RESEARCH PAPER

Phytochemical Characterization and Antioxidant Activity of *Callistemon Citrinus* Hydroalcoholic Extract with HPTLC Quantification of Quercetin

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ABSTRACT

The medicinal plant *Callistemon citrinus* is rich in a variety of plant-derived bioactive compounds. This study aimed to characterize the phytochemical profile of the hydroalcoholic extract from its leaves, evaluate the extract for *in vitro* antioxidant activity, and quantitatively detect quercetin, the active marker, via high-performance thin-layer chromatography (HPTLC). The study first detected 8 categories of phytochemicals through an initial qualitative screening. It then measured antioxidant activity using three classic methods, namely DPPH, ABTS, and FRAP. The Total phenolic content (TPC) and Total flavonoid content (TFC) were tested separately via the Folin-ciocalteau's method and the aluminium chloride colorimetric method, while the HPTLC quantitative analysis was completed synchronously. All experimental data obtained are precise and reliable. This study ultimately confirms that the extract has clear antioxidant potential, and the HPTLC method adopted can support the quality standardization work for this medicinal raw material. The hydroalcoholic extract was found to exhibit high antioxidant activity with IC_{50} values of DPPH and ABTS radical scavenging of 6.74±1.52 µg/mL and 9.98±0.63 µg/mL and FRAP value of 132.389 ± 5.853 µM Fe++/ml, respectively. The total phenolic content and total flavonoid content were measured as 18.192 ± 0.104 mg GAE/g and 16.351± 3.907 mg QE/g, respectively. HPTLC analysis revealed quercetin as one of the marker compounds with an Rf value of 0.567 having content of 74.85 µg/100 mg extract (0.0748 % w/w), exhibiting high linearity (R = 0.999928) and precision (CV = 0.29%). The results show that hydroalcoholic extract of *C. citrinus* possesses high antioxidant potential due to its phenolic and flavonoid composition.

Keywords: *Callistemon citrinus*, Phytochemical Screening, Antioxidant Activity, HPTLC Quantification, Quercetin

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INTRODUCTION

Medicinal plants have long served as a high-quality source of therapeutic agents, owing to their diverse phytochemical components and extremely low toxicity(1,2). *Callistemon citrinus* (Curtis) Skeels, a species of the Myrtaceae family commonly known as the crimson lemon bottlebrush tree, has attracted substantial research attention in the field of pharmacology (3). In folk medicine, this plant is used to treat a variety of conditions including cough and bronchitis (4–6). Phytochemical screening is a core preliminary research step for identifying and characterizing active ingredients linked to the pharmacological activities of medicinal plants. Existing studies have confirmed that *C. citrinus* contains a variety of bioactive metabolites such as flavonoids (quercetin, rutin), phenolic acids, tannins, triterpenoids, alkaloids, steroids, and essential oils including eucalyptol and α-pinene, exhibits multiple pharmacological properties including antioxidant, anti-inflammatory, and antibacterial activities(7,8). Due to imbalance between reactive oxygen species (ROS) and the body's antioxidant defence mechanisms the oxidative stress occurs in the body(9,10). It plays an important role in the pathogenesis of chronic kidney disease, cardiovascular disease, diabetes, neurodegenerative diseases, and cancer(11–14). Natural antioxidants derived from medicinal plants have attracted widespread attention in academic communities. Previous

studies have pointed out that the prominent antioxidant activity of *C. citrinus* originates from the phenolic and flavonoid components it contains(15,16).Phytochemical characterization and standardization are necessary prerequisites to guarantee the quality, efficacy, and reproducibility of herbal preparations(17,18). Reliable and low-cost high-performance thin-layer chromatography (HPTLC) can be used to detect markers in plant extracts. Quercetin, a flavonoid with strong antioxidant activity, is often used as a phytochemical marker (19–21). Our research was plans to conduct phytochemical characterization of the hydroalcoholic extract of *C. citrinus*, evaluate its antioxidant potential, and quantify its quercetin content via high-performance thin-layer chromatography (HPTLC), so as to provide support for the scientific validation and quality standardization of this species as a natural antioxidant source.

2. Materials and Methods

2.1 Chemicals

All analytical grade chemicals and solvents were utilized in the study and obtained locally.

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 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(22). The residue was dried and powdered sample is stored in a moisture free container for further use.

2.4 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(23,24).

2.5 Determination of antioxidant activity

2.5.1. DPPH Assay

For the purpose of identifying the free radical scavenging potential of both the extracts, we performed a DPPH test. Our sample extracts, marked as CCLE, were made alive using 99.9% methanol medium, spanning through concentrations of 62.5 to 1000 µg/ml from the stock solution of 1 mg/ml. Ascorbic acid served as the standard, being used in concentrations of 6.25 to 100 µg/ml. Both the daring samples and the steady standards were kept into 1 ml of buffer. The actual chemistry occurred when we added 1 ml of the DPPH solution of concentration 0.135 mM and shook the whole mixture. These colorful solutions were then put under cover in darkness for an incubation period of 30 minutes at 20°C. After their wake-up time, the absorbance of both was measured at the wavelength of 517 nm. Freshly prepared DPPH solution served as our constant control. In the end, the antioxidant success, presented in terms of percentage of radical scavenging activity (% RSA), was determined using the following formula:

% of Antioxidant Activity = [(Ac-As) ÷ Ac] × 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(25–27).

2.5.2. ABTS assay

The scavenging activity of free radicals of each extract was carried out using an ABTS method. This is done by preparing a 1 mg/mL extract of the extract CCLE in 99.9% methanol in different concentrations that range from 62.5-1000 µg/mL. The standard used here is ascorbic acid which is prepared in different concentrations that range from 6.25-100 µg/mL, and both are subjected to addition of 1 mL of buffer. Then, 1 mL of 7 mM ABTS solution was added and well mixed. It is allowed to incubate for 7 minutes. The absorbance was taken at 734 nm after

incubation. The absorbance of freshly prepared ABTS solution at 734 nm was used as control. The formula used to calculate % RSA was:

% of Antioxidant Activity = [(Ac-As) ÷ Ac] ×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 (6,28).

2.5.3. FRAP Assay

The FRAP activity of the extracts labelled CCLE was performed by mixing 100 µL of the extract (1 mg/mL Conc.) with 900 µL of the FRAP solution. At room temperature the mixture was allowed to incubate for 4 to 6 minutes. After which, absorbance readings were taken using UV-Visible double beam spectrophotometer (Systronic) at 700 nm. The same procedure was done using varying concentrations of $FeSO_4$ (100-1000 µM). FRAP activity was calculated based on the $FeSO_4$ standard and expressed in terms of $FeSO_4$ equivalents (µM Fe++) per mL of the sample (25,29,30).

2.6. Determination of Total Phenolic Content

By using the Folin-Ciocalteau's method total phenolic content was estimated. The Folin-Ciocalteu reagent (10%, v/v) (2.5 ml) was mixed with 0.1 ml of CCLE extract (1 mg/ml). This mixture was further supplemented with 2.0 ml of 7.5% (w/v) sodium carbonate solution. In the absence of light the mixture was left for 1.5 hours at room temperature after which its absorbance was measured at 765 nm using Systronic UV-Visible Double Beam Spectrophotometer. For gallic acid (10 to 100 µg/ml), the same procedure was used to calculate their concentration. Total phenolic content was calculated using the gallic acid standard curve and expressed in mg Gallic Acid Equivalents (GAE)/gram of sample (31–34).

2.7 Determination of Total Flavonoids Content

Quantification of total flavonoids was carried out through a colorimetric assay based on the development of a flavonoid-aluminum complex. In brief, the total flavonoid content (TFC) of the CCLE samples was quantified by reacting 1 mg/ml of the sample with 0.1 ml of 10% AlCl3, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water. At room temperature the mixture was kept for 30 minutes. Absorbance then taken at 415 nm with the help of UV-Visible double-beam spectrophotometer (Systronic). Similarly, quercetin was reacted in different concentrations (10-100 µg/ml). Considering quercetin as a standard the TFC was quantified, and the data was reported in mg QE/ml of the sample (22,35–37).

2.8 HPTLC Quantification

Pre-coated silica gel 60 F_{254} (50 × 100 mm) plates supplied by Merck (Darmstadt, Germany) for the HPTLC method were used as the stationary phase. The test substance, referred to as KA1, was dissolved in ethanol, and different amounts (5.0, 6.0, 8.0, and 9.0 µL) of the test substance solution were spotted on the plates using a CAMAG Linomat 5 automatic applicator (Serial No. 100632, CAMAG, Muttenz, Switzerland). The sample bands were placed 8.0 mm from the bottom edge of the plate, each having a length of 8.0 mm, with a distance of 10.4 mm between the tracks. In order to ensure the exact dosage of the samples, the dispensing rate was set to be 100 nL/s, and the pre-dosage was set to be 0.20 µL. The thin-layer chromatography analysis in this study was performed according to the following procedure: First, a 20×10 cm double-trough glass development chamber was used, with filter paper lined on its inner walls to ensure uniform gas conditions inside the chamber. After 20 minutes of pre-saturation, development was carried out using a mobile phase of toluene: ethyl acetate: methanol: formic acid at a volume ratio of 3: 3: 0.8: 0.2. The plate was removed when the solvent front reached 70 mm, and dried at room temperature for 5 minutes. Next, a TLC Scanner 3 manufactured by Swiss firm CAMAG, with serial number 140507, paired with visionCATS 3.2.23095.1 software, was used to complete automatic detection and densitometric scanning. The working parameters were set as follows: absorbance scanning mode, dual wavelengths of 254 and 366 nm, matched deuterium-tungsten lamp for detection, scanning speed of 20 mm/s, resolution of 100 µm per step, and slit size of 5×0.45 mm.

In this study, data preprocessing for the HPTLC experiments adopted Savitzky–Golay smoothing with a window size of 7 and baseline correction via the minimum slope method. All operations complied with CAMAG standards and the HPTLC analysis guidelines specified in references (38–40).

3. Result and Discussion

3.1. Phytochemical Screening

This study adopted a preliminary phytochemical screening method to test the hydroalcoholic extract of lemon bottlebrush (*Callistemon citrinus*). We detected six categories of chemical components, including phenols and alkaloids, whose abundance levels were marked with the ++/+ rating scale. Follow-up research plans to carry out in-depth investigations using high-performance thin-layer chromatography (HPTLC), and the findings of this study can provide support for subsequent related research.

Table 01: Preliminary Phytochemical Screening of Hydroalcoholic Extract of *Callistemon citrinus* Leaves (CCLE)

S. No.	Phytochemical Constituent	Test Name	Hydro-Alcoholic CCLE
1	Alkaloids	Dragendorff's Test	++
2	Carbohydrates	Molisch Test	+
3	Steroids and Sterols	Liebermann–Burchard Test	-
4	Glycosides	Legal Test	-
5	Saponins	Foam Test	+
6	Flavonoids	Shinoda Test	++
7	Tannins and Phenolic Compounds	Ferric Chloride Test	++
8	Triterpenoids	Tin–Thionyl Chloride Test	_
9	Proteins and Amino Acids	Biuret Test	+
10	Fixed Oils / Essential Oils	Spot Test	++
		Saponification Test	++

3.2. Antioxidant Activity

3.2.1. 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 73.92 % and reached an inhibition rate of 87.24 % at 1000 µg/mL concentration. Its half-maximal inhibitory concentration (IC50) was 6.74±1.52 µg/mL, indicating weaker antioxidant activity than the standard antioxidant ascorbic acid, which had an IC50 of 3.69±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.

Table 02: DPPH Free Radical Scavenging Activity A. Ascorbic Acid (Std) B. *C. citrinus* Leaves Extract (CCLE).

A.

S. No	Sample	Conc. (µg/ml)	Mean Abs	%RSA
1.	Ascorbic Acid	0	0.393	1.01
2.		0.78125	0.359	5.89
3.		1.5625	0.342	13.1
4.		3.125	0.316	20.08
5.		6.25	0.285	28.67
6.		12.5	0.252	36.53
7.		25	0.184	56.84

B.

S. No	Sample	Conc. (µg/ml)	Mean Abs	%RSA
1.	CCLE	0	0.351	9.04
2.		1	0.301	14.7
3.		10	0.247	39.8
4.		50	0.129	73.92
5.		100	0.091	84.24
6.		250	0.095	85.37

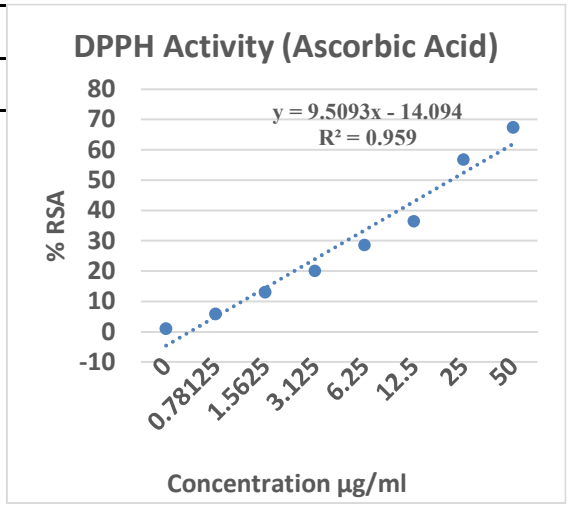

Figure 1. DPPH % RSA OF Ascorbic Acid.

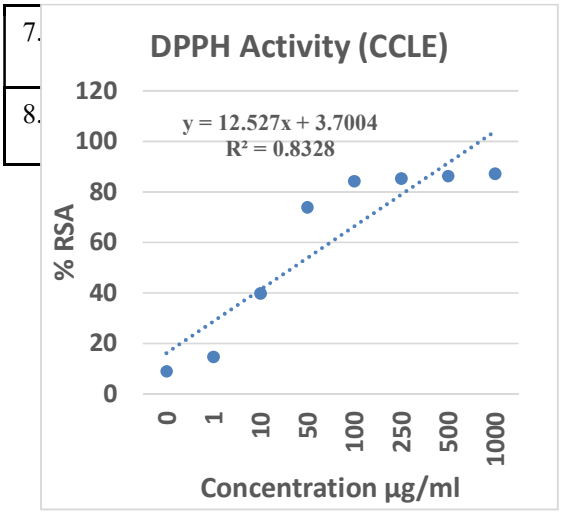

Figure 2. DPPH % RSA of CCLE.

3.2.2. 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 3.62±0.21 µg/mL for ascorbic acid, and an IC50 of 9.98±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. Relevant data are presented in Table 2.

Table 02: ABTS Free Radical Scavenging Activity A. Ascorbic Acid (Std) B. *C. citrinus* Leaves Extract (CCLE).

A.

S. No	Sample	Conc. (µg/ml)	Mean Abs	%RSA
1	Std-Ascorbic acid	0	0.181	2.18
2		0.78	0.162	8.61
3		1.56	0.132	36.98
4		3.125	0.059	84.04
5		6.25	0.052	90.12
6.		12.5	0.049	93.71
7.		25	0.045	96.22
8.		50	0.047	98.56
1.	Control	-	1.995	-

B.

S. No	Sample	Conc. (µg/ml)	Abs	%RSA
1	CCLE	0	0.201	0.82
2		1.5625	0.194	3.07
3		3.125	0.185	6.76
4		6.25	0.184	13.53
5		12.5	0.162	24.07
6.		25	0.141	35.53
7.		50	0.104	47.3
8.		100	0.083	56.53
1.	Control	-	1.995	-

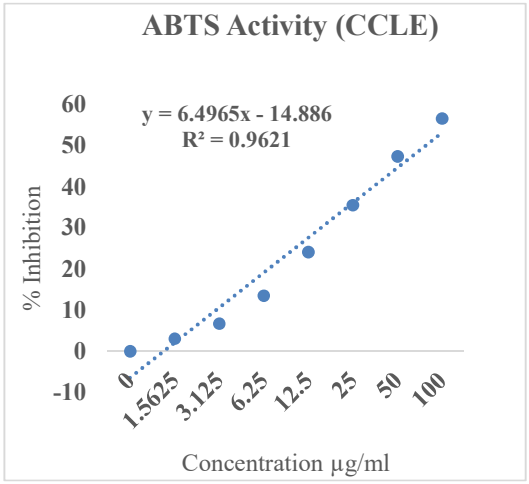

Figure 3. ABTS % RSA of Ascorbic Acid.

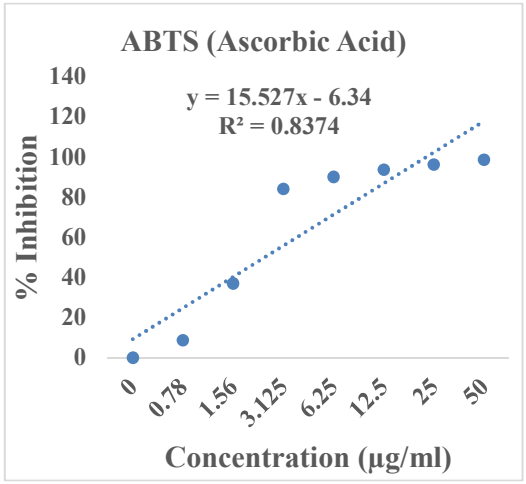

Figure 4. ABTS % RSA of CCLE

3.2.3. FRAP Assay: The ferric reducing antioxidant power assay (FRAP assay) was used to measure the reducing potential of CCLE, the hydroalcoholic leaf extract of the lemon bottle tree *Callistemon citrinus*. The measured FRAP value was 132.389 ± 5.853 µM Fe++/ml. At a wavelength of 700 nm, absorbance increased synchronously with the reduction of ferric iron. The calibration curve prepared with $FeSO_4$ had an R^2 of 0.9991, which confirms that the phenolic and flavonoid substances contained in the extract have antioxidant potential, and can serve as a source of natural antioxidants.

Table 06. FRAP Activity A. FeSO4 (Std.)

A.

Code	Conc. of FeSO4 (µM)	R1	R2	R3	Mean
c1	100	0.053	0.0158	0.0156	0.028
c2	200	0.144	0.141	0.14	0.142
c3	400	0.221	0.223	0.226	0.223
c4	800	0.449	0.449	0.435	0.444
c5	1000	0.592	0.596	0.592	0.593

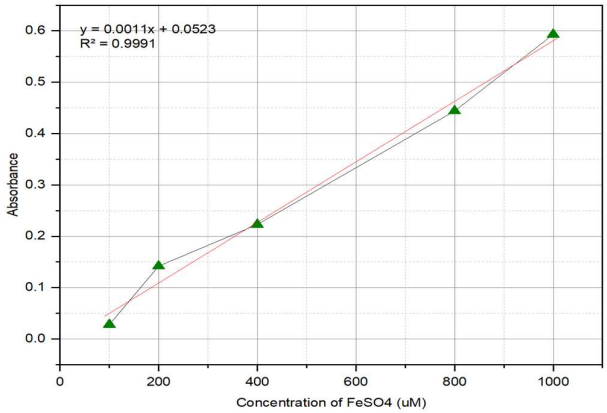

Figure 5. FRAP std Graph Absorbance Vs Conc. of FeSO4

3.3. Total Phenolic Content: The Folin–Ciocalteu 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.

Table 07. Total Phenolic content A. Galic acid (Std.)
A.

Sr.no	Conc. of Gallic Acid (µg/ml)	Absorbance at 765 nm
1	0	0
2	10	0.107
3	20	0.126
4	40	0.155
5	80	0.225
6	100	0.292

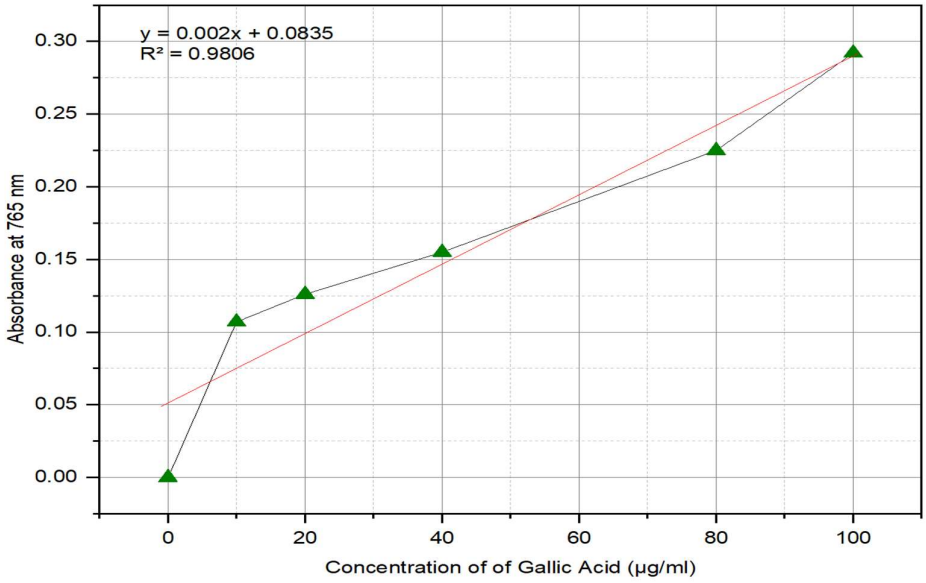

Figure 6. Std. Curve of Galic acid for Total Pnenolic Content Estimation.

3.4. 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. It is inferred that the flavonoid compounds in CCLE are responsible for the antioxidant activity observed in its ABTS and FRAP assays.

Table 08. Total flavonoid content (TFC) A. Quercetin (Std.)
A.

Sr no.	Concentration of Quercetin (µg/ml)	Absorbance at 415nm
1	0	0
2	10	0.149
3	20	0.211
4	40	0.283
5	80	0.466
6	100	0.698

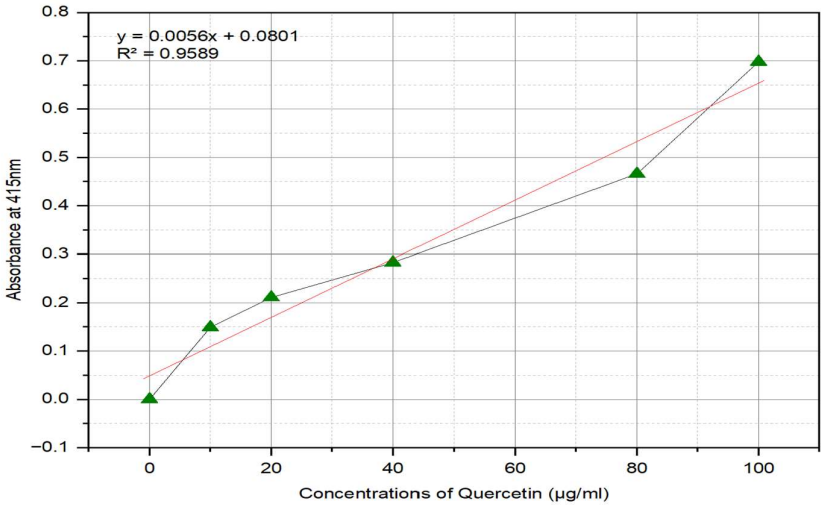

Figure7. Std. Curve of Quercetin for Total Flavonoid Content.

3.5. HPTLC Quantification: This study uses the hydroalcoholic extract of *C. citrinus* (CCLE) as its research subject, and adopts high-performance thin-layer chromatography (HPTLC) for detection. The measured retention factor (Rf) value of quercetin was 0.567, which falls within the standard range of 0.570–0.575, and the content of quercetin reached 74.85 µg/100 mg. This method presents good linearity and precision, and results from the ABTS assay confirm that CCLE has strong antioxidant potential.

Table 09. HPTLC quantification of the hydroalcoholic extract of *Callistemon citrinus* (CCLE).

S.no	**Lane**	**Sample**	**Volume Applied**	**R_f**	**AU (Absorbance Unit)**
1.	Lane 01	Quercetin	2 µL	0.558-0.598	0.0841
2.	Lane 02	Quercetin	4 µL	0.557-0.593	0.1192
3.	Lane 03	Quercetin	6 µL	0.555-0.592	0.1557
4.	Lane 04	CCLE	5 µL	0.548-0.590	0.1150

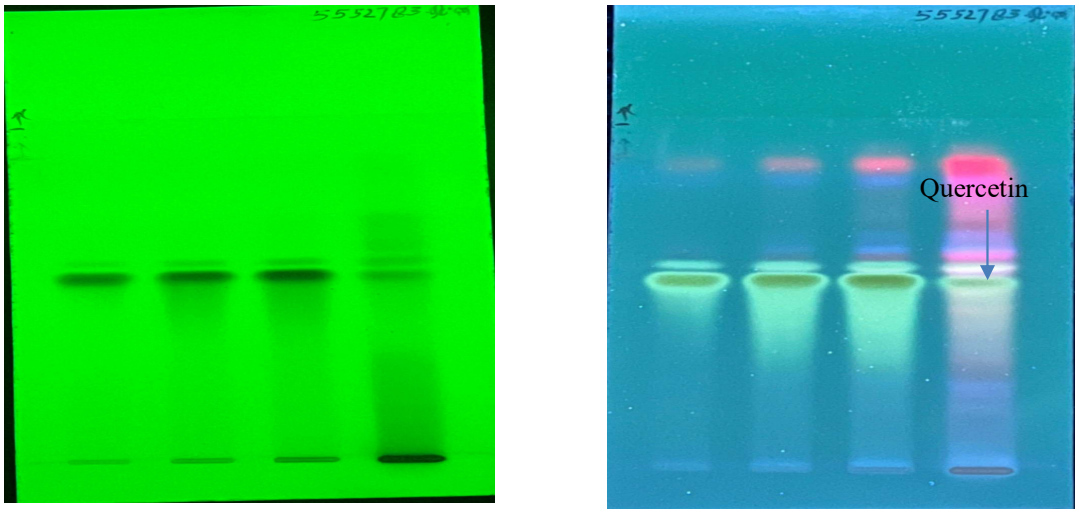

Figure 8. Quantification of Quercetin by HPTLC A. 254 nm B. 366 nm

4. Conclusion

This study carried out a series of activity assays on the hydroalcoholic extract prepared from the leaves of lemon bottlebrush *Callistemon citrinus*. It is confirmed that the extract is rich in phenolic and flavonoid compounds, with considerable contents of total phenols and total flavonoids. Three antioxidant assays, namely DPPH, ABTS, and FRAP, verified that the extract has strong antioxidant activity. The High-performance thin-layer chromatography (HPTLC) was used to quantify quercetin as a quality marker, which provides research support for the quality standardization of this extract, as well as its application as a natural antioxidant raw material in the fields of medicine and health products.

Ethical approval and consent to participate: This review article does not contain any studies with human participants or animals.

Consent for publication: Not applicable.

Conflict of interest: The authors have no conflict of interest to declare.

Authorship Contribution Statement: Shashi Ranjan Singh: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing – original draft. Omsatyam: Validation, Data curation. Arvind K. Patel: Formal analysis, Validation, Resources. Shankul Kumar: Investigation, Data curation, Visualization. Dharmendra Kumar: Supervision, Methodology, Validation. Laliteshwar Pratap Singh: Conceptualization, Supervision, Writing – review & editing.

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Availability of data and material: The Data analysed in this study are available upon request to the corresponding author.

Abbreviation	**Full Form**
ABTS	2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
AU	Absorbance Unit
CCLE	*Callistemon citrinus* Leaf Extract
CV	Coefficient of Variation

Abbreviation	**Full Form**
DPPH	2,2-Diphenyl-1-picrylhydrazyl
FRAP	Ferric Reducing Antioxidant Power
GAE	Gallic Acid Equivalent
HCl	Hydrochloric Acid
HPTLC	High-Performance Thin-Layer Chromatography
IC_{50}	Half Maximal Inhibitory Concentration
KOH	Potassium Hydroxide
NaOH	Sodium Hydroxide
QE	Quercetin Equivalent
ROS	Reactive Oxygen Species
RSA	Radical Scavenging Activity
Rf	Retardation Factor
SD	Standard Deviation
TFC	Total Flavonoid Content
TPC	Total Phenolic Content
UV-Vis	Ultraviolet–Visible Spectrophotometry
µg	Microgram
µg/mL	Microgram per Millilitre
µM	Micromolar
µM/g	Micromolar per Gram
w/w	Weight by Weight

References

1. Latif R, Nawaz T. Medicinal plants and human health: A comprehensive review of bioactive compounds, therapeutic effects, and applications. Phytochem Rev. 2025;1–44.
2. Halder M, Jha S. Medicinal plants and bioactive phytochemical diversity: a fountainhead of potential drugs against human diseases. Medicinal plants: Biodiversity, biotechnology and conservation. Springer; 2023. ص
3. Onohuean H, Cherop N, Nwozo SO, Najophe SE, Bukke SPN. A systematic review of the ethnopharmacology and phytochemistry of Callistemon citrinus "Myrtaceae": implication for phytomedicine future prospect. RPS Pharm Pharmacol Reports, 2025;4(1):rqae029. https://doi.org/10.1093/rpsppr/rqae029
4. Mathew S, Zhou X, Münch G, Raju R. Exploring the Anti-Inflammatory Potential of Australian Native Plants Based on their Ethnopharmacological Knowledge. Chem Biodivers. 2024;21(7):e202400492.
5. Gadhave TB, Bhandawane DD, Hajare PK, Jadhav MB. Review on Callilstemon citrinus: Recent Approaches in Novel Drug Delivery System. J Drug Deliv Ther. 2024;14(9).
6. Piñón-Simental JS, Ayala-Ruiz LA, Ortega-Pérez LG, Magaña-Rodríguez OR, Meléndez-Herrera E, Aguilera-Méndez A, Use of Callistemon citrinus as a gastroprotective and anti-inflammatory agent on indomethacin-induced gastric ulcers in obese rats. Oliveira S, PeerJ.2024;12:e17062.https://doi.org/10.7717/peerj.17062
7. Dongmo Lekagne JB, Fouelefack FR, Dakole Daboy C, Galani YJH, Fotio D, Nguefack J. Chemical composition, antifungal properties and seed treatment potential of essential oil fractions of Callistemon citrinus against two seed-borne fungi of rice: Alternaria padwickii and Bipolaris oryzae. J Plant Dis Prot. 2023;130(4):833–41.
8. Vashisath S, Kapoor S, Kumari V, Butt S, Kumar D. Comparative Studies of Essential Oil Composition and Biological Activities of Callistemon citrinus from Western Himalaya. Chem Biodivers. 2024;21(6):e202301129.
9. Ismail E, Mohamed A, Maboza E, Dhlamini MS, Adam RZ. Callistemon citrinus: A plant-mediated synthesis of sustainable Rhodium nanoparticles and their antimicrobial activity. Appl Res. 2024;3(5):e202300130.
10. Abisoye LR, Olisah C, Gideon A, Ocheme W, Omale S, Ilenikhena A, Research Evolution on Medicinal Plant from Myrtaceae Family (Callistemon genus): Five Decades of Research (a Bibliometric Review). 2023;
11. Reddy VP. Oxidative stress in health and disease. Biomedicines. 2023;11(11):2925.
12. Tiwana JK, Shah AK, Dhalla NS. The involvement of reactive oxygen species in causing chronic cardiovascular and neurodegenerative diseases and some cancers. Scr Med (Brno). 2024;55(2):199–217.
13. Anwar S, Alharbi HOA, Babiker AY, Rahmani AH. Oxidative Stress in Health and Disease: Mechanisms and Therapeutic Perspectives. Int J Mol Sci. 2026;27(6):2681.
14. Kishi S, Nagasu H, Kidokoro K, Kashihara N. Oxidative stress and the role of redox signalling in chronic kidney disease. Nat Rev Nephrol. 2024;20(2):101–19.
15. Tumilaar SG, Hardianto A, Dohi H, Kurnia D. A comprehensive review of free radicals, oxidative stress, and antioxidants: Overview, clinical applications, global perspectives, future directions, and mechanisms of antioxidant activity of flavonoid compounds. J Chem. 2024;2024(1):5594386.
16. Akbari B, Baghaei-Yazdi N, Bahmaie M, Mahdavi Abhari F. The role of plant-derived natural antioxidants in reduction of oxidative stress. BioFactors. 2022;48(3):611–33.
17. Veryer K, Saygideğer B, Süfer Ö, Saygideğer Y, Umay A, Bozok F. Biological Activities of Callistemon citrinus and Callistemon viminalis Essential Oils: EMT-Linked Antitumor, Antioxidant, and Antibacterial Effects. Chem Biodivers. 2026;23(1):e02563.
18. Ortega-Pérez LG, Piñón-Simental JS, Magaña-Rodríguez OR, Lopéz-Mejía A, Ayala-Ruiz LA, García-Calderón AJ, Evaluation of the toxicology, anti-lipase,

and antioxidant effects of Callistemon citrinus in rats fed with a high fat-fructose diet. Pharm Biol. 2022;60(1):1384–93.
19. Zymone K, Benetis R, Trumbeckas D, Baseviciene I, Trumbeckaite S. Different effects of quercetin glycosides and quercetin on kidney mitochondrial function—Uncoupling, cytochrome C reducing and antioxidant activity. Molecules. 2022;27(19):6377.
20. Shanmugasundaram D, Roza JM. Assessment of anti-inflammatory and antioxidant activity of quercetin–rutin blend (SophorOx^{TM})–an invitro cell based assay. J Complement Integr Med. 2022;19(3):637–44.
21. Cizmarova B, Hubkova B, Birkova A. Quercetin as an effective antioxidant against superoxide radical. Funct Food Sci ISSN 2767-3146. 2023;3(3):15–25.
22. Prajapati VK, Singh SR, Kumar U, Nain S. Analytical and bioactivity-based evaluation of Callistemon citrinus: HPTLC fingerprinting, marker quantification, and antioxidant potential of Hydroalcoholic extracts. J Chromatogr B. 2026;125065.
23. Nga Nt, Ly Nts, Yến Vth, Thảo Htt, Loan LQ, Dũng NH. Phytochemicals And Antioxidant Activities Of Methanolic Extract From Callistemon citrinus Leaves. Tạp chí Khoa học Trường Đại học Sư phạm TP Hồ Chí Minh. 2022;19(10):1631.
24. Dilbar N, Fatima S, Anwar M, Anwer Z, Ashraf H. Qualitative phytochemical screening of some selected medicinal desert species. Int J Appl Exp Biol. 2023;2:141–6.
25. Ghosh P, Goswami S, Roy S, Das R, Chakraborty T, Ray S. Comparative in vitro antioxidant and antibacterial activities of leaf extract fractions of Crimson bottlebrush, Callistemon citrinus (Curtis.) Skeels. The Microbe. 2025;8:100445.
26. Ortega-Pérez LG, Ayala-Ruiz LA, Magaña-Rodríguez OR, Piñón-Simental JS, Aguilera-Méndez A, Godínez-Hernández D, Development and Evaluation of Phytosomes Containing Callistemon citrinus Leaf Extract: A Preclinical Approach for the Treatment of Obesity in a Rodent Model. Pharmaceutics.
27. Esertaş ÜZÜ, Kobya O, Çağlak E, Kara A. Chemical and Biological Activities of Callistemon citrinus and Punica granatum. Biol Bull. 2023;50(Suppl 3):S338–50.
28. López-Mejía A, Ortega-Pérez LG, Magaña-Rodríguez OR, Ayala-Ruiz LA, Piñón-Simental JS, Hernández DG, Protective effect of Callistemon citrinus on oxidative stress in rats with 1,2-dimethylhydrazine-induced colon cancer. Biomed Pharmacother.
29. Benzie IFF, Devaki M. The ferric reducing/antioxidant power (FRAP) assay for non-enzymatic antioxidant capacity: concepts, procedures, limitations and applications. Meas Antioxid Act Capacit Recent trends Appl. 2018;77–106.
30. Rumpf J, Burger R, Schulze M. Statistical evaluation of DPPH, ABTS, FRAP, and Folin-Ciocalteu assays to assess the antioxidant capacity of lignins. Int J Biol Macromol. 2023;233:123470.
31. Larayetan R, Ololade ZS, Ogunmola OO, Ladokun A. Research Article Phytochemical Constituents, Antioxidant, Cytotoxicity, Antimicrobial, Antitrypanosomal, and Antimalarial Potentials of the Crude Extracts of Callistemon citrinus. 2019;
32. Bhandari NL, Khadka S, Dhungana BR, Bhandari G, Bhatt TD, Pandey DPP. Study of phyto-and physicochemical analysis, antimicrobial and antioxidant activities of essential oil extract of callistemon citrinus (curtis) skeels leaves. Adv J Chem B. 2021;3(2):109–19.
33. Laganà G, Barreca D, Smeriglio A, Germanò MP, D'Angelo V, Calderaro A, وآخ. Evaluation of Anthocyanin Profile, Antioxidant, Cytoprotective, and Anti-Angiogenic Properties of Callistemon citrinus Flowers. Plants (Basel, Switzerland).
34. Lamuela-Raventós RM. Folin–Ciocalteu method for the measurement of total phenolic content and antioxidant capacity. Meas Antioxid Act Capacit Recent trends Appl. 2018;107–15.
35. Nicolescu A, Bunea CI, Mocan A. Total flavonoid content revised: An overview of past, present, and future determinations in phytochemical analysis. Anal Biochem. 2025;700:115794.
36. Orsavová J, Juríková T, Bednaříková R, Mlček J. Total phenolic and total

flavonoid content, individual phenolic compounds and antioxidant activity in sweet rowanberry cultivars. Antioxidants. 2023;12(4):913.
37. Hagos M, Chandravanshi BS, Redi-Abshiro M, Yaya EE. Determination of total phenolic, total flavonoid, ascorbic acid contents and antioxidant activity of pumpkin flesh, peel and seeds. Bull Chem Soc Ethiop. 2023;37(5).
38. Srinivas A, Nehra S. Development of HPTLC method for simultaneous determination of quercetin and kaempferol in leaf extract of Hibiscus mutabilis. J Chromatogr B. 2024;1246:124277.
39. Muhammad II, Pandey DK. HPTLC quantification of quercetin from leaf extracts of C. occidentalis L. and its inhibitory activity against protease enzyme of P. falciparum. Vegetos. 2024;37(5):1804–16.
40. Ravindrakumar P, Vyas N, Sandip P. Urolithiasis: HPTLC method for quantitative detection of rutin and quercetin in an herbal plant. 2022;