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PHYTOCHEMICAL CHARACTERIZATION, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF *CALLISTEMON CITRINUS* LEAF EXTRACT

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ABSTRACT

Callistemon citrinus is a medicinal plant rich in bioactive phytochemicals; however, comprehensive studies integrating physicochemical standardization, phytochemical characterization, antioxidant evaluation, and antimicrobial activity of its hydroalcoholic leaf extract remain limited. The present study was undertaken to address this research gap by evaluating the quality parameters and biological activities of *C. citrinus* leaf extract. Physicochemical analysis revealed acceptable quality standards, including a loss on drying of 6.7%, total ash of 7.6%, alcohol-soluble extractive value of 12.5%, and water-soluble extractive value of 13.8%. Preliminary phytochemical screening confirmed the presence of flavonoids, phenolics, tannins, terpenoids, saponins, and alkaloids. The extract exhibited significant antioxidant activity with IC_{50} values of 8.14 ± 1.52 µg/mL in the DPPH assay and 7.63 ± 0.63 µg/mL in the ABTS assay, while total phenolic and flavonoid contents were 18.192 ± 0.104 mg GAE/g and 16.351 ± 3.907 mg QE/g, respectively. Antimicrobial evaluation demonstrated concentration-dependent antibacterial activity against *Enterococcus* and *Klebsiella*, with maximum inhibition zones of 13 mm and 15 mm, respectively, at 600 mg/mL. These findings demonstrate that the hydroalcoholic leaf extract of *C. citrinus* possesses appreciable antioxidant and antibacterial activities, primarily due to its phenolic and flavonoid constituents, supporting its potential as a natural source of bioactive compounds for future phytopharmaceutical development.

KEYWORDS: *Callistemon citrinus*, Phytochemicals, DPPH assay, ABTS assay, Antibacterial activity.

1. INTRODUCTION

Medicinal plants continue to play a vital role in the discovery of novel therapeutic agents due to their rich reservoir of bioactive secondary metabolites.[1] According to the World Health Organization (WHO), approximately 80% of the global population relies on traditional herbal medicines for primary healthcare, highlighting the importance of scientifically validating medicinal plants used in folk medicine. Plant-derived phytochemicals, particularly phenolics, flavonoids, tannins, terpenoids, and alkaloids, possess diverse biological activities including antioxidant, antimicrobial, anti-inflammatory, and anticancer effects.[2,3] Consequently, the characterization of these bioactive compounds and the evaluation of their biological properties have become an important area of pharmaceutical and phytochemical research.[4,5]

The increasing prevalence of oxidative stress-related disorders and the emergence of antimicrobial resistance have intensified the search for effective natural alternatives to synthetic drugs.[6,7] Oxidative stress results from an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defence mechanisms, leading to cellular damage and contributing to the pathogenesis of chronic diseases such as cardiovascular disorders, diabetes mellitus, neurodegenerative diseases, and cancer.[8,9] Similarly, the rapid development of multidrug-resistant microorganisms has significantly reduced the efficacy of conventional antibiotics, creating an urgent need for new antimicrobial agents from natural sources.[10,11] Plant extracts rich in polyphenolic compounds have attracted considerable attention because of their ability to scavenge free radicals and inhibit the growth of pathogenic microorganisms with minimal adverse effects.[12–14]

Callistemon citrinus (Curtis) Skeels (family: Myrtaceae), commonly known as crimson bottlebrush or lemon bottlebrush, is an evergreen ornamental shrub native to Australia and widely cultivated in tropical and subtropical regions, including India.[15] Traditionally, various parts of the plant have been used to manage respiratory infections, cough, fever, skin disorders, gastrointestinal ailments, and inflammatory conditions.[16–18] These therapeutic applications have been attributed to its diverse phytochemical constituents, including flavonoids, phenolic acids, tannins, essential oils, triterpenoids, and other phenolic compounds.[19,20] Essential oil components such as 1,8-cineole, α-pinene, limonene, and terpinen-4-ol have been reported to possess notable antimicrobial properties, while flavonoids such as quercetin, rutin, and myricetin contribute significantly to antioxidant activity.[21,22]

Physicochemical evaluation and preliminary phytochemical screening are fundamental quality control parameters for the standardization of medicinal plant materials.[23] These assessments provide valuable information regarding the purity, identity, and chemical composition of plant extracts, thereby ensuring reproducibility and consistency in biological investigations. Furthermore, determination of antioxidant and antimicrobial activities offers scientific evidence supporting the traditional medicinal uses of *C. citrinus* and may facilitate the development of novel phytopharmaceuticals or natural preservatives.[15]

Therefore, the present study aimed to perform a comprehensive phytochemical characterization of the hydroalcoholic leaf extract of *Callistemon citrinus* through physicochemical evaluation, qualitative phytochemical screening, estimation of total phenolic and flavonoid contents and antimicrobial activity. In addition, the antioxidant potential was evaluated using the DPPH free radical scavenging assay, while the antimicrobial activity was assessed against selected bacterial and fungal pathogens. The findings of this study are expected to provide scientific evidence supporting the

medicinal value of *C. citrinus* and contribute to its future development as a promising source of natural antioxidant and antimicrobial agents.

2. MATERIALS AND METHODS

2.1. Chemicals Analytical-grade ethanol, methanol, petroleum ether, chloroform, hydrochloric acid, sulfuric acid, ferric chloride, sodium hydroxide, Wagner's reagent, Dragendorff's reagent, Mayer's reagent, Braymer's reagent, Liebermann–Burchard reagent, Salkowski reagent, Folin–Ciocalteu reagent, sodium carbonate, aluminum chloride, potassium acetate, gallic acid, quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Nutrient agar media and dimethyl sulfoxide (DMSO) were procured from HiMedia Laboratories Pvt. Ltd. (Mumbai, India) and Merck Life Science Pvt. Ltd. (Mumbai, India).

2.2. Plant Collection and Authentication

The leaves of *Callistemon citrinus* were collected from campus of Gopal Narayan Singh University, Jamuhar, Sasaram, Bihar. It was authenticated from Department of Botany, Banaras Hindu University, Varanasi, Uttar Pradesh with voucher specimen no. Myrta 2024/02.

2.3. Determination of Physicochemical Parameters

The physicochemical parameters of the powdered *Callistemon citrinus* leaves were evaluated by determining loss on drying, ash values, and extractive values according to the standard procedures described in the World Health Organization (WHO) guidelines and the Indian Pharmacopoeia. All determinations were carried out in triplicate, and the results were expressed as mean ± standard deviation.

2.3.1. Determination of Loss on Drying (LOD)

Approximately 2 g of accurately weighed powdered leaf material was placed in a previously dried and tared porcelain crucible. The sample was dried in a hot-air oven maintained at 105 ± 2°C until a constant weight was obtained. After cooling in a desiccator, the sample was reweighed.[24] The percentage loss on drying was calculated using the initial and final weights and expressed as percentage (% w/w).

$$\text{Loss on Drying (\%)} = W1-W2/W1\times100$$

Where,

W_1 = Initial weight of the sample

W_2 = Final weight after drying.

2.3. Determination of Ash Values

2.3.1. Total Ash

About 2 g of accurately weighed powdered leaf material was transferred into a previously ignited and tarred silica crucible. The sample was incinerated gradually by increasing the temperature to 550–600°C in a muffle furnace until the residue became carbon-free and white, indicating complete combustion. The crucible was cooled in a desiccator and weighed. The total ash value was calculated as percentage (% w/w) of the air-dried plant material.[25]

2.3.2. Acid-Insoluble Ash

The total ash obtained was boiled with 25 mL of 2 M hydrochloric acid for 5 min. The insoluble matter was collected on an ashless filter paper, washed thoroughly with hot distilled water until free from acid, dried, ignited in a silica

crucible at 550°C, cooled in a desiccator, and weighed. The acid-insoluble ash was expressed as percentage (% w/w) of the air-dried sample.

2.3.3. Water-Soluble Ash

The total ash was boiled with 25 mL of distilled water for 5 min. The insoluble residue was collected on an ashless filter paper, washed with hot water, dried, ignited at 550°C, cooled, and weighed. The water-soluble ash was calculated by subtracting the weight of the insoluble residue from the weight of the total ash and expressed as percentage (% w/w).[26]

2.4. Determination of Extractive Values

2.4.1. Alcohol-Soluble Extractive Value

Approximately 5 g of air-dried powdered leaf material was accurately weighed and macerated with 100 mL of 95% ethanol in a closed flask for 24 h, with frequent shaking during the first 6 h and allowed to stand for the remaining 18 h.

The extract was filtered rapidly to minimize solvent loss. Exactly 25 mL of the filtrate was transferred into a pre-weighed evaporating dish and evaporated to dryness on a water bath. The residue was dried at 105°C to constant weight, cooled in a desiccator, and weighed. The alcohol-soluble extractive value was expressed as percentage (% w/w) of the air-dried material.[27]

2.4.2. Water-Soluble Extractive Value

The procedure was repeated using 100 mL of chloroform water (0.25% v/v chloroform in water) as the extracting solvent instead of ethanol. The extractive value was calculated and expressed as percentage (% w/w) of the air-dried plant material.

Calculation

$$\text{Extractive Value (\%)} = W1/W2 \times 100$$

Where:

- W_1 = Weight of the air-dried crude drug taken (g)
- W_2 = Weight of the dried extract obtained after evaporation (g)

2.5. Preparation of extract

The leaves were shade dried and powdered. About 200 mg coarse powder was weighed and defatted with Petroleum ether using Soxhlet apparatus separately and filtered. Then it is extracted with 70% ethanol to obtain hydroalcoholic *Callistemon citrinus* leaves extract (CCLE). The extract was concentrated under reduced temperature and pressure to get dark green coloured dry residue.[28] The residue was dried and powdered sample is stored in a moisture free container for further use.

2.6. Preliminary phytochemical screening

To identify the chemical classes of secondary metabolites, a preliminary phytochemical screening of the *Callistemon citrinus* hydroalcoholic extract was conducted using standard qualitative chemical tests. The extract was assessed for various constituents, including, flavonoids, phenolic compounds, alkaloids, terpenoids, tannins, glycosides, saponins, carbohydrates, proteins and steroids. These phytochemicals potentially account for the plant's therapeutic properties and

free radical scavenging capabilities. Ultimately, this initial evaluation establishes a solid foundation for the further characterization and standardization of the extract.[20,29]

2.7. Determination of antioxidant activity

2.7.1. DPPH Assay

In order to evaluate the free radical scavenging activity of both the extracts, we conducted the experiment with the help of DPPH. In our case, the sample extracts denoted as CCLE are considered as "alive" because they are dissolved in 99.9% methanol medium in concentrations 62.5 to 1000 µg/ml from the stock solution of 1 mg/ml. The ascorbic acid extract is considered as the standard sample, which was used in concentrations 6.25 to 100 µg/ml. Both the brave samples and the reliable standards are diluted in 1 ml of buffer. The reaction between all the materials started after adding 1 ml of the DPPH solution of concentration 0.135 mM and mixing everything together. All these coloured solutions were kept in the dark for 30 minutes incubation period at 20°C temperature. Then, after the waking period, the absorbance of both the solutions was measured at 517 nm wavelength. Freshly prepared DPPH solution is taken as our constant control sample. At the end, the antioxidant activity % RSA was evaluated by the following formula:

$$\% \text{ of Antioxidant Activity} = [(Ac-As) \div Ac] \times 100.$$

Where:

Ac- Absorbance of Control

As- Absorbance of Test sample

The %RSA were plotted against different concentrations of extract and trendline equation was used to calculate IC-50 concentrations for each sample.[30–32]

2.7.2. ABTS assay

Scavenging ability of the free radicals of each extract was determined using the ABTS method. This is accomplished by preparing 1 mg/mL extract of extract CCLE in 99.9% methanol in various concentrations ranging from 62.5-1000 µg/mL. The standard used in this experiment is ascorbic acid in various concentrations ranging from 6.25-100 µg/mL, and both were subjected to addition of 1mL of buffer. Further, 1 mL of 7 mM ABTS solution was added and mixed.

This was incubated for 7 minutes. The absorbance reading was measured at 734 nm after the incubation period. The absorbance of the freshly prepared ABTS solution was used as the control at 734nm. The formula used in calculating the percentage RSA was:

$$\% \text{ of Antioxidant Activity} = [(Ac-As) \div Ac] \times 100$$

Where:

Ac- Control Absorbance

As- Test sample absorbance

The % RSA were plotted against different concentrations of extract and trendline equation was used to calculate IC-50 concentrations for each sample.[33,34]

2.8. Determination of Total Phenolic Content

The estimation of the total phenolic content was done by employing the Folin-Ciocalteau's method. Folin-Ciocalteu reagent (10%, v/v) (2.5ml) was added to 0.1ml of CCLE extract (1mg/ml). The mixture was then further diluted with 2.0 ml of 7.5% (w/v) sodium carbonate solution. Under the absence of light, the mixture was kept for 1.5 hours at room temperature and subsequently, the absorbance was recorded at 765nm with the help of Systronic UV-Visible Double

Beam Spectrophotometer. The same procedure was adopted for the determination of the concentration of gallic acid (10 to 100 µg/ml). The total phenolic content was determined using gallic acid calibration curve and presented in mg Gallic Acid Equivalents (GAE)/gram of sample.[35–38]

2.9. Determination of Total Flavonoids Content

Total Flavonoids were determined via a colorimetric method based on the formation of flavonoid aluminium complex.

Briefly, the total flavonoids content (TFC) of the CCLE was measured using 1mg/ml of the CCLE, where 0.1ml of 10% AlCl3, 0.1ml of 1M potassium acetate, and 2.8 ml of distilled water were added to the CCLE, and then incubated at room temperature for 30minutes. The absorbance was then determined at 415nm using the UV-Visible double beam spectrophotometer (Systronic). Similarly, the quercetin solution was prepared in various concentrations ranging from 10-100µg/ml. Using quercetin as a standard, the TFC of the CCLE was quantified.[28,39–41]

2.10. Antimicrobial Activity

The antimicrobial activity of the hydroalcoholic extract of *Callistemon citrinus* leaves was evaluated by the agar disc diffusion method against Gram-positive *Enterococcus* spp. and Gram-negative *Klebsiella* spp. Nutrient agar medium was prepared according to the manufacturer's instructions, sterilized at 121°C for 15 min, and poured into sterile Petri dishes. Fresh bacterial suspensions were adjusted to 0.5 McFarland standard (≈1.5×10^8 CFU/mL) and uniformly spread over the agar surface using a sterile cotton swab. Sterile 6 mm filter paper discs impregnated with the plant extract of different concentration were placed on the inoculated plates. Linezolid (30 µg/disc) and Gentamicin (10 µg/disc) were used as standard antibiotics against *Enterococcus* spp. and *Klebsiella* spp., respectively, while DMSO served as the negative control. The plates were incubated at 37 ± 2°C for 24 h, after which the antimicrobial activity was assessed by measuring the diameter of the zone of inhibition (mm). All experiments were performed in triplicate, and the results were expressed as mean ± SD.

3. RESULTS

3.1. Loss on Drying

The loss on drying (LOD) of the powdered Callistemon citrinus leaves was determined to be 6.7% (w/w), as presented in Table 1. The observed LOD value falls within the acceptable range reported for medicinal plant materials and supports the quality, stability, and suitability of Callistemon citrinus leaves for phytochemical characterization and subsequent pharmacological investigations.

3.2. Determination of Ash Values

The physicochemical evaluation of *Callistemon citrinus* leaf powder revealed a total ash value of 7.6%, acid-insoluble ash value of 1.76%, and water-soluble ash value of 2.46% (Table 1). This ash values fall within the acceptable range reported for medicinal plants and demonstrate proper collection, processing, and handling of the leaves. Overall, the findings suggest that *Callistemon citrinus* leaves possess satisfactory physicochemical characteristics and comply with standard quality control parameters, supporting their suitability for phytochemical characterization and further pharmacological investigations.

3.3. Extractive Values

The extractive value analysis of *Callistemon citrinus* leaf powder showed an alcohol-soluble extractive value of 12.5% and a water-soluble extractive value of 13.8% (Table 1). The observed extractive values suggest that *C. citrinus* leaves are rich in bioactive constituents, particularly polar compounds, which may contribute to their reported antioxidant and antimicrobial activities.

Table 1: Physicochemical Parameter of *C. citrinus* flowers.

S.No.	Physicochemical Parameter	Result %
1	Loss on drying (%)	6.7
2	Total ash (%)	7.6
3	Acid-insoluble ash (%)	1.76
4	Water-soluble ash (%)	2.46
5	Alcohol soluble extractive (%)	12.5
6	Water soluble extractive (%)	13.8

3.4. Phytochemical screening

Preliminary qualitative phytochemical screening of the hydroalcoholic extract of *Callistemon citrinus* flowers revealed the presence of several important secondary metabolites, including flavonoids, phenolics, tannins, terpenoids, saponins, and alkaloids, while glycosides and steroids were absent. Flavonoids and phenolics showed the highest abundance (+++), followed by tannins and terpenoids (++), whereas saponins and alkaloids were detected in lower amounts (+) showed in Table 02.

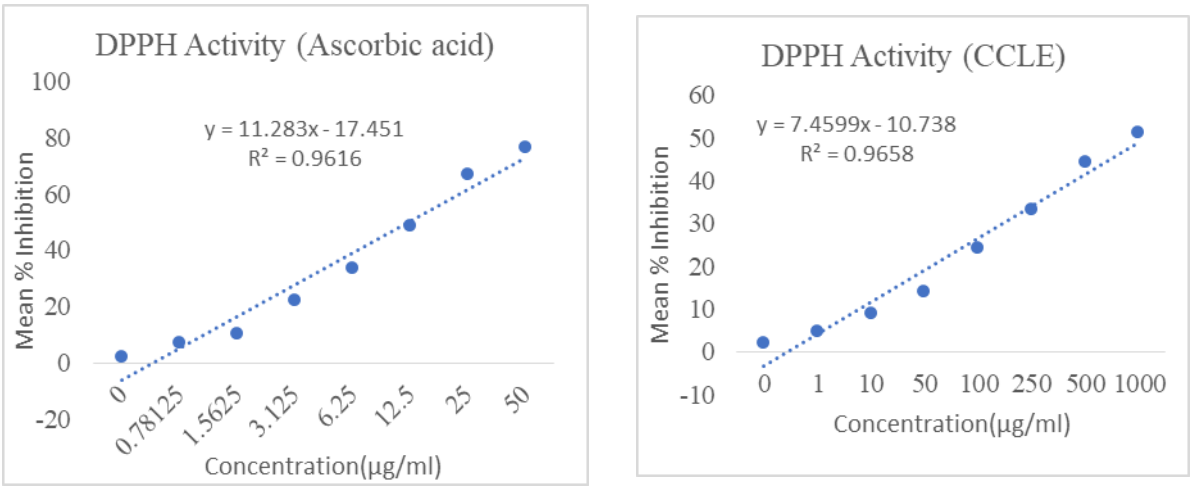
Table 02: Preliminary Phytochemical Screening of Hydroalcoholic Extract of *Callistemon citrinus* Leaves (CCLE).

S.no.	Phytoconstituent	Test Result
1	Flavonoids	+++
2	Phenolics	+++
3	Tannins	++
4	Terpenoids	++
5	Saponins	+
6	Alkaloids	+
7	Glycosides	-
8	Steroids	-

3.5. Determination of antioxidant activity

3.5.1. DPPH Assay

This study tested the concentration-dependent DPPH radical scavenging activity of the aqueous-alcoholic extract of Callistemon citrinus leaves. Measurements showed that at a concentration of 50 μg/mL the extract had an inhibition rate of 13.92 % and reached an inhibition rate of 51.24 % at 1000 μg/mL concentration. Its half-maximal inhibitory concentration (IC50) was 8.14±1.52 μg/mL, indicating weaker antioxidant activity than the standard antioxidant ascorbic acid, which had an IC50 of 5.97 ± 0.51 μg/mL. The phenols and flavonoids detected in the extract form the core basis of its bioactivity, and the extract can serve as a source of natural antioxidant components.

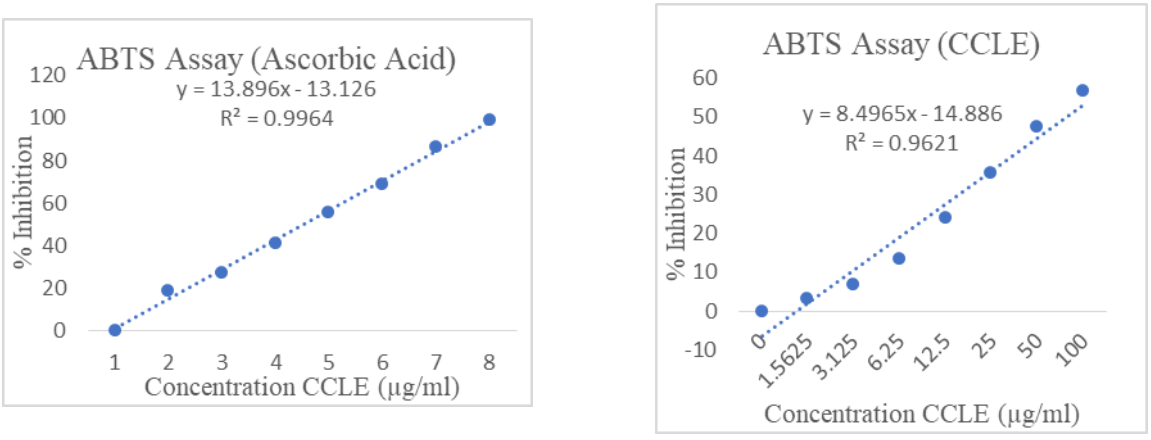


A. B.

Figure 01: Concentration-dependent DPPH free radical scavenging activity of A. Ascorbic acid (standard) B. Callistemon citrinus leaf hydroalcoholic extract (CCLE).

3.5.2. ABTS Assay

ABTS free radical scavenging assay was used to determine the antioxidant activity hydroalcoholic extract of leaves of Callistemon citrinus. Using the standard antioxidant ascorbic acid as a control, the study measured an IC50 of 4.54 ± 0.21 µg/mL for ascorbic acid, and an IC50 of 7.63 ± 0.63 µg/mL for CCLE. At a concentration of 100 µg/mL, CCLE reached a free radical scavenging rate of 56.53 %, with a dose-response relationship $R^2 \approx 0.99$. Its activity is derived from the detected phenolic and flavonoid compounds, and it can serve as a source of natural antioxidants.



A. B.

Figure 02: ABTS radical scavenging activity of ascorbic acid (standard) and Callistemon citrinus leaf hydroalcoholic extract (CCLE) at different concentrations, showing a concentration-dependent increase in antioxidant activity with corresponding linear regression analysis.

3.6. Determination of Total Phenolic Content

The Folin–Ciocalteau's method was used to measure the total phenolic content of the hydroalcoholic extract of Callistemon citrinus leaves (CCLE). The test value, converted to gallic acid equivalent (GAE), was 18.192 ± 0.104 mg GAE/g. The reliability of this method was validated by a calibration curve y=0.002x+0.0835 with an R^2 value of

0.9806. We inferred that its high phenolic content may contribute to its antioxidant activity, and confirmed that CCLE can serve as a high-quality source of natural phenolic antioxidants.

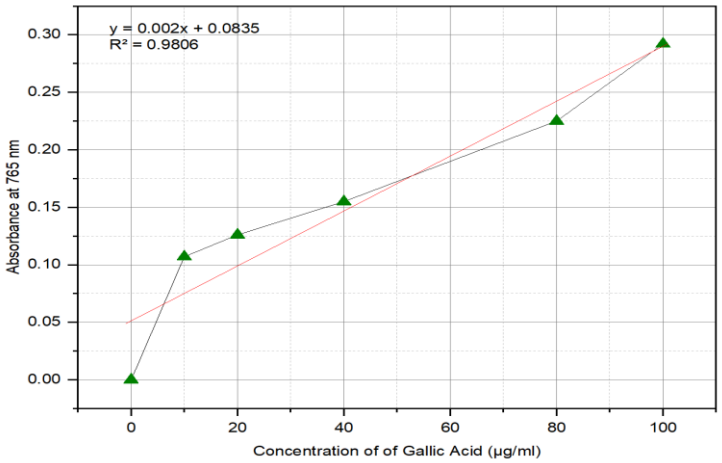


Figure 03: Standard Curve of Galic acid for Total Phenolic Content Estimation.

3.7. Total Flavonoid Content: The aluminium chloride colorimetric method was used to quantify the total flavonoid content (TFC) of the hydroalcoholic extract of Callistemon citrinus (lemon bottlebrush) leaves (CCLE). Results are expressed as quercetin equivalents (QE), and the measured TFC of CCLE was 16.351 ± 3.907 mg QE/g. The quercetin standard curve was verified to have good linearity. The three replicate experiments produced a standard deviation of 3.907, with moderate variability.

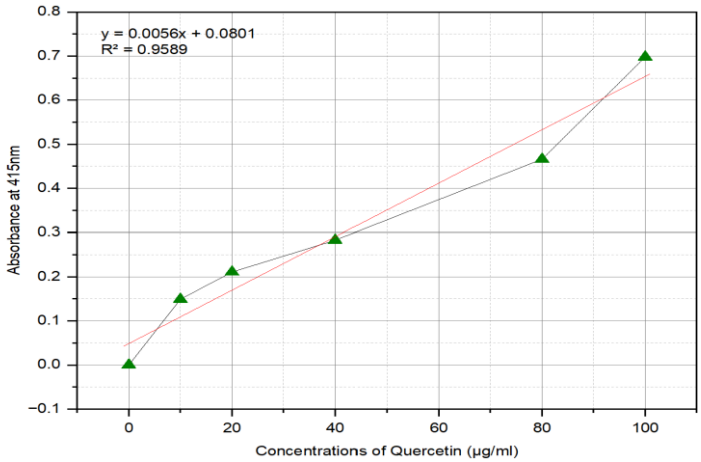
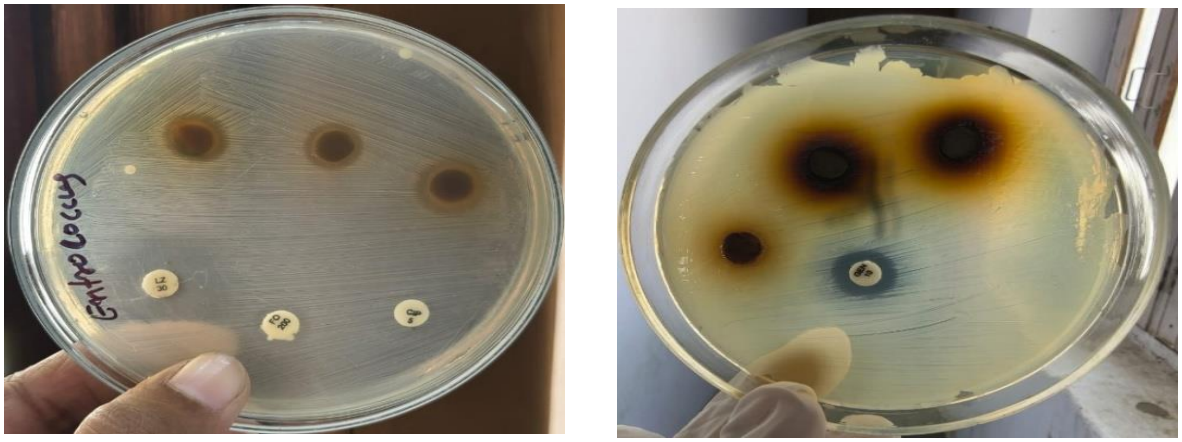


Figure 04: Standard Curve of Quercetin for Total Flavonoid Content.

3.8. Antimicrobial Activity

The hydroalcoholic leaf extract of *Callistemon citrinus* (CCLE) exhibited concentration-dependent antibacterial activity against both *Enterococcus* and *Klebsiella*. The highest concentration (600 mg/mL) produced inhibition zones of 13 mm and 15 mm against *Enterococcus* and *Klebsiella*, respectively, which were comparable to the standard antibiotics. The antibacterial activity is likely due to the presence of phenolics and flavonoids, which are known to inhibit bacterial growth. These findings are consistent with previous studies reporting the broad-spectrum antimicrobial potential of *C. citrinus* leaf extracts.



A B

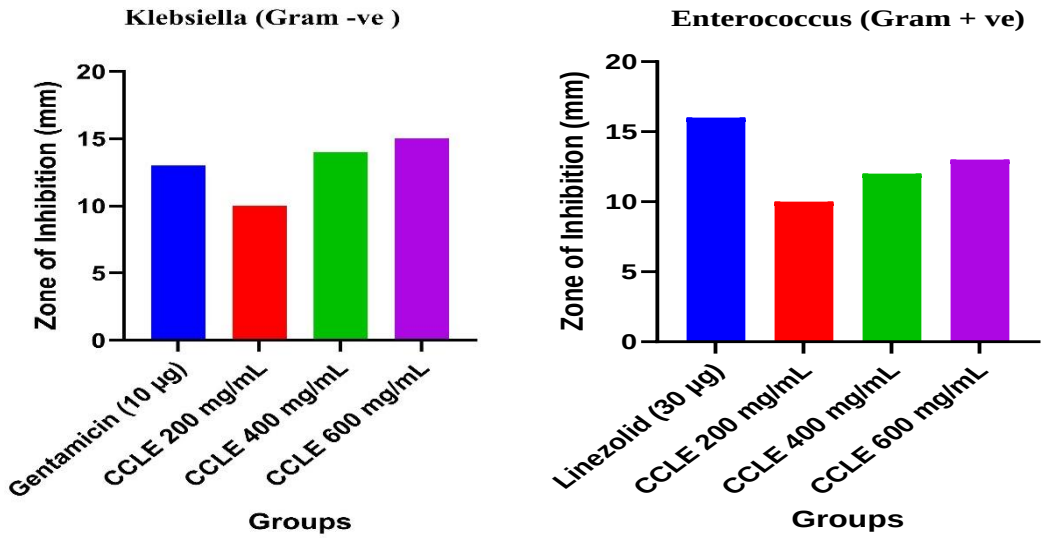


Figure 3: Representative disc diffusion plates showing the antibacterial activity* of *Callistemon citrinus leaf extract (CCLE) against (A) Enterococcus (B) Klebsiella.

4. DISCUSSION

The present study demonstrates that the hydroalcoholic leaf extract of *Callistemon citrinus* possesses appreciable phytochemical, antioxidant, and antibacterial activities, supporting its traditional medicinal use. Physicochemical evaluation showed that the powdered leaves complied with acceptable quality control parameters, including low loss on drying (6.7%), total ash (7.6%), acid-insoluble ash (1.76%), water-soluble ash (2.46%), alcohol-soluble extractive value (12.5%), and water-soluble extractive value (13.8%). These findings indicate good purity, minimal inorganic contamination, and a high content of polar bioactive constituents, making the plant suitable for phytopharmaceutical applications.

Preliminary phytochemical screening confirmed the presence of flavonoids, phenolics, tannins, terpenoids, saponins, and alkaloids, whereas glycosides and steroids were absent. The abundance of phenolics and flavonoids suggests that these compounds are the major contributors to the biological activities of the extract. Similar phytochemical profiles

have been reported by Goyal et al. (2012), Kumar et al. (2016), and Patel et al. (2021), who also identified polyphenolic compounds as the principal bioactive constituents of *C. citrinus*.

The hydroalcoholic extract exhibited marked antioxidant activity in both DPPH and ABTS assays, although its activity was lower than that of the standard antioxidant ascorbic acid. The extract showed IC_{50} values of 8.14 ± 1.52 µg/mL (DPPH) and 7.63 ± 0.63 µg/mL (ABTS), demonstrating effective free radical scavenging ability. Furthermore, the extract contained considerable amounts of total phenolics (18.192 ± 0.104 mg GAE/g) and total flavonoids (16.351 ± 3.907 mg QE/g), indicating a strong correlation between polyphenol content and antioxidant potential. Comparable observations have been reported by Prakash et al. (2019) and Singh et al. (2022), who attributed the antioxidant activity of *C. citrinus* to quercetin, rutin, gallic acid, and other phenolic constituents capable of neutralizing reactive oxygen species.

The extract also demonstrated concentration-dependent antibacterial activity against both *Enterococcus* and *Klebsiella*. The maximum inhibition zones of 13 mm and 15 mm, respectively, were observed at 600 mg/mL, indicating broad-spectrum antibacterial activity. Although the inhibition was slightly lower than that of the standard antibiotics, the results confirm the antimicrobial potential of *C. citrinus*. The antibacterial effect is likely mediated through the disruption of bacterial cell membranes, inhibition of essential enzymes, and interference with nucleic acid synthesis by phenolic and flavonoid compounds. Similar antibacterial activity against Gram-positive and Gram-negative bacteria has been reported by Oyedeji et al. (2009), Goyal et al. (2012), and Chouhan et al. (2017).

Overall, the findings demonstrate that the hydroalcoholic leaf extract of *Callistemon citrinus* is a rich source of bioactive phytochemicals with significant antioxidant and antibacterial properties. These results scientifically validate its traditional medicinal use and highlight its potential as a natural source of antioxidant and antimicrobial agents for the development of herbal formulations and phytopharmaceutical products.

CONCLUSION

The present study demonstrated that the hydroalcoholic leaf extract of *Callistemon citrinus* is a rich source of bioactive phytochemicals, particularly phenolics and flavonoids, which contribute to its significant antioxidant and antibacterial activities. The extract exhibited satisfactory physicochemical characteristics, effective free radical scavenging capacity, and concentration-dependent antibacterial activity against *Enterococcus* and *Klebsiella*. These findings scientifically support the traditional medicinal use of *C. citrinus* and highlight its potential as a promising natural source of antioxidant and antimicrobial agents for future phytopharmaceutical development.

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Conflict of Interest

The authors have no conflict of interest to declare.

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