

PHYTOCHEMICAL INVESTIGATION AND STUDY OF ANTIOXIDANT AND ANTI-INFLAMMATORY PROPERTIES OF CORYMBIA CITRIODORA

Shanta Joseph¹, Jag Narayan,^{2*} Avdhesh Kumar³

¹Department of Biotechnology Holy Cross Women's College Ambikapur, Sarguja (C.G.)

^{2*}Department of Biotechnology Holy Cross Women's College Ambikapur, Sarguja (C.G.)

³Department of Biotechnology Holy Cross Women's College Ambikapur, Sarguja (C.G.)

Abstract

Background and aim - *Corymbia citriodora* (lemon-scented gum) is traditionally used for treating inflammation and infections. Its leaves are rich in bioactive compounds like flavonoids and terpenoids, known for antioxidant and anti-inflammatory properties. Despite its medicinal use, further studies are needed to identify and confirm its key phytochemicals and biological activities.

Aim-To investigate the phytochemical composition of *Corymbia citriodora* and evaluate its antioxidant and anti-inflammatory properties to validate its traditional uses and explore therapeutic potential. **Methods**: The leaves of *Corymbia citriodora* were collected, dried, powdered, and subjected to solvent extraction using Methanol, Water, n-Hexane, and Acetone. Phytochemical analysis was conducted to detect major compounds. Qualitative tests identified groups like alkaloids, flavonoids, phenols, and terpenoids. Antioxidant activity was assessed using DPPH, Phosphomolybdenum assay, and FRAP assays to determine the extracts' free radical scavenging potential. Anti-inflammatory activity was evaluated through protein denaturation inhibition tests. All experiments were performed in triplicate, and statistical analysis was conducted using ANOVA, with significance set at $p < 0.05$.

Results: Qualitative phytochemical screening of various *Corymbia citriodora* extracts confirmed the presence of alkaloids, flavonoids, terpenoids, steroids, phenols, tannins, and glycosides. Antioxidant assays (FRAP, phosphomolybdenum, and DPPH) showed the highest activity in methanol extracts compared to water, n-hexane, and acetone. Methanol extracts also exhibited the most effective inhibition of heat-induced protein denaturation, followed by water, n-hexane, and acetone. At 100 $\mu\text{g/mL}$, inhibition percentages were 78 ± 0.27 (methanol), 71 ± 1.06 (water), $64 \pm 1.36\%$ (n-hexane), $53 \pm 1.04\%$ (acetone), and $81 \pm 1.36\%$ (diclofenac sodium).

Conclusions: The phytochemical evaluation of *Corymbia citriodora* revealed significant antioxidant and anti-inflammatory activities, highlighting its pharmacological potential. Advanced phytochemical strategies could help develop these components into marketable drugs

Key words: *Corymbia citriodora*, Antioxidant activity, Anti-inflammatory activity, Phytochemical analysis, Protein denaturation inhibition

Introduction

Corymbia citriodora exhibits substantial anti-inflammatory and antioxidant qualities, enhanced by its essential oils and extracts, highlighting its potential for therapeutic applications in managing oxidative stress and inflammation.(1) The essential oil of *Corymbia citriodora*'s chemical constituents and antioxidant properties, identifying 72 compounds and demonstrating in vitro antioxidant properties, but does not cover anti-inflammatory properties.(2) the antioxidant activity of *Corymbia citriodora* essential oil but does not specifically investigate its phytochemical composition or anti-inflammatory properties.(3)

Corymbia citriodora, commonly known as lemon-scented gum, is a prominent species in the Myrtaceae family, indigenous to Australia.(4) This evergreen tree is renowned not only for its distinctive aroma but also for its rich phytochemical composition, which holds significant therapeutic potential. Historically, various parts of *C. citriodora* have been utilized in traditional medicine to treat a plethora of ailments, hinting at its diverse bioactive compounds. Among its most notable constituents are essential oils, flavonoids, and phenolic acids, which are reputed for their medicinal properties.(5)

Oxidative stress and inflammation are critical factors in the pathogenesis of numerous chronic diseases, including cardiovascular disorders, cancer, and neurodegenerative conditions. Antioxidants and anti-inflammatory agents play a crucial role in mitigating these processes, thus offering therapeutic benefits. The exploration of natural sources for such compounds has intensified, driven by the growing preference for alternative medicine and the need for safer, more sustainable therapeutic options.(6)

This study aims to delve into the phytochemical profile of *C. citriodora*, with a particular focus on its antioxidant and anti-inflammatory properties. By employing advanced analytical techniques, we seek to isolate and characterize the key bioactive compounds present in this species. Furthermore, through in vitro and in vivo assessments, we intend to elucidate the mechanisms underlying the observed biological activities. This research not only contributes to the understanding of *C. citriodora*'s therapeutic potential but also underscores the broader significance of phytochemicals in modern medicine.

Through this investigation, we aspire to pave the way for future studies that may lead to the development of novel, plant-derived therapeutics, harnessing the natural bounty of *C. citriodora* to combat oxidative stress and inflammation-related diseases.

Materials and Method

Identification of the Plant

Corymbia citriodora (lemon-scented gum) was identified based on its smooth white to pinkish bark, elongated lemon-scented leaves, straight trunk, and cream-colored summer flowers.(7) Identification was confirmed using reference images and expert guidance.

Collection of Plant Materials

In March 2024, fresh *C. citriodora* leaves were collected locally, packed in polyethylene bags, transported to the lab, and stored at room temperature.

Cleaning of Plant Materials

The collected leaves were manually cleaned, washed, and separated from stems to ensure quality.

Drying

Plants are air-dried in the shade at room temperature for 5-7 days to remove moisture and prevent spoilage, ensuring safe storage.

Powdering

Fully dried leaves were ground using an electric grinder for 30 seconds, followed by an additional 3 minutes for fine powdering. The powdered samples were stored in airtight glass bottles for extraction.

Extraction using Soxhlet apparatus

Soxhlet extraction was performed on 10 g of powdered *C. citriodora* leaves using 100 ml of distilled water, methanol, acetone, and hexane as solvents. Boiling temperatures were maintained at 100°C, 64°C, 56°C, and 68°C, respectively, for 90 minutes under reflux. After extraction, the solvent was evaporated, and the extracts were filtered and dried in a hot air oven at 37°C for 24 hours, yielding semi-solid extracts.(8)

Qualitative phytochemical analysis

A comprehensive analysis of crude drugs involves examining both primary and secondary metabolites originating from plant metabolism. Various qualitative tests such as Various phytochemical tests were conducted, including **Alkaloids**: Wagner's Test, Dragendorff's Test, **Flavonoids**: Shinoda Test, NaOH Test, **Glycosides**: Keller-Killani Test, Molisch's Test, **Phenols**: Phenol Test, **Saponins**: Froth Test, Foam Test, **Terpenoids**: Salkowski Test, **Tannins**: Gelatin Test, Lead Acetate Test, **Anthraquinones**: Bontrager's Test, **Steroids**: Steroid Test are conducted to establish the chemical composition profiles of different extracts. Subsequently, these extracts undergo specific chemical tests according to prescribed methods to identify various phytoconstituents. (9) (10), (11), (12).

Antioxidant activity analysis

Ferric Iron Reducing Antioxidant Power (FRAP) assay

To evaluate the antioxidant potential of *Corymbia citriodora* extracts, the FRAP test was used. Prior to the experiment, all samples and reagents, such as ferric chloride solutions, TCA, potassium ferricyanide, and PBS, were newly made. Extracts made in distilled water, methanol, acetone, and hexane were diluted to quantities between 100 and 500 microliters. For 20 minutes, the reaction mixture—which included 2.5 mL of PBS, 2.5 mL of extract, and 2.5 mL of 1% potassium ferricyanide—was incubated at 50°C. 2.5 mL of 10% TCA was added to stop the reaction, and centrifugation was then run for 10 minutes at 3000 rpm. 2.5 mL of the resultant supernatant, 2.5 mL of distilled water, and 0.5 mL of a 0.1% ferric chloride solution were added, and the mixture was left to react for 10 minutes. A spectrophotometer was then used to measure absorbance at 700 nm. The positive reference was ascorbic acid, while the control was a blank sample. Antioxidant activity was evidenced by a rise in absorbance, which showed that ferric ions

(Fe³⁺) were reduced to ferrous ions (Fe²⁺). The findings provide a numerical assessment of the extracts of *Corymbia citriodora*'s antioxidant capacity (12, 13).

Phosphomolybdate assay

By creating a phosphomolybdenum complex, the Phosphomolybdate assay—which was modified from Prieto et al. (2)—measures overall antioxidant capability. 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate are combined to create the reagent.

For the experiment, 1 mL of the prepared reagent is combined with 0.1 mL of the sample (containing 100 µg of analyte) and incubated for 90 minutes at 95°C. A UV spectrophotometer is used to measure absorbance at 695 nm once the reaction mixture has cooled to room temperature during incubation. The reference standard is ascorbic acid, while the control is a blank sample. The absorbance data are used to calculate the total antioxidant capacity.

DPPH Assay

A popular technique for assessing the antioxidant potential of different substances is the DPPH test. The DPPH methanol solution will decolorize as a result of a reduction process caused by antioxidants' capacity to scavenge DPPH radicals. Blois (1958) originally described the technique, which quantifies the antioxidants' ability to reduce the DPPH radical (12). This work used Blois's (1958) DPPH technique to assess the extracts of *Corymbia citriodora*'s capacity to scavenge free radicals (12). In short, 3 mL of methanol-containing plant extracts at several concentrations (100, 200, 300, 400, and 500 µg/mL) was mixed with 100 µL of DPPH solution. After vortexing, the reaction mixtures were allowed to sit at room temperature for half an hour. At 517, absorbance was measured.

All samples were prepared and analyzed in triplicate, and the results are reported as mean ± SD.

Anti-inflammatory activity

Padmanabhan and Jangle (2012)(12) used a modified approach to assess the anti-inflammatory efficacy of extracts of *Corymbia citriodora* (made in methanol, distilled water, acetone, and hexane), using Diclofenac sodium as the reference. PBS and egg albumin were combined with extracts at a concentration of 100 µg/mL, and the mixture was incubated for 15 minutes at 27°C. After that, the mixture was heated to 70°C to denaturize the proteins. A spectrophotometer was used to detect the absorbance at 660 nm after cooling. The following formula was used to determine the percentage inhibition of protein denaturation:

$$= [(At - Ac) / Ac] \times 100 = \text{Inhibition}(\%)$$

where Ac is the absorbance of the control and At is the absorbance of the test sample.

Result

Phytochemical Analysis of leaf extracts of *Corymbia citriodora*

The leaf extracts were analyzed for phytochemical constituents to identify bioactive compounds, which could potentially contribute to drug discovery and development. In this study, hexane, ethanol, and methanol extracts of *Corymbia citriodora* were assessed for their qualitative and quantitative phytoconstituents.

Qualitative phytochemical analysis

S. No.	Phytochemical Test	Test performed	Result			
			Water	Methanol	n-Hexane	Acetone
1.	Test for alkaloid					
		Wagner's test	+	+	+	+
		Dragendorff's test	+	+	+	+
2.	Test for flavonoids					
		Shinoda's test	+	+	+	+
		NaOH test	+	+	+	+
3.	Test for glycosides					
		Killer Killani test	+	+	+	+
		Molish's test	+	+	+	+
4.	Test for phenol					
		Phenol test	+	+	+	+
5.	Test for saponin					
		Froth test	+	+	+	+
		Foam test	+	+	+	+
6.	Test for terpenoids					
		Salkowskis test	+	+	+	+
7.	Test for tannin					
		Gelatin test	+	+	+	+
		Lead acetate test	+	+	+	+
8.	Test for anthraquinone					
		Bontrager's test	+	+	+	+
9.	Test for steroids					
		Steroid test	+	+	+	+

(+) present; (-) absent

Table: preliminary qualitative phytochemical study of *C. citriodora* leaf extracts.

Antioxidant activity

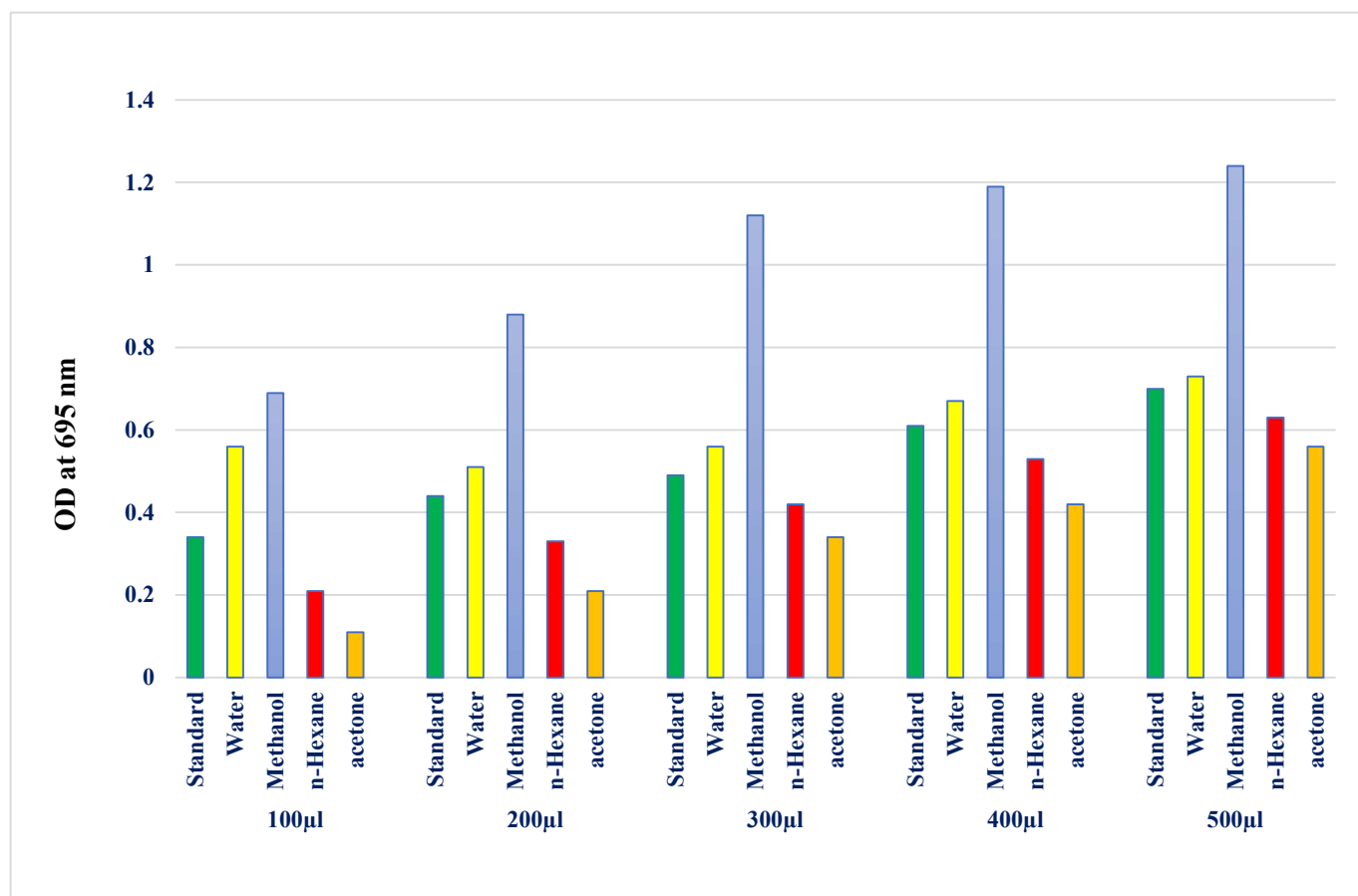
Oxidative stress, caused by an imbalance in redox status and excess free radicals, leads to cellular damage and adverse effects like inflammation and antiproliferation. Defense against oxidative stress is crucial to prevent diseases. Phytochemicals such as anthocyanins, flavonoids, and

phenolics exhibit antioxidant activity and are preferred over synthetic antioxidants. Their effectiveness depends on plant extract composition, growing conditions, and experimental methods. In this study, The FRAP, PM, and DPPH tests were used to evaluate the extract's antioxidant activity.

Ferric Ion Reducing Antioxidant Power (FRAP) assay

The antioxidant qualities of *Corymbia citriodora* leaf extracts in water, methanol, acetone, and n-hexane were evaluated in this study using the FRAP assay in conjunction with standard gallic acid. Methanol extracts of *Corymbia citriodora* leaves showed the highest antioxidant activity when compared to water, n-hexane, and acetone extracts. The graph displays the findings of the FRAP assay for the antioxidant activities of water, methanol, acetone, and n-hexane leaves extract of *Corymbia citriodora*.

Concentration (µg/ml)	Standard	Water	Methanol	n-Hexane	Acetone
100 µg/ml	0.34	0.56	0.69	0.21	0.11
200 µg/ml	0.44	0.51	0.88	0.33	0.21
300 µg/ml	0.49	0.56	1.12	0.42	0.34
400 µg/ml	0.61	0.67	1.19	0.53	0.42
500 µg/ml	0.77	0.73	1.24	0.63	0.56



Graph 1 FRAP assay of *Corymbia citriodora* leaf extracts

Phosphomolybdate assay:

When the phosphomolybdate assay was used in this study to test the methanol, water, acetone, and n-hexane leaf extracts of *Corymbia citriodora* along with standard ascorbic acid, the methanol extracts showed the highest antioxidant activity, followed by water, acetone, and n-hexane extract.

Concentration (µg/ml)	Standard	Water	Methanol	n-Hexane	Acetone
100 µg/ml	1.44	0.45	0.66	0.21	0.23
200 µg/ml	1.64	0.51	0.71	0.33	0.34
300 µg/ml	1.67	0.56	0.78	0.42	0.44
400 µg/ml	1.68	0.67	0.83	0.53	0.42
500 µg/ml	1.71	0.73	0.89	0.63	0.61

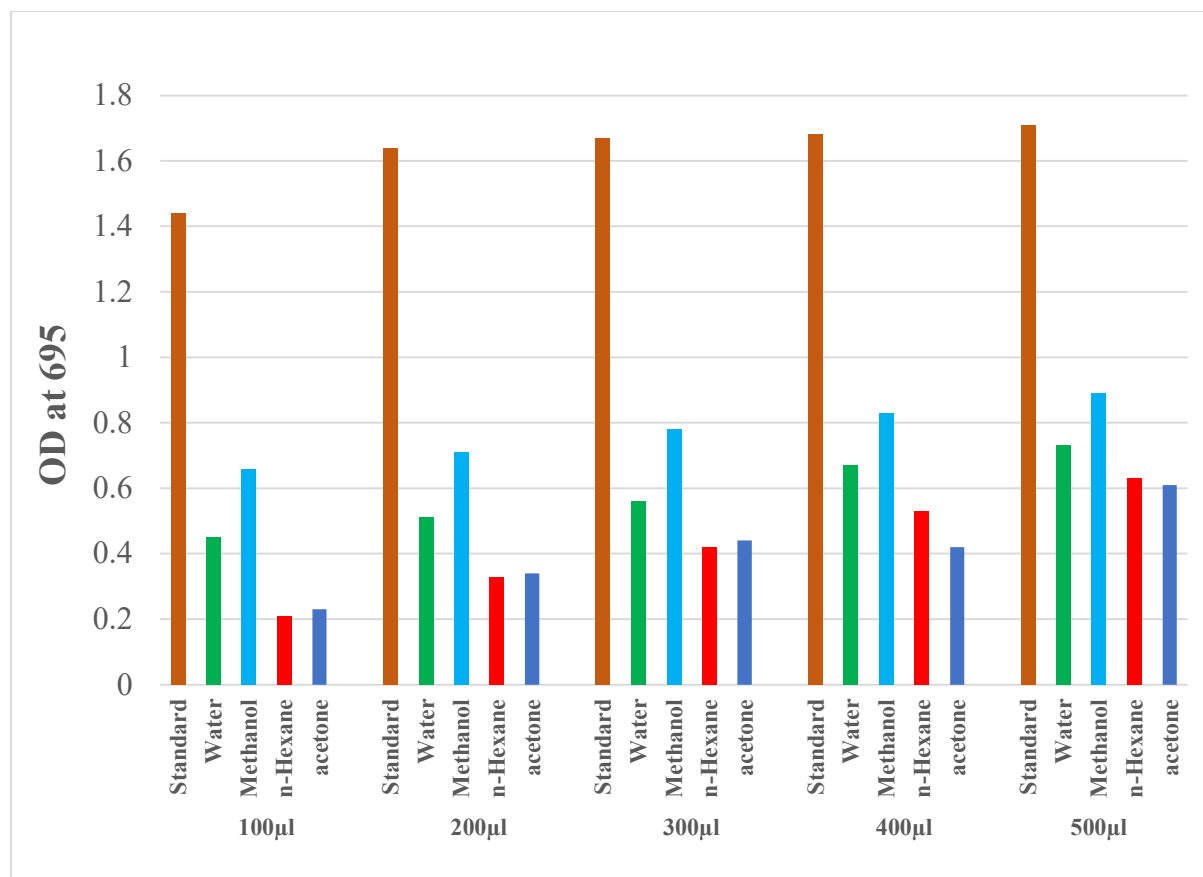


Fig Phosphomolybdate assay

DPPH assay

Ascorbic acid was used as a reference standard in the DPPH radical technique to assess the *Corymbia citriodora* leaf extracts. The range of concentrations was 100–500 µg/mL. The findings of *Corymbia citriodora*'s DPPH free radical percentage inhibition Methanol extracts outperformed

water n-hexane and acetone in terms of antioxidant activity at all investigated doses, including 100 µg/mL, 200 µg/mL, 300 µg/mL, 400 µg/mL, and 500 µg/mL.

Concentration (µg/ml)	Inhibition (in %)				
	Standard	Water	Methanol	n-Hexane	Acetone
100 µg/ml	39±1.12	24±2.01	33±1.19	21±1.01	11±2.06
200 µg/ml	45±2.01	27±1.44	48±0.96	33±1.35	21±1.12
300 µg/ml	49±1.45	32±0.67	56±0.86	41±0.36	34±0.24
400 µg/ml	74±0.68	39±0.54	61±1.22	49±0.67	42±0.35
500 µg/ml	81±0.58	52±0.95	70±0.98	59±0.77	56±0.86

***In vitro* anti-inflammatory assay**

Protein denaturation, caused by external stress (e.g., acids, bases, salts, solvents, or heat), leads to loss of function and inflammation. Anti-inflammatory activity was assessed by testing the ability of *Corymbia citriodora* leaf extracts (water, methanol, acetone, n-hexane at 100 µg/mL) to inhibit protein denaturation, using diclofenac sodium as a reference drug)(13).

Corymbia citriodora leaf extracts in methanol demonstrated effective inhibition of heat-induced protein denaturation, which was followed by water, n-hexane and acetone. It was found that extracts of methanol, water, n-hexane, and acetone leaves of *Corymbia citriodora* and the diclofenac sodium reference standard inhibited heat-induced protein denaturation by 78%, 71 %, 64%, 53%, and 81% percent at 100 µg/ml, respectively.

Treatment	Concentration	Inhibition (%) = $\frac{At - Ac}{Ac} \times 100$
Methanol-extract	100 µg/mL	78 ± 0.27
Water-extract	100 µg/mL	71 ± 1.06
Acetone-extract	100 µg/mL	53 ± 1.04
n-Hexane	100 µg/mL	64 ± 1.36
Diclofenac sodium	100 µg/mL	81 ± 1.36

Table Anti-inflammatory activities of *Corymbia citriodora* leaf extracts.

At is the test sample's absorbance, while Ac is the control's absorbance.

Discussion –

India is among the top 12 mega biodiversity centers globally, owing to its diverse climate, soil, altitude, and latitude. It houses the largest repository of medicinal plants, crucial for producing raw

materials for crude drugs, pharmaceuticals, and cosmetics. Herbal medicines have been a primary healthcare source worldwide, with 80% of the population relying on traditional medicine. India records around 20,000 medicinal plants (14) with traditional communities using 7,000–7,500 species for treating various diseases (15).

Phytochemical screening is essential for identifying bioactive compounds and advancing drug discovery. (16) Medicinal herbs comprise diverse phytochemicals similar as polyphenols, steroids, terpenoids, glycosides, alkaloids, tannins, and saponins, which exhibit significant chemical, toxicological, and pharmacological properties (17). These biologically active compounds provide health benefits (18) and play a key role in protecting human health when ingested in adequate amounts. Over 4000 phytochemicals have been cataloged, with around 150 studied in detail (19) This study analyzed methanol, water, acetone, and n-hexane extracts of *Corymbia citriodora* for phytochemical composition.

Antioxidant Assay

Quantitative analysis of total phenols and flavonoids in extracts does not fully represent their antioxidant potential. Various antioxidant assays are necessary to evaluate this potential. While natural antioxidants from tea, fruits, vegetables, and spices are widely used commercially, the search for novel plant-based antioxidants continues (20, 21). The antioxidant properties of plants are often attributed to phenolic and flavonoid compounds, whose biological actions are linked to their antioxidant activity. In this study, PM, DPPH, and FRAP scavenging assays were used to evaluate the total antioxidant properties of different extracts.

FRAP Assay

The FRAP method, introduced by (22), evaluates antioxidants' capacity to reduce by reducing ferric TPTZ to a colored complex. It is frequently employed to assess the antioxidant capacity of plant extracts (23). In this study, methanol leaf extracts of *Corymbia citriodora* having the highest antioxidant activity in contrast to water, acetone, and n-hexane extracts, and were comparable to the standard gallic acid. These values align with the antioxidant potential of various plant extracts (24).

Phosphomolybdate assay:

In our study, when methanol, ethanol, and chloroform leaf extracts of both *Corymbia citriodora* were assayed by phosphomolybdate assay along with standard ascorbic acid. methanol, extracts of *Corymbia citriodora* exhibited the highest antioxidant activity than water, acetone, and n-hexane extracts.

DPPH assay:

Free radicals cause oxidative damage linked to aging, cardiovascular diseases, cancer, and inflammation. The DPPH Assay were used to test the radical-scavenging activities for four *Corymbia citriodora* extracts, unaffected by side reactions like metal ion chelation (25). Compared to ascorbic acid as a positive control, scavenging activity risen in conjunction with concentrations

from 100 to 500 µg/mL. Methanol leaf extracts showed the highest antioxidant activity across all tested concentrations (100–500 µg/mL) compared to water, n-hexane, and acetone extracts.

***In-vitro* anti-inflammatory activity**

In this study, the in-vitro anti-inflammatory activity of *Corymbia citriodora* leaf extracts prepared in methanol, water, acetone, and n-hexane was assessed based on their ability to inhibit the denaturation of egg albumin. Among the tested extracts, the methanol extract exhibited the highest inhibition of thermally induced protein denaturation, followed by the water, acetone, and n-hexane extracts. The percentage inhibition of thermally induced protein denaturation was recorded as $81 \pm 1.36\%$, $78 \pm 0.27\%$, $71 \pm 1.06\%$, $64 \pm 1.36\%$, and $53 \pm 1.04\%$

for methanol, water, acetone, n-hexane extracts, and the reference drug diclofenac sodium, respectively. These results indicate that *Corymbia citriodora* extracts, particularly the methanol extract, are rich in phenolic compounds and demonstrate significant anti-inflammatory activity.

Conclusion

The remarkable results of the present phytochemical evaluation of *Corymbia citriodora* has shown antioxidant and anti-inflammatory activity. The studies conducted have potential pharmacognosy and its applications with many advanced phytochemical strategies to bring these components into a latent drug into the market.

Ethic approval: This study was conducted in accordance with the ethical guidelines of the Institutional Ethics Committee (IEC) of Holy Cross Women's College, Ambikapur, Sarguja, Chhattisgarh. No animals or microorganisms were used in this study.

Conflict of Interest: All authors have reviewed and approved the manuscript and confirm that there are no financial, professional, or personal conflicts that could have influenced the research outcomes.

Authors Contribution: JN conceived and designed the study, collected and organized the data. AK analyzed the data, and SJ drafted the manuscript and provided research materials. All authors contributed to data interpretation, provided critical feedback, approved the final draft, and are responsible for its content and originality..

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