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Research Article

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Phytochemical profile and antioxidant potential of leaf and bark extracts of *Cassine glauca* (Rottb.) Kuntze

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ABSTRACT

In the present study the phytochemical composition and antioxidant prospective of *Cassine glauca* leaf and bark extracts. Leaf and bark of *C. glauca* were extracted by methanol and chloroform through Soxhlet extraction. The extracts were analyzed for qualitative and quantitative phytochemical constituents, including phenolics, flavonoids, tannins, and other bioactive compounds. Antioxidant activity was determined through five different methods such as DPPH radical scavenging, reducing power, assay of molybdenum, hydrogen peroxide (H_2O_2) scavenging, and nitric oxide scavenging assays. Phytochemical analysis revealed that methanol extracts from both leaf and bark were more effective than chloroform, showing higher concentrations of phenolics, tannins and flavonoids. The leaf extract had a total phenol content of 47.458 mg GAE/g, tannins at 45.298 mg RE/g. and a flavonoid content of 160.106 mg TAE/g, and the bark extract showed lower but notable levels of these compounds. The leaf extract showed greater antioxidant activity when compared with bark extract in all experiments, includes DPPH radical scavenging activity, molybdenum assay, reducing power activity, nitric oxide scavenging activity and H_2O_2 scavenging activities. Methanol extracts of *C. glauca* leaf and bark exhibited significant antioxidant properties, with the leaf extract showed greater efficacy. The present study highlights a prospective source of *C. glauca as* a potential resource of natural antioxidants and suggests additional exploration of its therapeutic benefits in oxidative stress-related disorders. The results emphasize the effectiveness of methanol as a solvent for extracting potential bioactive compounds from *C. glauca*.

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INTRODUCTION

Traditional medicine has become increasingly important worldwide in recent years, particularly in developing and developed nations where numerous people depend on medicinal plants for their basic health care needs (Mosihuzzaman, 2012). Historical records emphasize the crucial role of herbal medicine specialists in promoting well-being (Iyiola & Wahab, 2023). The healing properties of medicinal herbs are linked to their various phytochemical compounds, including flavonoids, phenols, alkaloids, and terpenes (Raina *et al.*, 2014). Factors like population growth, limited synthetic pharmaceuticals, high costs, and adverse effects of man-made medications drive increased use of plant-based medicines. Phytomedicine involves using phytochemical extracts or phytocompounds to interact with cells, tissues, or organs to manage conditions such as diabetes, and cardiovascular diseases, and prevent oxidants, and infections (Hassan, 2012). Plant secondary metabolites are good potential antimicrobial agent and derived from different parts of the herbal plants (Sundari & Kavitha, 2024).

Medicinal plants are defined as plants with therapeutic value in any part such as leaves, stems, bark, roots, flowers, or fruits are employed in the creation of medicinal mixtures or as ingredients in industrialized pharmaceutical drugs. These plants are frequently utilized in traditional or alternative and complementary medicine for the prevention and treatment of particular illnesses and conditions (Chintamunnee & Mahomoodally, 2012). They may be located as wild species naturally present in terrestrial ecosystems with minimal human disturbance or cultivated domestically through selective breeding for commercial purposes, providing bioactive compounds essential for pharmaceutical drug production (Calixto, 2000).

Addressing oxidative stress with medicinal plants involves enhancing bioavailability, understanding interactions,

personalizing treatments, ensuring safety, and integrating with conventional medicine (Muscolo *et al*., 2024). By adopting a comprehensive approach, it is possible to harness the benefits of medicinal plants while overcoming challenges related to drug resistance and efficacy. Phytochemical compounds were identified through various sophisticated instrumentation methods such as HPLC, GC-MS and LC-MS (Kavitha *et al*., 2024).Various phytomolecules, including quercetin are help to prevent the oxidative damage in human beings by modulating glutathione levels, enzyme activity, and signal transduction pathways (Xu *et al*., 2019). To date, numerous bioactive compounds have been discovered and characterized from the medicinal plants that have been effectively utilized for biomedical applications. Several phytocompounds derived from natural products are currently undergoing clinical trials. Despite the increasing interest in conventional medicine, the demand for alternative medicine remains strong, prompting ongoing research in this field. Numerous chemical compounds produced by medicinal plants exist as valuable secondary metabolites (Dhanasekaran *et al*., 2025).

Cassine is a notable tropical genus that belongs to the Caesalpiniaceae family comprising around 600 species around the globe. *C. glauca* is an indigenous to India, tropical Asia and Australia. This species is distinguished by its smooth, evergreen characteristics and its swift growth into shrubs or small trees that can reach heights of 2 to 5 meters. The leaves are bipinnately compound, showcasing 4 to 6 pairs of ovate leaflets with notched tips. Its flowers are vibrant yellow and are found in axillary racemes. Historically, *C. glauca* has been utilized for numerous medicinal applications (Albrahim *et al*., 2021). Previous research has examined the antioxidant potential of various extracts derived from the aerial parts and seeds of *C. glauca* (Veerapur *et al*., 2017). Nonetheless, there is a scarcity of studies focused on the antioxidant properties of the leaves and bark of *C. glauca*. Hence, the current study aimed to perform qualitative and quantitative phytochemical analysis and to identify the active compounds present in this plant and explore the antioxidative characteristics of *C. glauca* to tackle oxidative stress, aiming to better understand its potential therapeutic benefits against oxidative damage.

MATERIALS AND METHODS

Collection of Plant materials

Leaves and bark of *Cassine glauca* (Rottb.) Kuntze were collected in Namakkal, identified with the help of plant taxonomist and its specimen sample was deposited at the Herbarium of PG and Research and Department of Botany, Arignar Anna Government Arts College, Namakkal (#NKL2019/12). The collected plant samples were shade dried, powdered and used for extraction.

Preparation of Solvent Extracts

Fine powdered plant samples (Leaf and bark) (20 g) were packed into the thimble of the extraction apparatus, which was placed in the Soxhlet tube and subjected to an 8-hour extraction process using heat. Two separate extractions were performed, one with 250 mL of methanol and another with chloroform. The concentrated vapors obtained from the extracts were further processed and dehydrated using a Rotary Vacuum Evaporator (Equitron, India) at a temperature of 60 °C under reduced pressure. The solvent was carefully evaporated, and the resulting dried crude residues were aseptically weighed, the extract value was then calculated using the formula: Weight of the dried extract/Weight of the plant material×100. The extracts were then re-dissolved in their respective solvents and stored at 4 °C in sterile, labeled, airtight containers for subsequent analysis. This meticulous process ensured the preservation and concentration of the plant extracts for subsequent investigations (Sreelatha & Padma, 2009).

Phytochemical Analysis

The leaf and bark extracts of *C. glauca* underwent qualitative analysis to detect flavonoids, alkaloids, phenolics, glycosides, tannins, coumarins, sugars, proteins, terpenoids, and amino acids. The methodology followed the procedures outlined by Harborne (1973) and Kokate (2005).

Phenolic Content

To assess the total phenolic content of the extracts, Folin-Ciocalteu reagent method was employed, and gallic acid was used as a reference standard. Solutions of gallic acid standards (ranging from 0.01 to 0.05 mg/mL) as well as plant extracts (0.1 and 1 mg/mL) were prepared with methanol. In each tube, 0.5 mL of either the standard or the extract was combined with 2.5 mL of diluted Folin-Ciocalteu reagent and 2 mL of a 7.5% sodium carbonate solution. After allowing the mixture to sit for at least 30 minutes at room temperature, the absorbance was measured at 760 nm using a spectrophotometer. The intensity of the blue color, which correlates to the phenolic content, was reported as mg of gallic acid equivalents (GAE) per gram of the extract. Measurements were conducted in triplicate to ensure accuracy.

Tannin Content

To assess the tannin levels in the leaf and bark extracts of *C. glauca*, the technique utilizing insoluble polyvinyl-polypyrrolidone (PVPP) was used as described. One milliliter of each extract, prepared in methanol at a concentration of 1 mg/mL, was combined with 100 mg of PVPP. The mixture was subjected to vortexing, followed by incubation at 4 °C for 15 minutes, and then centrifuged at 3,000 rpm for 10 minutes. The resulting clear solution, which contains non-tannin phenolics, was evaluated for total phenolic content using a similar method as was applied for the total phenolics. The tannin content was determined as the difference between the total phenolic content and the non-tannin phenolic content.

Flavonoid Content

The total flavonoid content in the leaf and bark extracts of *C. glauca*, followed the method outlined. For each extract, 1 mL of the sample was combined with 4 mL of distilled water and

0.30 mL of a 10% $NaNO_2$ solution. After 5 minutes, 0.30 mL of a 10% $AlCl_3$ solution was introduced, followed by the addition of 2.0 mL of a 1% NaOH solution. The mixture was mixed thoroughly, and the absorbance was recorded at 510 nm using a blank for comparison. The results were reported as quercetin equivalents (mg quercetin/g dried extract) derived from a standard curve for quercetin (0-12 mg/mL).

Antioxidant activity of Leaf and Bark Extracts of *C. glauca*

Based on the phytochemical study, methanol extracts from the leaves and bark of *C. glauca* were selected for the antioxidant activity *viz*. DPPH radical scavenging assay, molybdenum content assay, Reducing power assay, nitric oxide assay and hydrogen peroxide (H_2O_2) assay.

DPPH Radical Scavenging Assay

DPPH radical scavenging activity the methanol extracts from both parts of the plant was evaluated by the method of Ramasamy *et al.* (2022). A 0.004% DPPH solution was prepared in 95% methanol, and varying volumes (0.1 mL to 0.5 mL) of 10 mg/mL of each extract stock solution were tested. The DPPH solution was introduced, and the absorbance was recorded at 517 nm after 10 minutes. Ascorbic acid was used as the reference standard, and the DPPH free radical scavenging activity was evaluated and quantified using the equation below:

$$\%\ \text{of DPPH radicalscavenging activity} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample } b}{\text{Absorbance of control}} \times 100$$

Molybdenum Assay

The antioxidant activity of the extracts from both the leaves and bark was evaluated using the method described by Wan *et al.* (2017). 0.5 mL of an aliquot in each test sample was thoroughly mixed with A total of 4.5 mL of reagent (consisting of 28 mM sodium phosphate, 4 mM ammonium molybdate, and 0.6 M sulfuric acid) was combined with 0.5 mL of 45% ethanol, which served as the blank. The resulting mixture was incubated at 95 °C for 90 minutes, after which it was allowed to cool to room temperature. Absorbance was recorded at a wavelength of 695 nm with a UV-2450 spectrophotometer (Shimadzu, Japan). Higher absorbance indicated greater antioxidant activity.

Reducing Power Assay

The antioxidant enzyme-reducing capability of Fe^{3+} in the extracts from bark and leaves was examined as described by Irshad *et al.* (2012). 0.75 mL of the plant extracts at various concentrations was combined with the 0.75 mL phosphate buffer solution (0.2 m and pH 6.6). Then 0.75 mL of a 1% (w/v) potassium hexacyanoferrate ($K_3Fe(CN)_6$) solution. Both solutions were properly combined and incubated for twenty minutes at a temperature of 50 °C. To this mixture, 0.75 mL of a 10 percent TCA solution was added, followed by a combination of 1.5 mL of double distilled water and 0.1 mL of $FeCl_3$ solution at a concentration of 0.1% (W/V). After ten minutes the absorbance was taken at 700 nm. The highest absorbance indicated a high reducing power activity.

Nitric Oxide Scavenging Assay

The nitric oxide radical scavenging activity of TEMPO-functionalized dendrimers was assessed utilizing the method described by Ali *et al.* (2020) involving sodium nitroprusside. For this assay, a mixture was prepared consisting of 2 mL of a ten mM sodium nitroprusside solution and 0.5 mL of phosphate buffer (pH 7.4), combined with 0.5 mL of either TEMPO-functionalized dendrimers, vitamin C, or a 4-hydroxy TEMPO solution, and incubated at 25 °C for 150 minutes. Following this, 0.5 mL of nitrite was mixed with 1 mL of 0.33% sulfanilic acid ($C_6H_7NO_3S$) in 2% glacial acetic acid and allowed to react for 5 minutes. Afterward, 1 mL of 1% Naphthyl Ethylenediamine Dihydrochloride (NEDD) was introduced, and the reaction mixture was incubated at 25 °C for 30 minutes. The absorbance results were indicated by a pink solution measured at 540 nm. The percentage of nitric oxide inhibition was calculated using the relevant equation.

Percentage (%) of nitric oxide radical scavenging assay = [(A0-A1)/A0] X 100; where A0 was control, and A1 was absorbance of test sample.

Hydrogen Peroxide (H_2O_2) Scavenging Assay

The capability of the extracts to scavenge H_2O_2 was evaluated using a modified approach based on Banothua *et al.* (2017). A 40 mM H_2O_2 solution was created with a 0.05 M phosphate buffer solution (pH 7.4). Different concentrations of plant extracts (25, 50, 75, 100 and 125 µg/mL) were thoroughly combined with 0.6 mL of the H_2O_2 solution. After a ten-minute interval, the absorbance was measured at 230 nm using a UV-visible spectrophotometer (Shimadzu, Japan), with phosphate buffer without H_2O_2 serving as a blank. The percentage inhibition and IC_{50} values were determined by using the following equation.

Radical scavenging activity = $[(OD_{control} - OD_{sample})/OD_{control}]$ X 100, where optical density is the absorbance of samples.

Statistical Analysis

The results were analyzed statistically by using Statistica13.3 software. One-way analysis of variance (ANOVA) with Turkey's post hoc test was used to determine significant differences between the mean values. Values of $p<0.05$ were statistically significant.

RESULTS AND DISCUSSION

Recently, people have sought to manage health issues or reduce hazards by improving their diets. Plants and fruits contain a variety of phytochemical constituents with significant

antioxidant potential. Natural antioxidants are regarded as effective and promising substitutes for synthetic antioxidants in minimizing oxidation within intricate food systems (Wang *et al*., 2009). Phytochemical evaluations of plant extracts have identified the existence of phytoconstituents that demonstrate therapeutic and physiological effects (Banothua *et al*., 2017). In this study, an initial phytochemical analysis was conducted on both the leaf and bark extracts of *C. glauca*. In leaf extract, methanol is a superior solvent for extracting a diverse range of bioactive compounds compared to chloroform. A higher concentration of flavonoids, phenolics, and proteins was observed in the methanol extract, not only this, alkaloids, glycosides, terpenoids, tannins, coumarins, and amino acids were observed. This finding aligns with several previous studies that have examined the efficacy of methanol solvent in extracting valuable phytochemicals. For instance, Farswan *et al.* (2009) highlighted the broad spectrum of therapeutic compounds found in *C. glauca* extracts, emphasizing the importance of selecting appropriate solvents for effective extraction. Similarly Srinivas and Hosamath (2019) also observed the highest phytocompounds from the methanol extract of *C. glauca*.

In the chloroform extract of the leaf, alkaloids, flavonoids, glycosides, and phenolics were detected, but generally at lower concentrations. Flavonoids were present moderately (++), while phenolics were detected in smaller amounts (+). Sugars, tannins, terpenoids, and coumarins were absent, and proteins and amino acids were present at lower levels (Table 1). This represents a lower count of phytocompounds compared to the methanol extract. This finding is consistent with (Tiwari *et al*., 2023), who also observed a higher count of phytocompounds in methanol extracts compared to chloroform. Additionally, Fraga-Corral *et al.* (2020) highlighted that methanol's polar nature makes it more effective in extracting a wider range of phytocompounds.

The preliminary phytochemical screening of *C. glauca* bark extracts reveals that methanol is more effective than chloroform for extracting a diverse range of phytochemicals. Methanol consistently yielded higher concentrations of flavonoids, glycosides, phenolics, tannins, and terpenoids compared to chloroform. Alkaloids and proteins were present in both solvents, but flavonoids and glycosides were detected only in the methanol extract. Proteins and amino acids were present in both extracts, with methanol showing a higher concentration of proteins (++), while both extracts had similar concentrations of amino acids (+).To best of our knowledge there was no previous report on phytochemical composition of *C. glauca* bark extract. The absence of sugars in both extracts suggests either their minimal concentration or limitations in the detection methods used and low-concentration sugars in phytochemical screenings. The commonly utilized term antioxidant capacity refers to a measure used to describe samples obtained from mammals, food, beverages, plant health, and their constituents, which assess their capability to scavenge or neutralize free radicals (Amby *et al*., 2025).

The preliminary phytochemical screening of *C. glauca* leaf and bark extracts underscores methanol's superiority over chloroform in extracting a broader range of bioactive compounds. Methanol extracts were rich in flavonoids, phenolics, proteins, alkaloids, glycosides, terpenoids, tannins, and amino acids. They exhibit significant antioxidant and anti-inflammatory characteristics. Potential antimicrobial and anticancer effects, and support cardiovascular health and overall physiological functions (Tungmunnithum *et al*., 2018; Zandavar & Afshari Babazad, 2023; Bhavikatti *et al*., 2024). This aligns with the work (Farswan *et al*., 2009; Srinivas & Hosamath, 2019), who emphasized methanol's effectiveness in extracting diverse phytochemicals. Elevated concentrations of flavonoids, phenolics, and terpenoids indicate notable antioxidant, anti-inflammatory, and antimicrobial characteristics. Singh and Tiwari (2022) and Corral *et al*. (2020) further support these findings, noting methanol's polar nature as advantageous for extracting a wide array of therapeutic compounds. This research examined and compared the antioxidant properties and metabolite profiles of grapes from various regions and types to investigate the possible antioxidant mechanisms of flavonoid metabolites (Fei *et al*., 2025).

Quantitative Analysis of Phytochemicals on Bark Extract

The analysis of the phytochemicals in the bark extract of *C. glauca* shows considerable amounts of phenolics, flavonoids, and tannins. The total phenolic content is measured at 49.259±0.6547 mg of gallic acid equivalents (GAE) per gram of extract, indicating a strong presence of phenolic compounds recognized for their antioxidant properties. These phenolics

Table 1: Preliminary phytochemical analysis on leaf and bark extracts of *Cassine glauca*.

S. No.	Phytochemical constituents	Leaf		Bark	
		Methanol Extract	Chloroform Extract	Methanol Extract	Chloroform Extract
1	Flavonoids	+++	++	+	-
2	Alkaloids	+	+	++	++
3	Phenolics	++	+	++	+
4	Glycosides	+	+	+	-
6	Tannins	++	-	+++	+
7	Coumarins	+	-	-	-
8	Sugars	-	-	-	-
10	Proteins	+++	-	++	+
11	Terpenoids	+	-	+++	++
12	Amino acids	++	+	+	+

(+) low; (++) average; (+++) high; (-) absent

Table 2: Quantitative analysis of phenolics, flavonoids and tannins of bark and leaf extracts of *C. glauca*.

S. No.	Analytical parameters	Phytochemicals quantity in bark extract (mg)	Phytochemicals quantity in leaf extract (mg)
1	Total phenolics	49.259±0.6547 GAE[a]/g extract	47.458±0.5748 GAE[a]/g extract
2	Total flavonoids	54.24±0.5478 TAE[b]/g extract	160.106±0.3014 TAE[b]/g extract
3	Total tannins	140.307±0.6547 RE[c]/g extract	45.298±0.9874 RE[c]/g extract

GAE[a] - gallic acid equivalents, TAE[b] - Tannic acid equivalents, RE[c] - rutin equivalents

enhance the antioxidant capacity of the extract, playing a vital role in neutralizing free radicals and possibly reducing damage associated with oxidative stress.

The total flavonoid content is measured at 54.24±0.5478 mg of tannic acid equivalents (TAE) for each gram of extract, as shown in Table 2. This indicates a significant concentration of flavonoids in the extract. Flavonoids are known for their various biological activities, including anti-inflammatory and antioxidant effects, which further emphasize the potential therapeutic advantages of the extract. Additionally, the total tannin content stands at 140.307±0.6547 mg of rutin equivalents (RE) per gram of extract. According to estimates, approximately 50,000 to 70,000 plant species possess medicinal properties, emphasizing their vital importance in both traditional and contemporary healthcare systems (Ali *et al.*, 2025).

Quantitative Analysis of Phytochemicals on Leaf Extract

The analysis of the leaf extract from *C. glauca* shows considerable amounts of phenolics, flavonoids, and tannins. The overall phenolic content stands at 47.458±0.5748 mg equivalent to gallic acid (GAE) per gram of extract, highlighting a significant presence of phenolic compounds renowned for their antioxidant effects. Phenolics boost the antioxidant capacity of the extract, aiding in the neutralization of free radicals and potentially mitigating damage associated with oxidative stress. The total flavonoid content measures 160.106±0.3014 mg equivalent to tannic acid (TAE) per gram of extract and highlighting a rich concentration of flavonoids. These compounds are renowned for their antioxidants, biological activities, and anti-inflammatory effects, supporting the extract's potential therapeutic benefits. The total tannin content is 45.298±0.9874 mg rutin equivalents (RE) per gram of extract Table 2. While lower compared to phenolics and flavonoids, this substantial concentration of tannins is associated with various health benefits, including antimicrobial and antioxidant activities (Muniyandi *et al.*, 2019; Sundari *et al.*, 2024). Veerapur *et al.* (2017) performed a quantitative analysis of flavonoids in *C. glauca*. In contrast, the earlier study identified various phytocompounds, including flavonoids and phenolics, in *C. glauca*. According to the literature review, there has been no quantitative analysis of phytochemicals in the bark and leaf extracts of *C. glauca* (Faruq *et al.*, 2024; Adugna *et al.*, 2024). Key phytochemicals that demonstrate significant efficacy against these mites include phenol, flavonoids, terpenoids, tannins, and phenylpropanoids. They exert their effects by inducing neurotoxicity, disrupting digestion and metabolism, damaging the cuticle, causing oxidative stress, harming cell membranes, inhibiting respiration, and disrupting hormonal balance in these mites (Mustafa & Alsayeqh, 2025).

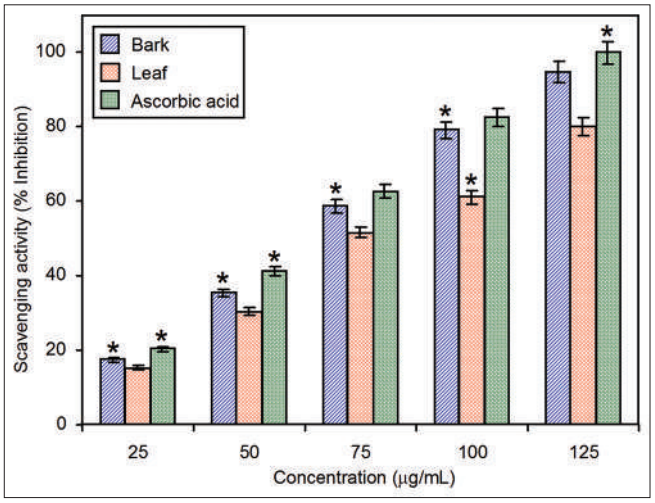

Figure 1: The DPPH assay of *Cassine glauca* bark and leaf extract at different concentrations

In the present study, the subsequent section of the analysis was the antioxidant activity of *C. glauca* leaf and bark extracts. Based on the phytochemical study, the highest count of phytochemicals containing methanol extract was selected for antioxidant activity with 5 different methods. The antioxidant properties of extracts from *C. glauca* were assessed and compared with ascorbic acid, which is a standard antioxidant, across various concentrations. The results revealed significant differences in antioxidant activity among the tested materials. At the lowest concentration of 25 µg/mL, ascorbic acid demonstrated the highest antioxidant activity with 20.4% inhibition, compared to 17.4% for the bark extract and 15.3% for the leaf extract. Finally, at 125 µg/mL, the bark extract reached 94.7% inhibition, surpassing the leaf extract's 80.3%, but ascorbic acid remained the most effective with 100% inhibition (Figure 1).

These findings suggest that while both *C. glauca* extracts exhibit strong antioxidant activity, the bark extract demonstrates particularly high activity at higher concentrations. Ascorbic acid, however, remains the most effective antioxidant across all concentrations. This is consistent with previous studies that have shown ascorbic acid's potent antioxidant properties due to its capacity to counteract free radicals and diminish oxidative stress (Bhattarcharya *et al.*, 2018). Additionally, research by El-Hashash *et al.* (2010) highlighted the effectiveness of various species of *Cassine* extracts. The increased activity of both extracts at elevated concentrations suggests that they could serve as an important source of antioxidants, aligning with findings from another study that emphasize the therapeutic potential of *C. glauca* (Kumar *et al.*, 2013; El-Hashash *et al.*, 2010).

The molybdenum assay measures antioxidant capacity by mixing a sample with a reagent containing molybdenum (VI) ions. Antioxidants in the sample reduce molybdenum (VI) to molybdenum (V), forming a green complex with phosphoric acid. This green color, quantified via spectrophotometry, reflects the sample's antioxidant activity. The assay thus gauges the sample's ability to neutralize oxidative stress. In the present study, using at 25 µg/mL of the bark extract exhibited the lowest activity at 3.3%, while the leaf extract showed 11.7%, and ascorbic acid demonstrated the highest activity at 15.6%. At 125 µg/mL, the bark extract reached its highest activity at 28.7%, the leaf extract showed 51.8%, and ascorbic acid remained the most effective with 71.4% (Figure 2). These results highlight that while both *C. glauca* extracts display increasing antioxidant activity with concentration, the leaf extract consistently outperforms the bark extract, and ascorbic acid maintains superior antioxidant activity across all tested concentrations. The findings are consistent with previous research that underscores the antioxidant potential of *C. glauca*. Based on the literature review, there is limited research on antioxidant determination using the molybdenum assay. El-Hashash *et al*. (2010) examined the antioxidant properties of different *Cassine* species utilizing this approach.

The reducing power is frequently utilized as a measure of electron-donating capability, a crucial method for assessing the antioxidative effects, antioxidant properties, or radical scavenging potential of the extracts. The reducing power of *C. glauca* extracts was evaluated and compared to ascorbic acid. At 25 µg/mL, the bark extract had a reducing power of 7.4%, the leaf extract 11.2%, and ascorbic acid 12.4%. At 50 µg/mL, the values increased to 12.3% for the bark extract, 19.4% for the leaf extract, and 25.4% for ascorbic acid. Finally, at 125 µg/mL, the bark extract showed 52.7%, the leaf extracts 58.2%, and the ascorbic acid 68.7% (Figure 3). These results reveal that while both *C. glauca* extracts showed that increasing reducing power with concentration, ascorbic acid consistently remains the most effective. To date, no studies have investigated the antioxidant activity of *C. glauca* using the reducing power method. The presented study correlated with estimation of antioxidants in *Benkara malabarica* (Kalusalingam & Balakrishnan, 2022b).

The nitric oxide (NO) assay evaluates antioxidant activity by measuring the ability to scavenge nitric oxide radicals, which are involved in various processes. In this assay, antioxidants inhibit the formation of nitrite from nitric oxide, reflecting their potential. In the present study, the next antioxidant method was the Nitric oxide method, at 25 µg/mL, the bark extract showed 4.7% inhibition of nitric oxide, while the leaf extract exhibited 6.9%, and ascorbic acid demonstrated a higher inhibition of 8.3%. At 100 µg/mL, the bark extract reached 27.8%, the leaf extract 35.7%, and ascorbic acid 36.7%. Finally, at 125 µg/mL, the bark extract showed 45.3% inhibition, the leaf extracts 49.8%, and the ascorbic acid 52.5% (Figure 4). These results indicate that both *C. glauca* extracts demonstrate increasing antioxidant activity with concentration, with the leaf extract consistently showing higher activity compared to the bark extract. Ascorbic acid remains the most effective inhibitor of

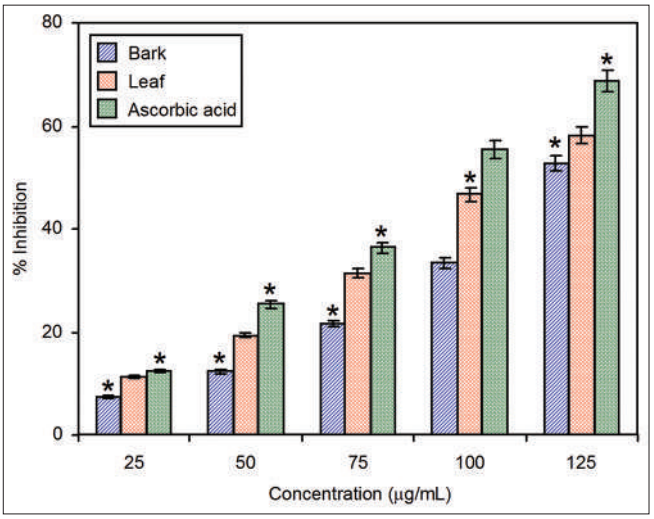

Figure 3: The reducing power assay of *Cassine glauca* bark and leaf extract at different concentrations

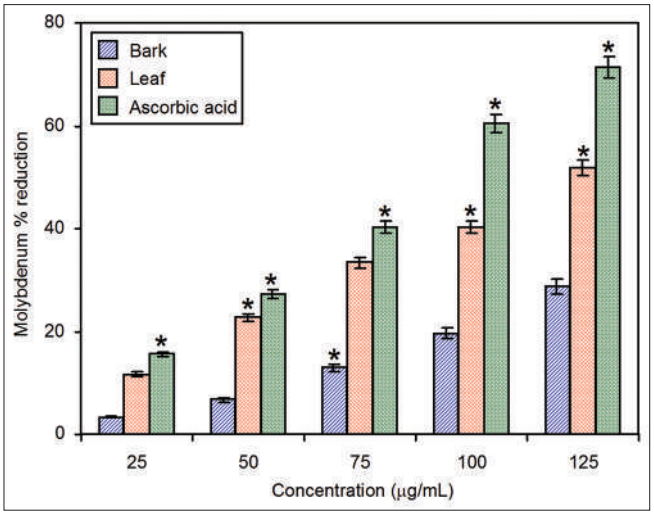

Figure 2: The percentage of molybdenum scavenging activity of *Cassine glauca* bark and leaf extract

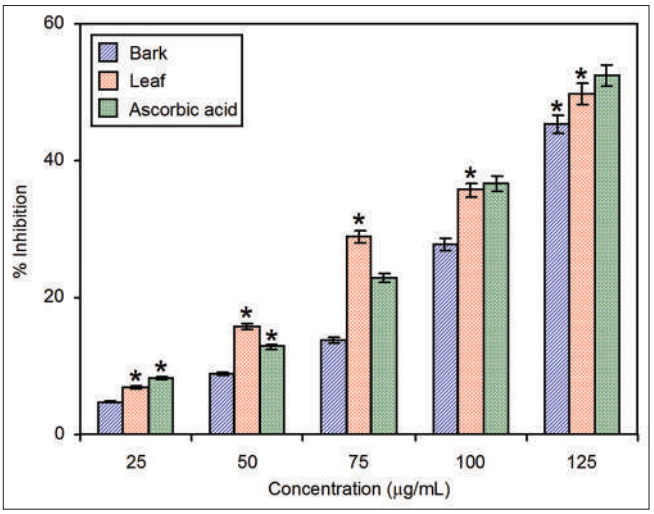

Figure 4: The nitric oxide scavenging activity of *Cassine glauca* bark and leaf extract at different concentrations

nitric oxide across all concentrations. Both *C. glauca* extracts demonstrated increased inhibition with higher concentrations, with the leaf extract showing superior activity compared to the bark extract.

The antioxidant properties of *C. glauca* extracts were evaluated using the hydrogen peroxide (H_2O_2) assay and compared to ascorbic acid. At 25 µg/mL, the bark extract exhibited 10.3% inhibition of H_2O_2, while the leaf extract showed 14.7%, and ascorbic acid demonstrated a higher inhibition of 28.7%. Finally, at 125 µg/mL, the bark extract demonstrated 53.4% inhibition, the leaf extracts 75.4%, and ascorbic acid 81.4% (Figure 5). These results indicate that both *C. glauca* extracts showed increasing antioxidant activity with concentration, with the leaf extract consistently exhibiting higher activity compared to the bark extract. Ascorbic acid remained the most effective inhibitor of H_2O_2across all concentrations.

These findings validate the H_2O_2 assay as a reliable technique for evaluating the antioxidant potential of natural extracts, as it effectively measures the ability of antioxidants to mitigate oxidative stress by neutralizing hydrogen peroxide. The observed trends are consistent with previous research that highlights the antioxidant properties of *C. glauca* and confirms the effectiveness of the H_2O_2 assay in assessing the antioxidant activity of various substances. This technique is both efficient and highly accurate, making it ideal for the quick measurement of H_2O_2 scavenging capacity in standard antioxidants as well as in natural antioxidants found in plant extracts (Fernando & Soysa, 2015).

Overall, the study confirms that both *C. glauca* extracts exhibit significant antioxidant activity, with methanol extracts demonstrating superior efficacy, because of their greater concentration of bioactive substances. The leaf extract generally shows better performance than the bark extract in antioxidant assays, while ascorbic acid remains the most potent antioxidant across all tested methods. Among the five methods used, the DPPH assay consistently provided the highest antioxidant activity, making it the most effective for evaluating antioxidant potential. Its sensitivity to radical scavenging activity resulted in the highest inhibition levels, underscoring its effectiveness compared to the other methods. A variety of new compounds including oxylipins, lignans, saponins, flavonoids, phenolics, and their derivatives have been identified. This highlights the plant's potential as a medicinal resource within alternative medicine systems such as Ayurveda, Siddha, and Unani for addressing various metabolic disorders, enabling the plant's roots to demonstrate a range of pharmacological effects like regulating glucose and fatty acid cycles, along with antioxidant and anti-inflammatory properties (Devadasu & Martin, 2025).

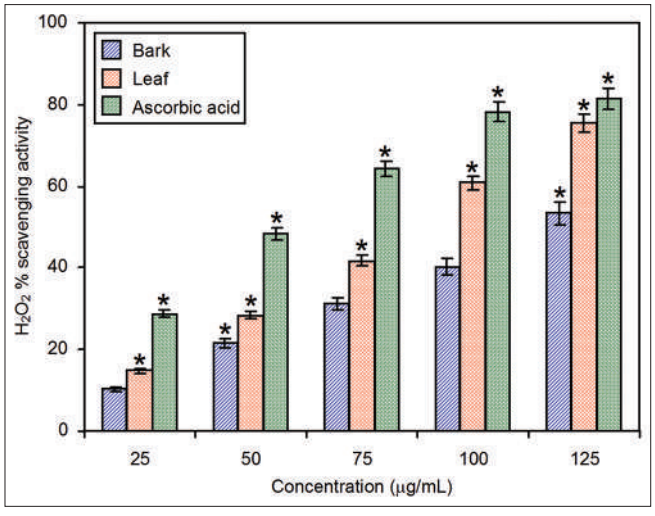

Figure 5: The Hydrogen Peroxide scavenging assay of *Cassine glauca* bark and leaf extract at different concentrations.

CONCLUSION

The phytochemical analysis of *C. glauca* extracts from both leaf and bark reveals considerable quantities of bioactive ingredients, such as phenolics, flavonoids and tannins, along with the leaf extract showing higher concentrations than the bark extract. Methanol extraction proves to be more effective than chloroform, highlighting its superiority in obtaining these valuable compounds. The high levels of flavonoids, phenolics and tannins underscore the extracts' potential as natural antioxidants and anti-inflammatory substances, which are essential for reducing oxidative stress and inflammation linked to chronic conditions like cardiovascular diseases, cancer, and neurodegenerative conditions. The remarkable antioxidant properties of the leaf extract bolster its promise as a treatment for disorders associated with oxidative stress. The research suggests that both leaf and bark extracts possess significant pharmaceutical potential, necessitating additional studies to isolate and identify the specific bioactive compounds and investigate their mechanisms of action and clinical uses.

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