out using n-hexane, ethanol, methanol and distilled water. Qualitative phytochemical analysis revealed the presence of carbohydrates as the primary metabolite in polar extracts, while secondary metabolites such as tannins, reducing sugars, saponins and flavonoids were predominantly detected in aqueous extract. The total flavonoid content was estimated to be 0.05 mg/100 g. The results highlight the significance of utilizing lesser-studied plant parts and support the sustainable use of *C. citratus* as a source of natural bioactive compounds for pharmaceutical and nutraceutical applications.

Keywords: Bioactive compounds, flavonoids, lemon grass

Introduction

The increasing demand for natural antioxidants and plant-based therapeutic agents has intensified scientific interest in underutilized plant parts rich in bioactive compounds (Hrelia et al., 2025). *Cymbopogon citratus* (lemon grass), a perennial aromatic grass of the family Poaceae, is widely recognized for its essential oil-bearing leaves with extensive medicinal applications (Parveen et al., 2025). However, during commercial processing and household use, lemon grass stems are often discarded or used minimally, despite their biochemical richness. Exploring the phytochemical composition of lemon grass stems offers an opportunity to transform agricultural by-products into valuable sources of health-promoting compounds (Zeppa et al., 2025). Bioactive compounds such as phenolics, flavonoids, tannins and terpenoids play a pivotal role in plant defence

mechanisms and contribute significantly to their pharmacological activities (Sharma et al., 2025). In lemon grass stems, these secondary metabolites are known to exhibit antioxidant, antimicrobial and anti-inflammatory properties, which are essential in mitigating oxidative stress-related disorders (Kiani et al., 2022). Among these compounds, flavonoids are particularly important due to their ability to neutralize free radicals, inhibit lipid peroxidation and modulate cellular signaling pathways involved in disease prevention (Zahra et al., 2024). Assessment of flavonoid content in lemon grass stems not only provides insight into their therapeutic potential but also supports the sustainable utilization of the whole plant. From scientific validation of the bioactive profile of stems, the present study contributes to the development of natural antioxidant sources for pharmaceutical, nutraceutical and functional food applications. Such an approach aligns with current trends in sustainable agriculture, waste reduction and the discovery of eco-friendly alternatives to synthetic antioxidants.

Methodology

Collection of plant sample

The stems of *Cymbopogon citratus* were collected from the vicinity of the Mahanadi River in the Cuttack district of Odisha, India. The plant material was taxonomically identified by the authors (Figures 1 & 2; Nayak and Kumar, 2023). Phytochemical extraction was carried out using the Soxhlet extraction method with four different solvents of varying polarity, following standard procedures (Kumar et al., 2013; Marndi et al., 2024). Qualitative detection of selected secondary metabolites was performed using established phytochemical screening methods as described by Devi et al., (2022), Jena et al., (2024) and Marndi et al., (2024).

Qualitative phytochemical analysis

Primary metabolites

Test for protein: One ml of the filtrate of the stem 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 carbohydrates: One ml of the filtrate of the stem 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 an orange-to-red precipitate. The appearance of an orange or red precipitate indicates the presence of carbohydrates.



Figure 1: Plant sample for experimental work

Test for starch: To 1 ml of the filtrate of the stem 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 stem 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 stem 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 stem 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: Morphology of *C. citratus* in the wild areas

Secondary metabolites

Test for tannin: About 1 ml of the stem 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 stem 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 stem 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 became colorless. This color change in appearance confirms the presence of flavonoids.

Test for terpenoids: About 1 ml of the stem 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 stem 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 stem 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 stem extract was taken. 1 ml of chloroform and 1 ml of concentrated sulphuric acid was 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 stem extract was taken and 3 to 4 drops of Dragendroff's reagent were added. The formation of a reddish-brown precipitate confirms 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 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 phytochemical screening of *C. citratus* stem extracts for primary metabolites in Table 1 indicated that carbohydrate was the only primary metabolite detected. Ethanol, methanol and aqueous extracts showed positive results for carbohydrates, whereas the non-polar n-hexane extract showed no detectable primary metabolites. This observation suggested that carbohydrates present in the stems are predominantly polar in nature and were efficiently extracted using polar solvents. The absence of primary metabolites in the non-polar extract highlighted the solvent-dependent extraction efficiency and supports the use of polar solvents for nutritional and biochemical studies.

Table 1: Qualitative phytochemical analysis for the detection of primary metabolites in *C. citratus* stem extracts

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

Table 2: Qualitative phytochemical analysis for the detection of secondary metabolites in *C. citratus* stem extracts

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

The qualitative screening for secondary metabolites (Table 2) revealed notable variations among different solvent extracts. Ethanol and methanol extracts showed the presence of tannins and reducing sugars, indicating moderate polarity of these compounds. The aqueous extract exhibited a richer phytochemical profile, revealing the presence of tannins, saponins, flavonoids and reducing sugars. In contrast, the n-hexane extract did not show any of the tested secondary metabolites, confirming that these bioactive constituents are largely polar in nature. The higher diversity of secondary metabolites in the aqueous extract suggested that water is an effective solvent for extracting bioactive compounds from *C. citratus* stems, particularly those associated with antioxidant and therapeutic activities.

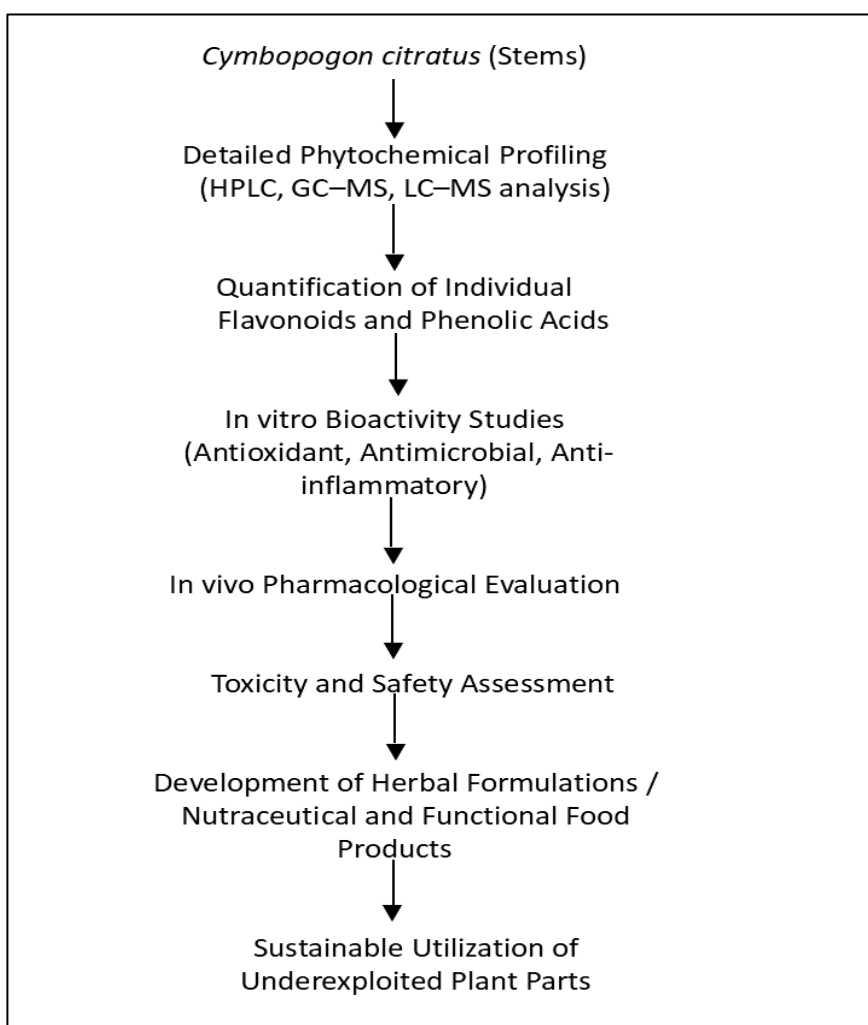


Figure 3: Illustration of *C. citratus* (stems) for future research scope

Table 3: Quantification of total Flavonoid content

<i>Cymbopogon citratus</i> (stems)	Bioactive compound	Content (mg/100g)
	Flavonoid	0.05

The quantitative estimation of total flavonoid content in *C. citratus* stem is presented in Table 3. The flavonoid content was recorded as 0.05 mg/100 g of sample. Although the detected level is relatively low, the presence of flavonoids confirms the antioxidant potential of the stems. Flavonoids are known for their ability to scavenge free radicals, reduce oxidative stress and contribute to anti-inflammatory and antimicrobial activities (Zahra et al., 2024). The detected flavonoid content, along with the qualitative presence observed in the aqueous extract, supports the therapeutic relevance of lemon grass stems. Present study emphasizes that even lesser-studied plant parts, such as stems, possess bioactive compounds that can contribute to pharmaceutical and nutraceutical applications in future aspects (Figure 3).

Conclusion

The present scenario demonstrates that the stems of *Cymbopogon citratus* possess a range of bioactive compounds, with solvent polarity playing a crucial role in extraction efficiency. Qualitative phytochemical screening confirmed the presence of carbohydrates as primary metabolites in polar extracts and revealed important secondary metabolites such as tannins, saponins, flavonoids and reducing sugars, particularly in aqueous extracts. Quantitative estimation indicated a measurable flavonoid content, supporting the antioxidant potential of the stems despite their relatively low concentration. These results emphasize that plant parts often considered underutilized or discarded, such as stems, can serve as a valuable source of bioactive compounds. The study contributes to the growing body of knowledge on *C. citratus* and promotes the sustainable utilization of the whole plant. Further exploration of these bioactive constituents may enhance their application in pharmaceutical, nutraceutical and functional food industries.

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