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Phytochemical Profiling of *Cymbopogon flexuosus* Plant Leaves

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Abstract

The qualitative and quantitative phytochemical composition of *Cymbopogon flexuosus* plant leaves were investigated using standard methods. Eight Phytochemical families were detected. Amongst those detected are phenolic acid, saponins, glycosides, cyanogenic glycosides, alkaloids, anthocyanins, flavonoids and sterols. Seven phenolic acids were quantitatively detected with ferulic acid (10.28±0.55 mg/100 g), chlorogenic acid (8.37±0.01 mg/100 g) and caffeic acid (2.93±0.04 mg/100 g) being the highest. Among the nine saponins detected sarsasapogenin (7.12±0.10 mg/100 g) and tigogenin (5.34±0.02 mg/100 g) were observed to be high. Nine glycosides and six cyanogenic glycosides were found in the plant leave and salicin (1.36±0.01 mg/100 g) and galliridoside (7.12±0.10 mg/100 g) were detected to be the highest respectively. Seventeen alkaloids and five anthocyanins were found in the plant leaf. For the alkaloids, akuammidine (4.21±0.01 mg/100 g) was seen to be the highest while cyaniding-3-sophoroside-5- glucoside (32.96±55.46 mg/100 g) was observed to be the highest for anthocyanins. Six and seven flavonoids and sterols were detected in the plant leaf respectively. Among them, glycitein with value of 19.01±0.06 mg/100 g and sitosterol at 16.25±0.01 mg/100 g were found to also be the highest respectively. The rich contents of many bioactive molecules detected in *Cymbopogon flexuosus* leaf suggest the strong nutraceutical potential of this plant leaves, further suggesting their likely use as functional food and as therapeutics in the management and prevention of diseases.

Keywords: *Cymbopogon flexuosus*; Phytochemicals; Leaves; Phenolic acids; Antioxidants

1. Introduction

Plants are important sources of foods and natural drugs. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, mainly based on their use in traditional medicines or phytomedicines (Nwauche *et al.*, 2018)

Green leafy vegetables occupy an important place among the food crops as they provide adequate amount of vitamins and minerals for human consumption. In addition to their nutritional value, vegetables also contain phytochemicals which exhibit some protective and disease preventive effects, thus, making them serve a dual function against a number of biochemical, physiological and metabolic disorder (Kalu *et al.*, 2019)

Lemongrass (*Cymbopogon flexuosus*) is a perennial grass belonging to the genus *Cymbopogon* of the family *Poaceae*. This genus contains between 102 and 104 species, of which few have been reported in Africa, India, Australia, Europe and North America, respectively, while the rest are distributed in South Asia (Verma *et al.*, 2022). The plant is found mainly in the tropics and sub-tropics of America, Asia and Africa, extending from grassland and mountains to arid regions (Mwithiga *et al.*, 2022). Among the different *Cymbopogon* species, *Cymbopogon flexuosus* and *Cymbopogon*

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citratus are the most esteemed species in terms of essential oil production and their phytochemical and pharmacological properties (Mwithiga *et al.*, 2022). The essential oils of these two species hold immense commercial value in cosmetics, pharmaceuticals, flavors, soaps, fragrances, perfumery, detergents and tobacco products (Ganjewala & Gupta, 2013). Lemongrass leaves are used in preparing herbal tea, whereas stems are used in the making of curries, fish, soup, beef products and poultry products.

The genus *Cymbopogon* is well-documented to yield essential oils of high quality that are widely applied in pharmaceutical products. Specifically, *C. flexuosus* is considered one of the primary species extensively cultivated in different regions of the globe owing to its essential oil's high citral concentration, which ranges between 65 and 85 %, but may vary because of myriad of factors like plant species, altitude and climatic conditions (Haque *et al.*, 2018). The *C. flexuosus* leaves and stems are mainly used in making soups, herbal teas, curries and beef products (Rao *et al.*, 2015). Lemongrass is also a valuable agricultural export crop that contributes substantially to both income generation and the inflow of foreign currency (Shabbara *et al.*, 2022). The lemongrass industry presents opportunities for research in cultivation, bioactive compounds, genetic diversity and post handling (Faheem *et al.*, 2022). The market size and distribution of medicinal and aromatic plants (MAPs), including *C. flexuosus* differs vastly from region to region as well as from country to country.



Figure 1 The Stem and Pristine Leaves of the *Cymbopogon flexuosus* plant

Several illnesses have been treated using lemongrass in medicine produced in traditional ways. According to Nambiar and Matela (2012), lemongrass is used worldwide to treat gastrointestinal problems, fevers, menstrual irregularities, malaria, and pneumonia. The aerial portions of lemongrass are frequently employed in traditional medicine through infusions or stews. According to scientific research, this plant is recommended for treating fever, mental disorders, inflammation, digestive disorders, and other human health issues. According to Heinerman's Encyclopedia of Healing, drinking one cup of lemongrass tea every four hours can help decrease fever, along with herbs and spices.

The phytochemical components of the leaves, which are essential oils containing terpinolene, geranyl acetate, myrcene, and terpinol methylhistamine, were recently analyzed (Asaolu *et al.*, 2009). The analysis demonstrated the presence of phytochemicals, such as flavonoids and phenols, in lemongrass. Each of these phytochemicals is famous for various protective and advantageous properties. Additionally, pentylenetetrazol-induced colonic seizures brought on by maximal electroshock were delayed by required oil and congested tonic allowances. These were examples of the spread of seizures being halted or promoted. One of the essential components of lemongrass extracts is an essential oil, which acts as a co-ingredient in perfumes and cosmetics. Due to its high citral content, it is crucial for several chemical syntheses (Kiani *et al.*, 2022). Accordingly, previous studies on different lemongrass extracts depicted further significant therapeutic potentials, including anti-cancer and anti-mutagenicity properties. Anxiolytic, anti-diabetic, antioxidant, non-toxic, and anti-fungal characteristics are additional benefits (Shahi *et al.*, 2012). The aerial parts of lemongrass have been infused and used in traditional medicines worldwide. The biological properties of lemongrass leaves include anti-hypolipidemic, anti-atherosclerotic, antioxidant, immune-stimulating, anti-hypertensive, and anti-tumor properties.

Lemongrass has also been used to cure various diseases and problems, including rheumatism, menstrual disorders, infections, and other multiparty issues. It has been determined that lemongrass extraction with alcohol at a concentration of 60 µg/mL has significant results, including DPPH foraging ability (85%), superoxide (76%), hydroxyl (70%), nitric oxide (78%), and ABTS assay (77%) of free radicals via in vitro analysis, and it also has a reasonable anti-lipid peroxidative effect (57%), as depicted by Nambiar and Matela, (2012).

In a competitive market, therapeutic plant parts are used often. Their uses include pharmaceuticals, food, cosmetics, and perfumery markets. In pharmaceuticals, extracts of plant parts are particularly valid due to their usage of active ingredients for the development of medicine, and also as sources of raw material (Rao et al., 2015). Lemongrass can be used as a stomach smoother, as it lowers cholesterol levels when used medicinally. Each day, lemongrass tea or powder can be consumed in quantities of 1–4 cups; this helps with vomiting, bladder issues, congestion, headaches, coughing, fever, stomachaches, digestive issues, and diarrhea, and increases respiration, acting as a potential hypocholesterolemic agent. Prior to this, Lonkar, *et al.*, (2013), researched the processed lemongrass powder sold in stores as “Sereh powder”. There are several recipes for lemongrass-based items, including lemongrass meat, sweet lemongrass blend, and lemongrass rice. It also has many uses in the food, medicine, and flavoring industries. It cannot be kept as fresh for a long time in ambient conditions, because its quality and, therefore, its taste will deteriorate. Therefore, it can only be stored in powdered form.

2. Material and methods

2.1. Reagents/Chemicals

Reagents and chemicals used are of analytical grade.

2.2. Collection and Identification of Plant Sample

The fresh leaves of *Cymbopogon flexuosus* were harvested from First Avenue, Egbelu- Ozodo at Obio/Akpor LGA, Rivers State and were identified by a Plant taxonomist from Department of Plant Science and Biotechnology in faculty of science, Rivers State University. A voucher specimen of the plant sample was lodged in the herbarium.

2.3. Preparation of Leave sample

The leaves were washed, air-dried for 7 days and ground into powdered form using electric blender (Philips NL 9206AD-4 Drachten) and sieved with a sieve of 1mm mesh size and thereafter, preserved in air tight clean storage bottles.

2.4. Determination of Quantitative Phytochemical Composition of the Plant

The phytochemical analysis of plant materials is crucial for understanding their potential therapeutic properties. *Cymbopogon flexuosus*, commonly known as lemongrass, is widely recognized for its medicinal and aromatic properties. This section delves into the methodologies used to ascertain the phytochemical components of *Cymbopogon flexuosus* leaves, providing insights into the specific compounds responsible for its therapeutic effects.

2.5. Quantitative Phytochemical Screening

2.5.1. Preparation of Samples for HPLC

Ten (10) mg of powdered plant extracts of sample was dissolved in 10 ml of ethanol to get final concentration of 1mg/ml subsequently the solution was filtered using 0.45µm syringe filter (millipore) for sterilization. 1 mg of each standard was dissolved individually in 1ml of ethanol and sterile filtered through 0.45 µm syringe filter (millipore) before subjecting to HPLC analysis.

2.5.2. Procedure

The prepared samples of extracts and standards were used for HPLC. Binary system (Waters) equipped with PDA detector connected to system processor was used for analysis. The system used Empower software with standard certification for analysis of the results. A maximum pressure of 2500 psi and minimum of 1500 psi was maintained. The HPLC of solvents was run at 200 nm to 600 nm wavelength using reverse phase C-18 column. During the run, a flow rate 1ml/min was maintained using binary mode of gradient system. Various combinations of the solvents 20:80, 80:20, 60:20, 50:50 of ethanol and water were used respectively. Ultimately for achieving best resolution of peaks the experiment was performed at 50:50 ratio of the solvent (ethanol and water). To identify the compounds, several standards of the secondary

metabolites were used. The peaks were identified by comparing the retention time (RT) of the standard compounds with that of different peaks obtained in HPLC analysis of extracts.

2.6. Qualitative phytochemical screening (Nwauche *et al.*, 2023)

2.6.1. Test for Alkaloids

0.2g of evaporated extract was boiled with 5ml of 2% HCl on a steam bath for 5mins; the mixture was filtered after cooling. The filtrate was shared into three test tubes (A, B and C). 1ml portion of the filtrate was treated with two drops of Mayer's Reagents for observation. To confirm the result, one 1ml portion of the filtrate was treated with Drage dorff's reagent to observe the reaction.

2.6.2. Test for Tannins

About 0.5g of the dried powdered samples was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and observed for brownish green or a blue-black coloration.

2.6.3. Test for Phenols

5ml of the extract was piped into a 3ml test tube and then 10ml of distilled water added to it. 2ml of ammonium hydroxide solution and 5ml of concentrated amyl alcohol was added and left for thirty 30mins. Result from the observation was recorded.

2.6.4. Test for Flavonoids

0.5g of extracts was introduced into a test tube, 10ml ethyl acetate solution was added and heated in a boiling water for 1min, the mixture was filtered and 4ml of filtrate shaken with 1ml of 1% aluminum chloride solution and left to stand for 10mins. Then observe for the formation of yellow coloration which indicated a positive test for flavonoids.

2.6.5. Test for Saponins

About 2 g of the powdered sample was boiled in 20ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

2.6.6. Test for Terpenoids

An amount of 0.8g of the dry, wet and oil extracts were taken in test tubes, then into it was poured 10ml of methanol, shake well and filtered to obtain 5ml extracts of each sample. Then 2ml of chloroform were mixed with each extract of the sample and 3ml of sulphuric acid were added to the samples extract. Formation of reddish-brown color indicates the presence of terpenoids in the samples.

2.6.7. Test for Glycosides

To 2ml of the extract in the test tube 5ml of distilled water and 2ml of concentrated H₂SO₄ was added and boil for 15mins using water bath. Allow cooling and then neutralizing with 2ml of 20% KOH, added 1ml of equal volume of Fehline solution A and B and boil for 15mins. The formation of brick red coloration confirmed the presence of glycoside.

2.7. Statistical Analysis of Data

All data were analyzed for statistical differences by means of one-way ANOVA and post hoc LSD, on SPSS 27. In all, $p < 0.05$ was considered significant. Data are presented as mean \pm S.D (standard deviation).

3. Results

3.1. Phytochemical Composition of *Cymbopogon flexuosus* Leaf

Tables 1a to 1d show the quantitative phytochemical composition of *Cymbopogon flexuosus* leaf. Table 1a presents the analysis of phenolic acids and saponins, with significant concentrations observed. The phenolic acids include ferulic acid at 10.28 ± 0.55 mg/100 g, chlorogenic acid at 8.37 ± 0.01 mg/100 g, and caffeic acid at 2.93 ± 0.04 mg/100 g. The saponins detected include sarsasapogenin at 7.12 ± 0.10 mg/100 g and tigogenin at 5.34 ± 0.02 mg/100 g. Table 1b shows the glycosides and cyanogenic glycosides found in the plant leaf, including salicin (1.36 ± 0.01 mg/100 g) and galliridoside at 7.12 ± 0.10 mg/100 g respectively. Table 1c focuses on the alkaloids and anthocyanins present in the leaf,

with values such as 4.21 ± 0.01 mg/100 g for akuammidine and 32.96 ± 55.46 mg/100 g for cyaniding-3-sophoroside-5-glucoside respectively.

Table 1d shows the flavonoids and sterols, revealing flavonoids like glycitein at 19.01 ± 0.06 mg/100 g and sterols such as sitosterol at 16.25 ± 0.01 mg/100 g

Table 1a Quantitative Phytochemical Analysis of Phenolic Acids and Saponins in *Cymbopogon flexuosus* Leaf

Phytochemicals	Constituents	Concentration (mg/100 g)
Phenolic acids	Protocatechuic acid	2.23 ± 0.01
	4-hydroxybenzoic acid	3.43 ± 0.01
	Vanillic acid	2.01 ± 0.02
	Gallic acid	1.66 ± 0.02
	Caffeic acid	2.93 ± 0.04
	Ferulic acid	10.28 ± 0.55
	Chlorogenic acid	8.37 ± 0.01
Saponins	Sarsasapogenin	7.12 ± 0.10
	Narthogenin	3.62 ± 0.11
	Diosgenin	2.66 ± 0.01
	Neotigogenin	1.44 ± 0.01
	Tigogenin	5.34 ± 0.02
	Neochlorogenin	4.73 ± 0.02
	Hecogenin	1.88 ± 0.01
	Sapogenin	1.66 ± 0.01
	Saponine	4.34 ± 0.17

Table 1b Quantitative Phytochemical Analysis of Glycosides and Cyanogenic Glycosides in *Cymbopogon flexuosus* Leaf

Phytochemicals	Constituents	Concentration (mg/100 g)
Glycosides	Arbutin	0.31 ± 0.05
	Linamarin	0.10 ± 0.00
	Salicin	1.36 ± 0.01
	Artemetin	0.40 ± 0.01
	Methyl Linamarin	0.27 ± 0.01
	Amygdalin	0.55 ± 0.01
	Ouabain	0.37 ± 0.00
	Digoxin	0.22 ± 0.01
	Lotaustralin	0.46 ± 0.01
Cyanogenic glycosides	Galliridoside	7.12 ± 0.10
	7-chloro-6-Desoxy-Haraogide	2.40 ± 1.99
	Quabain	0.13 ± 0.05

	Taraxacoside	0.23±0.05
	Verbacoside	0.28±0.03
	Lavandulifolioside	0.22±0.04

Table 1c Quantitative Phytochemical analysis of Alkaloids and Anthocyanins in *Cymbopogon flexuosus* Leaf

Phytochemicals	Phytochemicals	Concentration (mg/100 g)
Alkaloids	9-Octadecenamide	2.36±0.01
	Vicine	1.62±0.02
	Dihydro-oxo-demethoxyhaemanthamine	3.88±0.01
	Augustamine	1.25±0.01
	Oxoassoanine	2.07±0.01
	Sparteine	1.57±0.01
	Cinchonidine	2.31±0.02
	Cinchonine	1.04±0.01
	Crinane-3- α -ol	2.13±0.00
	Buphanidrine	1.87±0.02
	Alpha allocryptopine	3.22±0.04
	Coptisine	2.55±0.12
	Starchdrine	7.05±0.05
	Tetrahydrocoptisine	2.18±0.02
	Crinamidine	2.47±0.02
	Akuammidine	4.21±0.01
Echitamidine	2.86±0.01	
Anthocyanins	Peonidin-3-sophoroside-5-glycoside	19.03±32.01
	p-hydroxybenzolated (cyanidine-3-sophoroside	1.37±0.05
	Caffeolated (cyaniding-3-sophoroside-5- glucoside	32.96±55.46
	p-hydroxybenzolated (Peonidin-3-sphoroside	1.06±0.01
	Cyanidin-3-glucoside	0.86±0.01

Table 1d Quantitative Phytochemical analysis of Flavonoids and Sterols in *Cymbopogon flexuosus* Leaf

Phytochemicals	Constituents	Concentration (mg/100 g)
Flavonoids	Daidzein	0.98±0.01
	Coumesterol	3.63±0.06
	Genistein	5.71±0.08
	Glycitein	19.01±0.06
	Daidzin	13.11±0.28

	6-O-Acetyl daidzin	6.75±0.09
Sterols	Cholesterol	2.78±0.01
	Cholestanol	11.32±0.01
	Ergosterol	7.64±0.01
	Campesterol	14.54±0.02
	Stigmasterol	1.32±0.01
	Savenasterol	4.90±0.02
	Sitosterol	16.25±0.01

4. Discussion

The quantitative phytochemical analysis of *Cymbopogon flexuosus* leaf extract revealed a rich composition of bioactive compounds, including phenolic acids, saponins, glycosides, alkaloids, flavonoids, and sterols (Tables 1a–1d). These compounds are known for their antioxidant, anti-inflammatory, and antimicrobial properties, which play crucial roles in the gastroprotective effects of the plant. The presence of significant concentrations of phenolic acids such as ferulic acid (10.28±0.55 mg/100 g) and chlorogenic acid (8.37±0.01 mg/100 g) is essential. Phenolic acids are well-established antioxidants that scavenge free radicals and reduce oxidative stress which is a primary cause of gastric ulceration (Farzaei *et al.*, 2015). Ferulic acid has been shown to protect the gastric mucosa by reducing lipid peroxidation and enhancing antioxidant defenses (Ermis *et al.*, 2023). Chlorogenic acid, on the other hand, has been demonstrated to reduce inflammation and promote wound healing in various studies (Bagdas *et al.*, 2020).

In a related study by Nambiar and Matela, (2012), the therapeutic potential of phenolic acids and flavonoids in *Cymbopogon flexuosus* was extensively documented. They reported that these phytochemicals contribute to the plant's use in treating gastrointestinal disorders, particularly through their antioxidant effects. Similarly, some researches have shown that *Cymbopogon flexuosus* extracts possess significant anti-inflammatory properties due to their high citral content, which is a common phytochemical in lemongrass species.

The presence of saponins, including sarsasapogenin (7.12±0.10 mg/100 g) and tigogenin (5.34±0.02 mg/100 g), may also play a role in the plant's anti-ulcer effects. Saponins are known for their ability to reduce inflammation and enhance mucosal defense mechanisms (Shahzad *et al.*, 2024). Their presence in the extract suggests that they contribute to the healing of gastric ulcers by stimulating the production of protective mucus in the stomach (Shahzad *et al.*, 2024).

The detection of alkaloids such as 9-Octadecenamide (2.36±0.01 mg/100 g) and anthocyanins like caffeoylated cyanidin-3-sophoroside (32.96±55.46 mg/100 g) further supports the therapeutic potential of *Cymbopogon flexuosus*. Alkaloids are known for their antimicrobial and anti-inflammatory activities, which could help mitigate *Helicobacter pylori* (H. pylori)-induced ulcers (Baker, 2020). Anthocyanins, on the other hand, have potent antioxidant properties that protect the gastric mucosa from oxidative damage (Mattioli *et al.*, 2020).

5. Conclusion

These phytochemical profile of *Cymbopogon flexuosus* leaf gotten from this research suggest strong nutraceutical potential of this plant. Further research in it therapeutic uses in the management and prevention of disease as a result of its rich phytochemical composition could be explored. It may be a potent pharmaceutical which will help to alleviate some certain kind of diseases and infections such as cancer, cardiovascular diseases, diabetes mellitus, ulcer, cough, hypertension, piles, asthma, malaria etc.

Compliance with ethical standards

Disclosure of conflict of interest

Authors have declared that no competing interests exist.

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