RESEARCH ARTICLE

Investigation of Phytochemical Composition and *In-vitro* Antioxidant Activity of *Canthium parviflorum* Leaf Extract

Kalaivanan S*, Srinivasan R

Faculty of Pharmacy, Bharath Institute of Higher Education and Research, Selaiyur, Chennai, Tamil Nadu, India.

Received: 01st January, 2024; Revised: 27th January, 2024; Accepted: 16th May, 2024; Available Online: 25th June, 2024

ABSTRACT

This study provides a thorough investigation of *Canthium parviflorum* leaves, integrating macroscopical, phytochemical, and *in-vitro* antioxidant analyses to unveil the plant's therapeutic potential. The preliminary phytochemical analysis identifies a diverse array of bioactive compounds in *C. parviflorum*, encompassing alkaloids, terpenoids, phenols, flavonoids, tannins, glycosides, saponins, gum, and mucilage, known for their potential pharmacological activities. Physico-chemical parameters characterize the leaves, with a moisture content of 6.9% w/w indicating water content and ash values providing insights into the inorganic composition. The hydroalcohol extract exhibits an extraction efficiency of 26.91%, emphasizing the success of the extraction process. The focus extends to the quantification of phenolic and flavonoid content in the hydroalcoholic extract. The substantial phenolic content at 76.13 mg GAE/gm suggests antioxidant potential, and the quantified flavonoid content of 4.95 mg quercetin equivalents per gram extract further underscores the presence of bioactive compounds. The study seamlessly integrates *in-vitro* antioxidant analyses, revealing concentration-dependent scavenging activities against superoxide and hydroxyl radicals. These findings support the plant's potential therapeutic applications, emphasizing its antioxidant capabilities. The comprehensive exploration of *C. parviflorum*'s macroscopical and microscopical features, phytochemical composition, and *in-vitro* antioxidant properties contributes to a robust foundation for its potential integration into herbal medicine. The identification of bioactive compounds underscores its significance as a valuable natural resource. In conclusion, this study not only enriches our understanding of *C. parviflorum* but also paves the way for future investigations into its medicinal applications, fostering advancements in herbal medicine and natural product research.

Keywords: *Canthium parviflorum,* Phytochemical screening, Traditional medicine, Pharmacognosy.

International Journal of Pharmaceutical Quality Assurance (2024); DOI: 10.25258/ijpqa.15.2.18

How to cite this article: Kalaivanan S, Srinivasan R. Investigation of Phytochemical Composition and *In-vitro* Antioxidant Activity of *Canthium parviflorum* Leaf Extract. International Journal of Pharmaceutical Quality Assurance. 2024;15(2):666-670.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Pharmaceutical drugs have long been a cornerstone of medicine, but herbal remedies remain crucial as well, with 80% of the world's population still using them for healing. As a result of this widespread reliance on herbal products, pharmaceutical companies have delved into extensive research into the therapeutic potential of plant-based products. World Health Organization (WHO) highlights the necessity for stringent quality control measures to ensure the safety and effectiveness of herbal remedies.[1] It is emphasized by the WHO that samples should be characterized qualitatively and quantitatively, biomarkers measured and fingerprint profiles created using both qualitative and quantitative methods.

Generally, herbal medicines are safe and have little side effects.[2] A major concern regarding the safety, quality, and efficacy of medicinal plants and herbal products has been raised with the commercialization of herbal medicine. There are a number of factors that influence the consistency and accuracy of herbal raw materials, such as plant identity and seasonal changes. A full compliance with WHO guidelines not only improves herbal product quality, but also minimizes the risks associated with their use.[3]

The current study examines phytochemical preliminary studies, quantitative analysis of phytochemical constituents, and *in-vitro* research on antioxidant activity in extracts of *Canthium parviflorum* leaves.

MATERIALS AND METHODS

Collection and authentication of plants

The leaves of *C. parviflorum*, a member of the Rubiaceae family, were gathered from the Tirumala hills in Tirupati. They underwent identification and authentication by Dr. K. Madhavachetty, Asst. Professor in the Department of Botany at Sri Venkateswara University, Tirupati.

**Author for Correspondence: ki935831@gmail.com*

Plant extraction

C. parviflorum leaves were air-dried and subsequently ground into a coarse powder. Following this, hydroalcoholic (50% ethanol+ 50% water) was used to extract the powder sequentially using the soxhlet apparatus.[4] In subsequent experiments, the extract (26.91% w/w) was stored in desiccators for future use after being distilled under reduced pressure to remove the solvents.

Preliminary Phytochemical Screening

According to Kokate, the preliminary screening was conducted according to the established protocol.[4,5]

Total Phenol and Flavonoid Content Estimation[6]

Phenolic content

The study described a method for determining the total phenolic content.[7,8] In this method, a leaf extract was extracted using hydroalcohol. The measurement was done using the Folin-cio-calteu reagent, with gallic acid as the standard reference. The calibration of gallic acid was performed using hydroalcohol. The sample solution was diluted with distilled H_2O, then dissolved in a volumetric flask containing 10 mL of H_2O after 1-mg of extract was added. After mixing the mixture, 1.5 mL of Folin-Ciocalteu reagent was added, and it was left for five minutes before being analyzed. Using distilled water, the volume was adjusted to 25 mL after adding 4 mL of a 20% Na_2CO_3 solution. Following 30 minutes of oxidation, a Shimadzu 1800 spectrophotometer was used to measure the absorbance of the blue color. By using gallic acid as a reference standard, phenolic content was determined and percentages calculated.

Flavonoids estimation

By using an aluminum chloride colorimetric method, the flavonoid content in *C. parviflorum* leaves was evaluated. In this study, quercetin, 10 µg/mL, was used as the reference standard. A 100 mL solution of hydroalcohol containing 1-mg of quercetin was used to prepare this standard. The standard was diluted with hydroalcohol to yield conc. (1–10 µg/mL).[9,10] With the standard solution, 1-mL of hydro alcohol was mixed with 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1M potassium acetate, and 2.8 mL of H_2O for the flavonoid analysis. A Shimadzu 1800 spectrophotometer was used to measure the absorbance of the resulting solution after 30 minutes of incubation. Using 10% $AlCl_3$ and equal volumes of distilled water, we prepared a blank sample. Using 1.0 mL of the extract solution containing 4 mg of the extract, a similar procedure was followed to determine flavonoid content. Using standard solutions, a calibration curve was derived to determine the flavonoid content.

In-vitro antioxidant assay

Following established procedures, we used two assay methods to assess the antioxidant activity of the extracts *in-vitro*.[11]

Superoxide radical scavenging assay

The reaction mixture consisted of 0.5 mL phosphate buffer (pH 7.4), 1.0 mL of NADH, 0.156 mM NBT, 0.1 mL of PMS, and 3 mL of ascorbic acid at various concentrations (10–50 g/mL, dissolved in 90% ethanol). An appropriate blank was used to quantify the amount of formazan produced after the mixture was incubated for one hour at 25°C.[12]

Hydroxyl radical scavenging assay

To prepare the reaction mixture, 0.1 mL of 10 mM 2-deoxy-2-ribose, 0.33 mL of 50 mM phosphate buffer (pH 7.4), 0.1 mL of ferric chloride, 0.1 mL of EDTA, 0.1 mL of 1 mM H_2O_2, 0.1 mL of 1 mM ascorbic acid, and 1.0 mL of extracts at various concentrations (ranging from 5–50 µg/mL) were added. It was then incubated at 37°C for 45 minutes. A trichloroacetic acid solution containing 0.5% v/v in 0.025 mol/L sodium hydroxide solution with 0.2% w/v BHA and a thiobarbituric acid solution containing 0.5% v/v in NaOH solution were added afterward. To induce the pink chromogen to develop, the mixture was incubated at 95°C for 15 minutes. A blank solution was used to measure absorbance at 532 nm after cooling.[13] For all the aforementioned methodologies, the computation of the %inhibition was determined by using a formula.

$$\% \, inhibition = \frac{A\,(control) - A\,(test)}{A\,(control)} X100$$

Where,
A (control) = Control absorbance
A (test) = Test Absorbance (Extract / standard drug).

RESULTS

Preliminary Phytochemical Study

According to the preliminary phytochemical analysis (Table 1), the hydroalcoholic extract of *C. parviflorum* (HAECP) contains alkaloids, terpenoids, phenols, flavonoid, tannin content, glycosides, saponins, gum as well as mucilage.

Phenolic and flavonoid content determination

- *Phenol content*

An estimate of the total phenolic content of the extract was derived from the gallic acid standard (Figure 1). Hydroalcoholic extracts of *C. parviflorum* were analyzed for phenolic content in Table 2. Data clearly indicates that the hydroalcoholic extract of *C. parviflorum* contains significant amounts of total phenolics.

- *Flavonoid content*

As shown in Figure 2, total flavonoids in plant extracts were quantified in milligrams of quercetin equivalents per gram extract. Analyzing the flavonoid content of *C. parviflorum* hydroalcoholic extract was conducted in triplicate, as shown in Table 2.

Antioxidant Activity

Tables 3 and 4 present the superoxide and hydroxyl radical scavenging activity of the hydroalcoholic extract of *C. parviflorum* (HAECP) in comparison to ascorbic acid at different concentrations (10–50 µg/mL). Superoxide radical scavenging activity is an essential indicator of the antioxidant

Table 1: Preliminary phytochemical analysis

Phytoconstituents	*Hydroalcoholic Extracts (Leaves of C. parviflorum)*
Alkaloids	+
Carbohydrates	+
Proteins	-
Terpenoid	+
Phenolic compound	+
Flavonoid	+
Tannin content	+
Glycoside	+
Gum and mucilage	+
Saponin	+
Steroid	+
Fixed oil & fat	+

+ 'Indicates' Presence; - 'Indicates' Negative

Table 2: Quantification of flavonoids and phenolic content in *C. parviflorum*

S. No	*Phenolic equivalent (GAE/gm)*	*Flavonoid equivalent (Quercetin/gm)*
1	76.13 ± 0.295	4.95 ± 0.283

*value are mean ± SEM of three findings

potential of a substance, showcasing its ability to neutralize superoxide radicals in biological systems. At 10 µg/mL concentration, HAECP demonstrated a superoxide radical scavenging activity of 41.36 ± 3.53, while ascorbic acid exhibited a slightly lower activity of 39.36 ± 3.87. As the concentration increased, so did the scavenging activity for both HAECP and ascorbic acid. Notably, at 50 µg/mL, HAECP displayed a significant superoxide radical scavenging activity of 55.55 ± 4.22, exceeding the activity of ascorbic acid at the same concentration, which was 54.98 ± 2.08.

At 10 µg/mL concentration, HAECP exhibited a hydroxyl radical scavenging activity of 46.07 ± 4.88, slightly surpassing the activity of ascorbic acid at 45.77 ± 3.16. As the concentration increased, the hydroxyl radical scavenging activity of both HAECP and ascorbic acid showed a noticeable upward trend (Table 4). At the highest concentration of 50 µg/mL, HAECP demonstrated a significant hydroxyl radical scavenging activity of 85.11 ± 4.94, compared to 86.08 ± 3.57 for ascorbic acid. These results indicate that HAECP possesses concentration-dependent hydroxyl radical scavenging capabilities, highlighting its potential as an effective antioxidant (Figure 3). The comparison with ascorbic acid, a well-established antioxidant, underscores the promising antioxidant activity of HAECP.

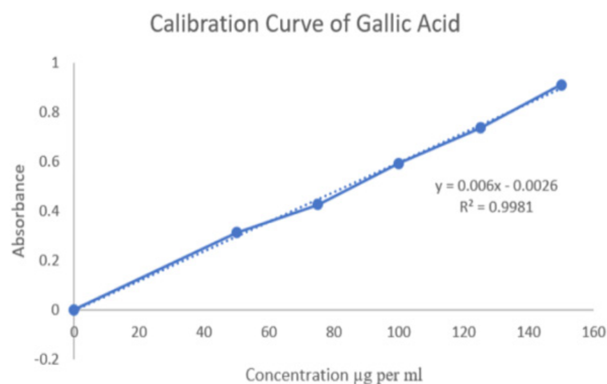

Figure 1: Gallic acid calibration curve

DISCUSSION

The preliminary phytochemical analysis reveals a diverse array of bioactive compounds in *C. parviflorum*. Alkaloids, terpenoids, phenols, flavonoids, tannins, glycosides, saponins, gum, and mucilage are detected. These compounds are known for their potential pharmacological activities, and their presence suggests that *C. parviflorum* may have therapeutic properties.[14] The hydroalcohol extract of *C. parviflorum* yields

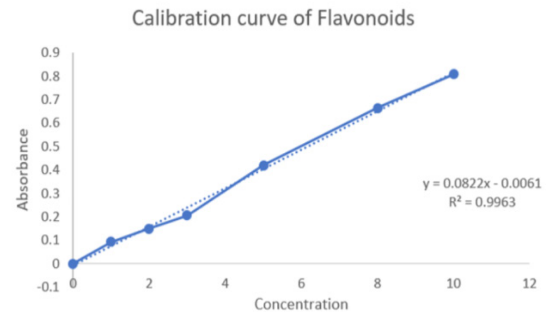

Figure 2: Quercetin calibration curve

Table 3: Superoxide radical scavenging activity of *C. parviflorum*

Group (µg/mL)	*Superoxide radical scavenging activity*	
	HAECP	*Ascorbic acid*
10	41.36 ± 3.53	39.36 ± 3.87
20	45.91 ± 4.47	42.33 ± 2.25
30	49.16 ± 4.32	48.58 ± 3.33
40	52.27 ± 3.18	51.87 ± 2.45
50	55.55 ± 4.22	54.98 ± 2.08

Table 4: Hydroxyl radical scavenging activity of *C. parviflorum*

Group (µg/mL)	*Hydroxyl radical scavenging activity*	
	HAECP	*Ascorbic acid*
10	46.07 ± 4.88	45.77 ± 3.16
20	67.91 ± 3.13	67.46 ± 3.63
30	71.13 ± 3.41	72.22 ± 4.55
40	78.14 ± 4.32	79.76 ± 3.06
50	85.11 ± 4.94	86.08 ± 3.57

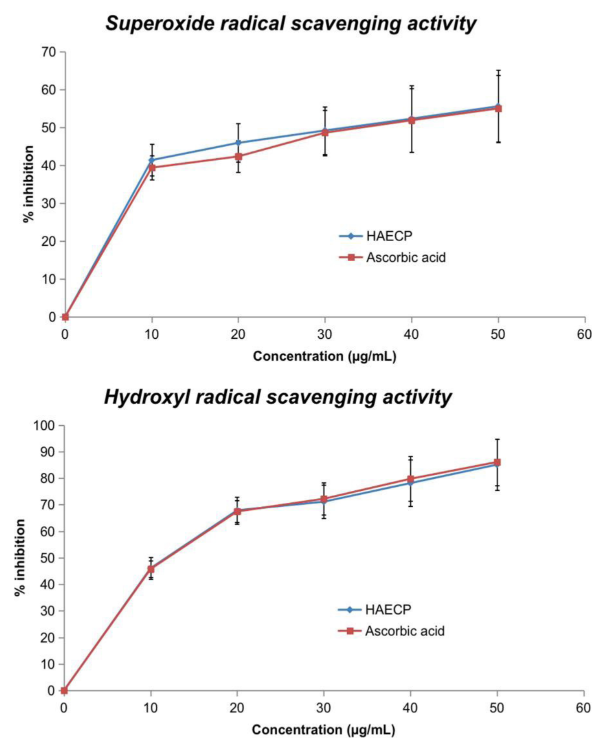

Figure 3: *In-vitro* antioxidant activity of extract of *C. parviflorum*

26.91%, indicating the efficiency of the extraction process.[15] This value is significant for evaluating the potential yield of bioactive compounds. The hydroalcoholic extract of *C. parviflorum* is rich in phenolic compounds, with a content of 76.13 mg GAE/gm based on gallic acid equivalents. Phenolic compounds are known for their antioxidant properties and potential health benefits. The flavonoid content, quantified at 4.95 mg quercetin equivalents per gram extract, further emphasizes the presence of bioactive compounds with potential antioxidant effects.[16,17]

The preliminary phytochemical analysis of *C. parviflorum* has provided valuable insights into its chemical composition, revealing the presence of diverse bioactive compounds such as alkaloids, terpenoids, phenols, flavonoids, tannins, glycosides, saponins, gum, and mucilage. These compounds are well-known for their potential pharmacological activities, suggesting that *C. parviflorum* holds therapeutic properties.[14] The hydroalcoholic extract of *C. parviflorum* demonstrated an extraction efficiency of 26.91%, a significant value that indicates the success of the extraction process[15] and implies a substantial yield of bioactive compounds.

Particularly noteworthy is the richness of phenolic compounds in the hydroalcoholic extract, quantified at 76.13 mg GAE/gm based on gallic acid equivalents. Phenolic compounds are recognized for their antioxidant properties,[16,17] contributing to potential health benefits. The flavonoid content, measured at 4.95 mg quercetin equivalents per gram of extract, further underscores the presence of bioactive compounds with potential antioxidant effects. As herbal drugs portray themselves as alternatives to synthetic drugs, proper standardization lies as the key to reproducibility of the pharmacological activity.[18,19]

Now, integrating the findings from the antioxidant activity *in-vitro* work, it becomes evident that *C. parviflorum*'s rich phenolic and flavonoid content aligns with its potent antioxidant capabilities. The scavenging activities against superoxide and hydroxyl radicals, as demonstrated in the *in-vitro* assays, corroborate the potential health benefits suggested by the phytochemical analysis. The concentration-dependent increase in radical scavenging activities further supports the notion that *C. parviflorum* may serve as an effective antioxidant. The comprehensive analysis combining preliminary phytochemical screening and antioxidant activity *in-vitro* results supports the potential therapeutic value of *C. parviflorum*. The presence of various bioactive compounds, particularly phenolic and flavonoid compounds, underscores its antioxidant potential.

CONCLUSION

This study suggests significant potential for using hydroalcohol extract from *C. parviflorum* leaves as a therapeutic approach for the treatment of various diseases. Proper standardization, as emphasized in the discussion, remains crucial for the reproducibility of pharmacological activities, positioning *C. parviflorum* as a promising herbal alternative with potential health benefits. These findings contribute valuable insights for further research into the medicinal potential of *C. parviflorum* and highlight its significance in the realm of herbal medicine and natural products.

REFERENCES

1. World Health Organization. World Health Organization-WHO. 2000.
2. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. 2nd ed. Delhi, India: Council of Scientific & Industrial Research, 1956:173.
3. Ahmed, Salman & Hasan, Mohtasheem. Standardization of crude drugs: A precise review. World Journal of Pharmaceutical Research, 2015; 4: 155-174.
4. J.B. Harborne, Methods of Extraction and Isolation, in: Phytochemical methods, third ed., Chapman and Hall, London, 1998: 60-66.
5. Kokate CK. Practical pharmacognosy. 1st ed. New Delhi: Vallabh prakashan; 1986.
6. Singleton V.L., Rossi J.A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagent. Am. J. Enol. Vitic. 1965;16:144–158.
7. Singleton VL and Joseph A. Rossi Jr. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. Am. J. Enol. Vitic. 1965; 16(3): 144-158.
8. Maurya S & Singh D. Quantitative Analysis of Total Phenolic Content in Adhatoda vasica Nees Extracts. International Journal of PharmTech Research. 2010; 2(4): 2403-2406.
9. Neupane, P. and Lamichhane, J., 2020. Estimation of total phenolic content, total flavonoid content and antioxidant capacities of five medicinal plants from Nepal. Vegetos, 2020; 33: 360-366.
10. Fattahi S, Zabihi E, Abedian Z, Pourbagher R, Motevalizadeh

Ardekani A, Mostafazadeh A, Akhavan-Niaki H. Total Phenolic and Flavonoid Contents of Aqueous Extract of Stinging Nettle and *In-vitro* Antiproliferative Effect on Hela and BT-474 Cell Lines. Int J Mol Cell Med. 2014; 3(2):102-7.

11. Ya Li, Sha Li, Sheng-Jun Lin, Jiao-Jiao Zhang, Cai-Ning Zhao, Hua-Bin Li. Microwave-Assisted Extraction of Natural Antioxidants from the Exotic Gordoniaaxillaris Fruit: Optimization and Identification of Phenolic Compounds. Molecules, 2017; 22(1481):1-16.
12. More, G.K., Makola, R.T. In-vitro analysis of free radical scavenging activities and suppression of LPS-induced ROS production in macrophage cells by *Solanum sisymbriifolium* extracts. Sci Rep 10
13. Sabir, S.M., Abbas, S.R., Shahida, S. *In-Vitro* antioxidant, anti-lipid peroxidative activities and In-Silico study of *Terminalia chebula* bioactive compounds. ClinPhytosci, 2020; 6: 83.
14. WC Evans. Pharmacognosy, 16th edition, London, W.B. Saunders Company Ltd., 2009: 541-570
15. Avinash Kumar Reddy, G., TrilokMitra, M., Shilpa, T., Shabnam, S., SatishBabu, K., Jyothi M Joy. Variation of Phenols, Flavonoids and Antioxidant Potential in Various Parts of Foeniculumvulgare on Drying. International Journal of Chemical and Pharmaceutical Sciences, 2012; 3(1):74-79.
16. Messaoud C, Boussaid M. *Myrtus communis* berry color morphs: A comparative analysis of essential oils, fatty acids, phenolic compounds, and antioxidant activities. Chem Biodivers. 2011;8:300–10.
17. Ya Li, Sha Li, Sheng-Jun Lin, Jiao-Jiao Zhang, Cai-Ning Zhao, Hua-Bin Li. Microwave-Assisted Extraction of Natural Antioxidants from the Exotic Gordoniaaxillaris Fruit: Optimization and Identification of Phenolic Compounds. Molecules, 2017; 22(1481):1-16.
18. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. 2nd ed. Delhi, India: Council of Scientific & Industrial Research; 1956: 173.
19. Rani S. Standardisation of Kushta: Classical and modern approaches. International Journal of Unani and Integrative Medicine. 2018:89-98.