Trop J Nat Prod Res, September 2024; 8(9):8275-8281 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Biochemical Composition, Polyphenol and Flavonoid Content, Antibacterial Activity of Leaf and Flower Extracts of *Clerodendrum paniculatum* Distributed In Thua Thien Hue Province, Viet Nam

Nguyen T. Q. Trang*, Hoang X. Thao, Bui T. T. Anh

Department of Biology, University of Education, Hue University, 34 Le Loi, Hue 530000, Vietnam.

ARTICLE INFO

Article history:
Received 25 August 2024
Revised 04 September 2024
Accepted 18 September 2024
Published online 01 October 2024

ABSTRACT

Clerodendrum paniculatum is a medicinal plant commonly used in Vietnam and many other places worldwide due to its antibacterial, anti-inflammatory, and antioxidant properties. In this study, we investigated the biochemical composition, total flavonoid and polyphenol contents, and antibacterial activity of the leaves and flowers of *C. paniculatum* found in Thua Thien Hue, Vietnam. The results indicated that some phytochemicals in the powdered leaf and flower of *C. paniculatum* are safe and suitable for medicinal use, while the flavonoid and polyphenol compounds in the plant materials were also quantified. The leaves and flowers contain antioxidant compounds, reducing sugar, vitamin C, and catalase enzymes. Methanol leaf and flower extracts of *C. paniculatum* contain significant amounts of total flavonoids and polyphenols, with the flowers having higher levels than the leaves. The flavonoid and polyphenol contents in the flower extract were 17.22 mg catechin/g extract and 373.33 mg GAE/g extract, compared to 6.14 mg catechin/g extract and 266 mg GAE/g extract in the leaf extract. Both leaf and flower extracts exhibited significant antibacterial activity against the test bacteria *E. coli* and *B. subtilis*, with stronger effects against *E. coli*. The flower extract showed better antibacterial effects than the leaf extract, with inhibition zone diameters at a concentration of 750 mg/ml being 15.33 mm for *E. coli* and 15.00 mm for *B. subtilis*. This research provides valuable scientific information for the use of this medicinal plant in Thua Thien Hue.

Keywords: Antibacterial activity, Phytochemicals, *C. paniculatum*, Flavonoids, Polyphenols

Introduction

Clerodendrum paniculatum L. (*C. paniculatum*) is a species of plant in the genus *Clerodendrum*.[1] It is also commonly known by other names such as Red Glorybower, Pagoda flower, and Red Bleeding Heart. *C. paniculatum* L. is found in many countries around the world, including India, Laos, Cambodia, Thailand, Indonesia, and Vietnam.[2] In Vietnam, this medicinal plant is mainly distributed in areas such as Quang Tri, Thua Thien-Hue, Quang Nam-Da Nang, Kontum, Dak Lak, and An Giang. The plant grows scattered along mountain slopes and in bushes. It is a valuable herb in folk medicine, though few people are aware of its wonderful benefits. Many species in the genus *Clerodendrum* have been used extensively in traditional medicine for centuries to treat various diseases and metabolic disorders in humans.[3] This plant is reported to be cultivated both as an ornamental and as a medicinal herb in several parts of the world.[4] In India, China, and Japan, this plant is used to treat conditions such as rheumatism, hepatitis, cirrhosis, neuralgia, and skin diseases like scabies, boils, itching, sores,[5] and typhoid fever.[6] Traditional Thai medicine uses it as an antipyretic, anti-inflammatory agent, and ingredient in herbal baths.[6]

*Corresponding author. E mail: ntqtrang@hueuni.edu.vn nguyenthiquynhtrang@dhsphue.edu.vn
Tel: +84982021127

Citation: Trang NTQ, Thao HX, Anh BTT. Biochemical Composition, Polyphenol and Flavonoid Content, Antibacterial Activity of Leaf and Flower Extracts of *Clerodendrum paniculatum* Distributed In Thua Thien Hue Province, Viet Nam. Trop J Nat Prod Res. 2024; 8(9): 8275-8281 https://doi.org/10.26538/tjnpr/v8i9.4

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

In Indonesia and Iceland, this plant is also used to treat eye pain, jaundice, body aches, snake bites, and wounds.[7] Additionally, *C. paniculatum* is used to treat conditions such as leukorrhea, vaginal discharge, irregular menstruation, uterine inflammation, and several other women's diseases.[4]

Currently, numerous studies worldwide have demonstrated that various parts of the plant, including roots, leaves, and flowers contain numerous bioactive compounds. Yasir *et al*. analyzed the phytochemical composition of the flowers, leaves, and stems of *C. paniculatum* using different extracts (methanol, ethanol, and hexane). The results revealed that the extracts contained secondary metabolites such as terpenoids, flavonoids, alkaloids, and tannins.[6] Similarly, Hebbar *et al*. demonstrated that flower and leaf extracts of *C. paniculatum* in three solvents - distilled water, hexane, and ethanol - also contained saponins, alkaloids, and terpenoids.[4] Furthermore, several other studies have underscored the plant's potential biological activities,[2] such as antibacterial,[8] anti-inflammatories,[9] antioxidant,[10] cancer treatment,[11] insecticidal, lipid-lowering,[12] anthelmintic, anti-mutagenic, cytotoxic,[13] and anti-ageing properties.[14] These findings have continued to foster substantial advancements in the in-depth study of this plant.

In Vietnam, although *C. paniculatum* has been widely used in the community, mainly based on folk experience, research on this plant remains limited. There has been no report on the chemical composition and biological activity of *C. paniculatum* distributed in Thua Thien Hue province, Vietnam. Therefore, this study aims to investigate the chemical compositions, polyphenol and flavonoid content, as well as the antibacterial activity of the leaves and flowers of *C. paniculatum* from Thua Thien Hue.

Materials and Methods

Plant material

C. paniculatum leaf and flower were collected from Thua Thien-Hue province in September 2023 (Latitude: 16°27'22.63" N; Longitude: 107°32'3.68" E). The sample was identified by Dr. Hoang Xuan Thao (Biology Department, Hue University of Education, Hue University, Vietnam, 530000). The plant samples are stored in a biological laboratory archive of the University of Education, Hue University, Vietnam, 530000 (Voucher no.: Cle.pa-9-2023).

Bacteria strains

Two strains of bacteria were tested, including *Escherichia coli (E. coli)* and *Bacillus subtilis (B. subtilis)*. These bacteria were obtained from the Biology lab at University of Education, Hue University, Vietnam, 530000.

Preparation of sample

Disease-free leaves and flowers of the same age from the *C. paniculatum* plant in Thua Thien Hue province were selected. These samples were then washed, dried to a constant mass, ground into a powder, and stored in sealed containers with desiccant for future use.

Preparation of the extract

C. paniculatum dried leaf and flower powder (100 g) were soaked in 500 mL of 70% methanol (Merck) for 24 hours. The extract was then filtered using the Whatman No. 41 filter paper. This process was repeated three times to thoroughly extract the active compounds from each part of the *C. paniculatum*. Finally, a rotary evaporator (Hei-VAP Value, Heidolph - Germany) was used to concentrate the filtrates and obtain the crude extracts.[15]

Moisture content Determination

The moisture content of the dried plant samples (flowers and leaves) was determined using a previously described method.[16] Briefly, 5 g of the plant materials were weighed into previously cleaned and dried dishes and heated at 100 - 105°C in a drying oven (MOV- 112, Japan), until constant weight. The dishes were removed into a desiccator to cool. The dishes were covered with a lid and weighed. The process was repeated until the sample weights were constant or until the weight difference between the initial weight before drying and the final weight after drying did not exceed 0.005 mg. The moisture content (X%) of the plant materials was calculated according to formula 1:

$$X\% = \frac{p-a}{p} \times 100\% \quad (1)$$

Where: p - The weight of the sample before drying (g); a - The weight of the sample after drying (g). The result is rounded up to 0.01. The difference between the results of two simultaneous determinations should not exceed 0.5%.

Determination of total ash content

The total ash content of the samples was determined using the ashing method at a temperature of 550 - 600°C.[17] Appropriate numbers of porcelain dishes with pre-labelled markings were selected for the experiment. The dishes were washed with distilled water, dried in an oven at 100°C, cooled to room temperature, and weighed to a constant mass (m_1). The dried leaf and flower powder of the *C. paniculatum* (m_2) were accurately weighed, and placed into the prepared porcelain dishes for ashing. The samples were initially carbonised on an electric stove and then placed into a muffle furnace, where the ashing process was carried out at 750-800°C for 5 hours until white ash was obtained (organic substances were burned off, leaving only inorganic substances). The samples were removed into a desiccator and allowed to cool. The sample dishes were weighed to obtain a constant mass (m_3). The ash content is the remaining substance after ashing. The total ash content was calculated according to formula 2:

$$\% \text{ Total ash} = \frac{m3-m1}{m2} \times 100\% \quad (2)$$

Where: m_1 - Weight of the porcelain dish (g); m_2 - Initial weight of the sample used for ashing (g); m_3 - Weight of the porcelain dish and sample after ashing (g).

Determination of total protein content

The protein content was determined using the Bradford method[18] by measuring the optical density (OD) of the protein extract on a spectrophotometer (G10S UV-Vis BIO, USA) at a wavelength of 595 nm, and calculating the protein content according to a bovine serum albumin (BSA-Promega) standard curve at a concentration of 0-300 mg/mL. The standard curve equation was: $y = 0.0042\ x - 0.002$, $R^2 = 0.9905$. Where; y - OD value; x - Protein content in the sample measured. Protein content was calculated by the formula 3:

$$A = \frac{x \times V \times a}{1000} \quad (3)$$

Where: A - Protein content in 100 g of sample (g/100g); x - Protein content according to the standard curve (mg/mL); V - sample solution volume (mL); a - solution dilution factor.

Determination of reducing sugar content

The reducing sugar content was determined by the Bertrand method.[19] Approximately 2 g of the sample was weighed into a porcelain mortar, and ground with 60 mL of hot distilled water (approximately 70-80°C). The mixture was heated in a water bath for 20 minutes. It was then allowed to cool to room temperature. The impurities were removed with $Pb(C_2H_2O_2)_2.3H_2O$, and a little HCl solution was added, and heated in a water bath for about 3 minutes. This solution was used for quantitative analysis. To 5 mL of the extract solution, 10 mL of Fehling A and B reagents were added, and boiled for about 3 minutes, until a red precipitate (Cu_2O) appeared in the flask. The flask was allowed to cool. Then the Cu_2O precipitate was dissolved with 5 mL of $Fe_2(SO_4)_3$ in H_2SO_4 and titrated with 0.1 N $KMnO_4$ until a light pink colour persisted for 20-30 seconds. From the amount of 0.1 N $KMnO_4$ used for titration, the amount of sugar in the sample was computed. A blank experiment was conducted by replacing the sugar solution with distilled water.

Determination of lipid content

The lipid content was determined by the Soxhlet method.[20] 2 g of dried, ground sample, was accurately weighed and then wrapped with filter paper (which has been dried to a constant weight) and the lipid content was determined. The sample was extracted with ethanol ether after which the solvent was allowed to evaporate completely and the sample was dried to a constant weight. The lipid content in the sample was calculated according to formula 4:

$$X = \frac{(a-b)}{c} \times 100 \quad (4)$$

Where: X - lipid content (%). a - weight of the sample package before extraction (g). b - weight of the sample package after extraction (g). c - weight of the extracted sample (g).

Determination of Catalase enzyme Activity

The activity of the Catalase enzyme was determined by titration with $KMnO_4$.[21] 2 g of ground sample was weighed into a porcelain mortar with 0.3 g $CaCO_3$, and 20 mL of distilled water was added and carefully grounded to form a homogeneous solution. The sample solution was transferred into a 50 mL volumetric flask and was made up to the mark. The mixture was thoroughly shaken and was filtered after 30 minutes. 20 mL of the filtrate was each added to two 100 mL Erlenmeyer flasks. The flasks were boiled for 2-3 minutes to inactivate the enzyme and then cooled. 20 mL of distilled water and 3 mL of 1% H_2O_2 solution were added to each flask and the flasks were left to stand at room temperature for 30 minutes. Then 4 mL of 10% H_2SO_4 solution was added to the flasks and titrated with 0.1N $KMnO_4$ solution until a stable light pink colour appeared for 1 minute. The formula for calculating catalase activity is as follows: 1 mL of 0.1 N $KMnO_4$ corresponds to 1.7 mg H_2O_2. The enzyme activity was calculated according to formula 5:

$$X = \frac{(a-b) \times f \times 1{,}7}{w} \quad (5)$$

Where: X- catalase activity in mg H_2O_2 per 1 g of decomposed sample; a volume of 0.1N $KMnO_4$ solution used for titration in the experimental flask (mL); b- volume of 0.1N $KMnO_4$ solution used for titration in the experimental flask (mL); f- correction factor for 0.1N $KMnO_4$; amount

of H_2O_2 in mg, equivalent to 1mL of $KMnO_4$ 0.1N; w- weight of the sample material (g)

Determination of vitamin C content

The vitamin C content was determined by titration using iodine solution.[22] 2 g of the sample was weighed and ground thoroughly with 5 mL of 5% HCl solution to obtain a homogeneous solution. The entire solution was transferred into a volumetric flask rinsing with hot distilled. The solution in the volumetric flask was made up to 100 mL mark with distilled water. The flask content was vortexed and filtered through a filter funnel. 15 mL of the filtrate was measured into a 100 mL Erlenmeyer flask and titrated with 0.01N iodine solution (5-10 drops of 1% starch solution) until a stable blue colour was observed. The vitamin C content was calculated according to formula 6:

$$X = \frac{Vi \times V \times 0{,}00088 \times 100}{Vf \times w} \quad (6)$$

Where: X% - Vitamin C content in the sample; V_i - Volume of 0.01N I_2 solution used for titration (mL); Vf - Volume of the sample solution analyzed (mL); V - Diluted sample solution, w - Weight of the sample analyzed (g); 0.00088 - Grams of vitamin C corresponding to 1 mL of 0.01N iodine solution.

Determination of total flavonoid

The total flavonoid content was determined by a colourimetric assay using $AlCl_3$ in an alkaline medium as described by Ribarova *et al*. with some modifications.[23] The Leaf and flower extracts were diluted to a concentration of 1 mg/mL with methanol, and catechin (Sigma) standard solutions at concentrations of 25, 50, 100, 200 and 300 µg/mL. 1 mL of the samples (test or standard) were pipetted into a 10 mL volumetric flask. Distilled water was used as the blank. 4 mL of distilled water was added to the sample, followed by 0.3 mL of 10% $NaNO_2$. The tubes were properly mixed and allowed to stand for 5 minutes. Then, 0.3 mL of 10% $AlCl_3$ was added, properly mixed and allowed to stand for another 6 minutes. Then 2 mL of 1M NaOH and 2.4 mL of distilled water were added to the solution, mixed and left to stand for another 10 minutes. The absorbance (Abs) of the sample solution was measured at 510 nm at room temperature by a UV-Vis spectrophotometer (G10S UV-Vis BIO, USA). The experiment was carried out in triplicates. The Abs values were recorded and a calibration curve was plotted to determine the flavonoid content in the extract samples. The flavonoid content of the extract was determined based on the catechin calibration curve equation: y = ax + b. Total flavonoid content was calculated according to formula 7:

$$Total\ flavonoid\ content = \frac{x \times V1 \times n}{m \times V2\ x1000}\ (mg\ catechin/g) \quad (7)$$

Where: V_1- initial sample volume; V_2 - Reaction sample volume; m - Weight of the extracted sample diluted in V_1 (g); n - Dilution factor (if applicable); x - Flavonoid content determined from the catechin calibration curve equation (µg/mL); 1000 - conversion factor from mg to µg.

Determination of total polyphenol

The polyphenol content was determined using the Folin-Ciocalteu method, as described by Feduraev *et al.* with some modifications.[24]

The extract samples were diluted with methanol to a concentration of 0.5 mg/mL, and standard solutions of gallic acid (Sigma) at concentrations of 25, 50, 100, 150, and 200 µg/mL were prepared. 0.1 mL of the sample to be analyzed (either the gallic acid standard or the test sample) was pipetted into a 10 mL volumetric flask. 0.3 mL of 0.2 M Folin-Ciocalteu reagent was added to the sample solutions and mixed. The mixtures were incubated in the dark for 10 minutes. Then, 6 mL of 6.75% Na_2CO_3 solution was added properly mixed and again incubated in the dark for 30 minutes. The absorbance (Abs) of the solution at 765 nm at room temperature was measured with a UV-Vis spectrophotometer (G10S UV-Vis BIO, USA). The experiment was carried out in triplicates. The Abs values were recorded to plot a calibration curve to determine the polyphenol content in the extract samples.

The polyphenol content of the extract is calculated based on the Gallic acid calibration curve equation: y = ax + b. Total polyphenol content was calculated according to formula 8:

$$C = \frac{c \times V}{m} \quad (8)$$

Where: C - Total polyphenol content was expressed as Gallic Acids Equivalent in mg/g (mgGAE/g extract); c - Value x from the gallic acid calibration curve (µg/mL); V - Volume of the extract solution (mL); m - Weight of the extract in volume V (g).

Determination of Antibacterial activity

The antibacterial activity of the extract was evaluated using the disk diffusion method as described by Bauer *et al*.[25] Each experiment was performed three times. The indicator bacterial solution with a density of 10^6 cfu/mL was spread on the LBA plate. After spreading, the plates were inverted for 15 minutes to dry. Then a sterilised cork borer was used to punch 6 holes in each agar plate and a sterilised needle was used to remove the agar plugs. The leaf and flower extracts of *C. paniculatum* were diluted with 70% methanol at the following concentrations: 750, 500, 250, and 100 mg/mL each. 100 µL of the extract under investigation was added into each hole on the agar plate previously spread with bacteria. Ampicillin was used as the positive control standard antibiotic agent, while Liquid LB was used as the negative control. The plates were incubated at 37°C for 24 hours and the diameter of the inhibition zones was measured.[26] To be considered sensitive, the bacteria must have a clear zone of inhibition greater than 12 mm. The sensitivity of the tested bacterial strains to leaf and flower extracts of *C. paniculatum* was compared with that of ampicillin and Liquid LB.[15]

Statistical analysis

All experiments were performed in triplicate. The experimental values are expressed as the mean ± standard error. The means were compared using Duncan's test and ANOVA. The analytical values were considered statistically significant at $p < 0.05$, based on Excel 2010 and IBM SPSS Statistics 20.

Results and Discussion

The results on various phytochemicals in the leaves and flowers of *C. paniculatum* are presented in Table 1. The moisture content of the dried powdered leaf and flower of *C. paniculatum* ranged from 9.64% to 10.70%, respectively. This moisture level is conducive for the long-term preservation of the raw materials without mould or sensory changes, making it favourable for the extraction process.[17]

The total ash content is an important criterion in evaluating the quality of medicinal herbs because it reflects the content of inorganic minerals present in the herbs. Checking the ash content helps ensure the quality and safety of medicinal herbs before they are used in medicine. According to the Vietnamese Pharmacopoeia, the total ash content of medicinal herbs typically ranges from 5% to 18%, depending on the type of herb.[17] The total ash content (total mineral salts) in the leaves was 6.25%, and 5.13% in the flowers (Table 1). Compared to the standards of the Vietnamese Pharmacopoeia, the ash content of powder samples of the leaves and flowers of *C. paniculatum* is within the permissible limit.

The lipid content in the leaves and flowers is quite low, at 8.47% and 9.27%, respectively. The samples have low lipid content which is very favorable for extracting bioactive components. If the lipid content is high, it will increase the viscosity of the extract, making the filtration and purification process difficult. Additionally, extracts containing lipids are unstable and tend to have an unusual odour.[17] Therefore, it is possible to extract bioactive substances from the leaves and flowers of *C. paniculatum*. The leaves and flowers of *C. paniculatum* provide relatively low amounts of protein. The protein content of the flowers is 0.35 g/100 g, which is significantly higher than in the leaves, at 0.09 g/100 g (Table 1).

The antioxidant capacity of plants is demonstrated through the activity of antioxidants such as the enzyme catalase, glutathione, reducing

sugars, superoxide dismutase, vitamin C, and so on. These are important components that help the body neutralise free radicals generated during metabolism and protect biological systems to some extent.[27] The results of the study on some of these indicators in the leaves and flowers of *C. paniculatum* are presented in Table 2. Catalase is not only an important biological catalyst but also plays a special role in many aspects of health and biological processes. In addition to inhibiting the degradation of nerve cells and apoptosis, catalase is involved in regulating inflammatory and ageing processes, as well as helping control the growth of a range of tumours. The presence of catalase is also crucial for supporting intracellular drug delivery and cholesterol quantification.[15] The results from Table 2 show that the catalase enzyme activity varies among different parts of the plant, ranging from 0.014 to 0.032 U/mg protein. The flowers exhibit higher enzyme activity compared to the leaves, approximately 128% higher.

Reducing sugars plays an essential role in plant life activities, affecting many important aspects such as cell structure, metabolism, synthesis of bioactive compounds, and energy storage.[28] The results in Table 2 indicate that the reducing sugar content in the leaves and flowers of *C. paniculatum* is quite high, with no significant difference between the two studied parts, ranging from 11.60 g/100 g (in flowers) to 13.00 g/100 g (in leaves) (Table 2). Our findings are higher compared to the study by Le Nguyen Thanh Dong *et al*. on *C. japonicum*, where the reducing sugar content in the leaves was 9.07 g/100 g.[29] This indicates a good redox potential of the leaves and flowers of *C. paniculatum* distributed in Thua Thien Hue province, similar to the conclusions of Hebbar *et al*. on the same species in India.[4]

Vitamin C (ascorbic acid) has many functions that contribute to the overall health of the human body. Ascorbic acid can reduce the risk of hypertension and cancer, increase hydroxyproline excretion in urine, boost immunity, regenerate tissues, and aid in the metabolism of drugs.[15] All studied parts of *C. paniculatum* contain a small amount of vitamin C. In the leaves, the ascorbic acid content is 0.772%, while in the flowers, it is 0.860% (Table 2).

Table 1: Proximate parameters in the leaves and flower samples of *C. paniculatum*

Analysis Type	Unit	Result	
		Leaf	Flower
The moisture content of the dry powder	%	$10.70^{a} \pm 0.15$	$9.64^{a} \pm 0.11$
Total Ash content		$5.25^{c} \pm 0.04$	$3.24^{d} \pm 0.01$
Lipid content		$9.27^{a} \pm 0.79$	$8.47^{a} \pm 0.21$
Protein content	g/100g	$0.09^{b} \pm 0.01$	$0.35^{a} \pm 0.02$

Note. Different letters on the same column indicate a statistically significant difference in the sample mean with $p < 0.05$ (Duncan's test). Values were obtained in triplicate and data were expressed as Mean ± SE.

Table 2: The antioxidant content in the leaves and flowers of *C. paniculatum*

Analysis Type	Unit	Result	
		Leaf	Flower
Catalase enzyme	U/mg protein	$0.014^{b} \pm 0.009$	$0.032^{a} \pm 0.018$
Reducing sugar content	g/100g	$13.00^{a} \pm 0.52$	$11.60^{ab} \pm 0.20$
Vitamin C	%	$0.860^{a} \pm 0.059$	$0.772^{a} \pm 0.019$

Note: Different letters on the same row indicate a statistically significant difference in the sample mean with $p < 0.05$ (Duncan's test). Values were obtained in triplicate and data were expressed as mean ± SE.

The total flavonoid and polyphenol content in the leaf and flower extract samples was determined based on the standard curves of catechin and gallic acid, respectively. From the initial absorbance and concentration of the standards, linear curves were plotted to show the correlation between the standard concentrations and their absorbance in the solution. Using the standard curve equations $y = 0.0012x + 0.0143$, $R^2 = 0.9737$ (Figure 1) and $y = 0.0002x + 0.0046$, $R^2 = 0.991$ (Figure 2), the total flavonoid and polyphenol content in the leaf and flower extracts of *C. paniculatum* were determined. The study results are shown in Table 3. Flavonoids are a large group of natural compounds commonly found in plants, especially in higher plant species.[7] They are present in most parts of the plant, including roots, stems, leaves, fruits, and flowers, with a particular abundance in flowers. Flavonoids have potent biological activity, including antioxidant capacity, enzyme inhibition, antibacterial, anti-inflammatory, anticancer, and antioxidant properties.[28]

The total flavonoid content of the extract from the leaves and flowers of *C. paniculatum* is 6.14 and 17.22 (mg catechin/g extract), respectively. The flowers have a significantly higher value than the leaves, similar to the results of Yasir *et al*.[6] on the same subject. Polyphenols have been studied and proven to have many positive effects on human health. Some important effects include antioxidant, antibacterial, anticancer and anti-inflammatory properties, as well as the ability to protect cells from damage, protect cardiovascular health, and support the digestive process.[7] The polyphenol content of extracts from different parts of the plant varies. Specifically, the polyphenol content in the leaves is 266.00 mg GAE/g extract, while in the flowers it is 373.33 mg GAE/g extract, which is 40.35% higher than in the leaves.

Hebbar *et al*. also reported the total phenolic content in leaf and flower extracts of *C. paniculatum* in aqueous, ethanol, and hexane extracts. The results showed that the total phenolic content in the different solvents extract of the flower was 104.7 µg GAE/g, 100.6 µg GAE/g, and 142.6 µg GAE/g, respectively. The aqueous extract of the leaf contained 110.0 µg GAE/g of total phenolics.[4] Thus, the *C. paniculatum* plant distributed in Thua Thien Hue, Vietnam, has a much higher total polyphenol content. This result is also higher than that of some other herbal plants such as *Hibiscus sabdariffa*,[30] *Elaeagnus angustifolia* L.[31] These results showed that the leaf and flower extracts of *C. paniculatum* contained significant amounts of bioactive compounds including Flavonoids and Polyphenols. The flower extract had significantly higher values than the leaves. This shows the potential for bioactivities such as antioxidants and anti-inflammatory properties in this plant and promotes further research in the future.

The antibacterial ability of extracts from the leaves and flowers of the *C. paniculatum* plant was evaluated based on the diameter of the inhibition zones at different extract concentrations (100, 250, 500, 750 mg/mL). The results are presented in Table 4. Table 4 shows that extracts from both the leaves and flowers have antibacterial properties against *E. coli* and *B. subtilis*. The diameter of the inhibition zones

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

increased progressively from 100 mg/mL to 750 mg/mL. Specifically, for the flowers, the inhibition zone diameters for *E. coli* and *B. subtilis* ranged from 8.67 to 15.33 mm and 3.67 to 15.00 mm, respectively. In contrast, for the leaves, these results were 5.67 to 14.67 mm (for *E. coli)* and 5.00 to 14.33 mm (for *B. subtilis*), corresponding to concentrations from 100 to 750 mg/mL. Notably, at a concentration of 750 mg/mL, the inhibition zones for both leaf and flower extracts were nearly comparable to those of ampicillin. This indicates that extracts from the leaves and flowers of the *C. paniculatum* plant have the potential to be effective antibacterial agents against these bacteria. Moreover, the leaf and flower extracts exhibited stronger antibacterial activity against *E. coli* than *B. subtilis*. The antibacterial activity of the flower extracts was significantly higher than that of the leaf extracts for both tested bacteria. The higher content of antioxidants, polyphenols, and total flavonoids in the flowers compared to the leaves may explain the antibacterial activity observed in these two parts. Shruthi *et al*.[9] reported that *C. paniculatum* extract exhibited higher antibacterial activity against *E. coli*, with an inhibition zone diameter of 15 mm. Similarly, Sumayya and Gopinathan reported that *C. infortunatum* root extract was effective against *Pseudomonas aeruginosa* and *E. coli* (16 mm) and *Salmonella typhi* (13 mm).[28] These findings confirm the strong antibacterial potential of species within the genus *Clerodendrum*.

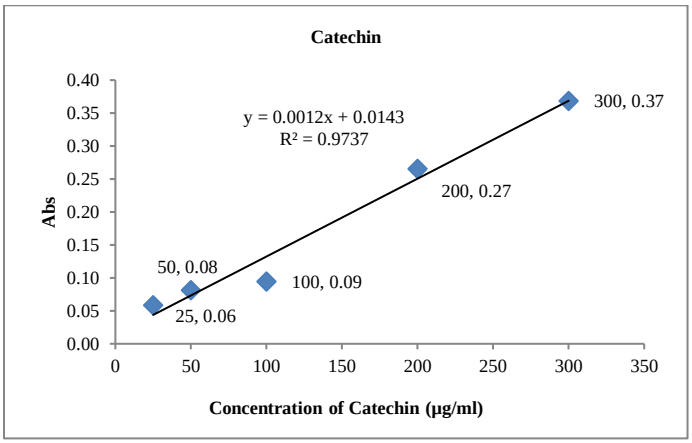

Figure 1: Catechin standard calibration curve

Table 3: Total polyphenol and total flavonoid contents in the leaf and flower extracts of *C. paniculatum*

Parts of C. *paniculatum*	Total flavonoid content (mg Catechin/g of extract)	Total polyphenol content (mg GAE/g of extract)
Leaf	$6.14^{b} \pm 0.73$	$266.00^{b} \pm 1.53$
Flower	$17.22^{a} \pm 0.03$	$373.33^{a} \pm 0.33$

Note: Different letters on the same column indicate a statistically significant difference in the sample mean with $p < 0.05$ (Duncan's test). Values were obtained in triplicate and data were expressed as Mean ± SE.

Table 4: Diameter of Inhibition Zones Induced by Extract of the leaf and flower extracts of *C. paniculatum* and ampicillin antibiotic (mm)

Tested Strains	Parts of *C. paniculatum*	Concentrations of extract (mg/ml)				Ampicillin (µg) (Positive control)	LB Liquid (Negative control)
		100	250	500	750	1000	
E. coli	Leaf	$5.67^{b} \pm 0.33$	$10.33^{b} \pm 0.33$	$12.33^{b} \pm 0.33$	$14.67^{b} \pm 0.33$	$15.67^{a} \pm 0,33$	-
	Flower	$8.67^{a} \pm 0.33$	$11.67^{a} \pm 0.67$	$13.67^{a} \pm 0.33$	$15.33^{a} \pm 0.33$	$16.00^{a} \pm 0.58$	-
		100	250	500	750	100	-
B. subtilis	Leaf	$5.00^{a} \pm 0.58$	$9.33^{a} \pm 0.33$	$12.67^{a} \pm 0.67$	$14.33^{a} \pm 0.67$	$21.00^{a} \pm 0.58$	-
	Flower	$3.67^{b} \pm 0.33$	$8.33^{a} \pm 0.33$	$10.33^{b} \pm 0.33$	$15.00^{a} \pm 0.58$	$21.67^{a} \pm 0.33$	-

Note: Different letters on the same column indicate a statistically significant difference in the sample mean with $p < 0.05$ (Duncan's test); "-": no zone of inhibition. Values were obtained in triplicate and data were expressed as Mean ± SE.

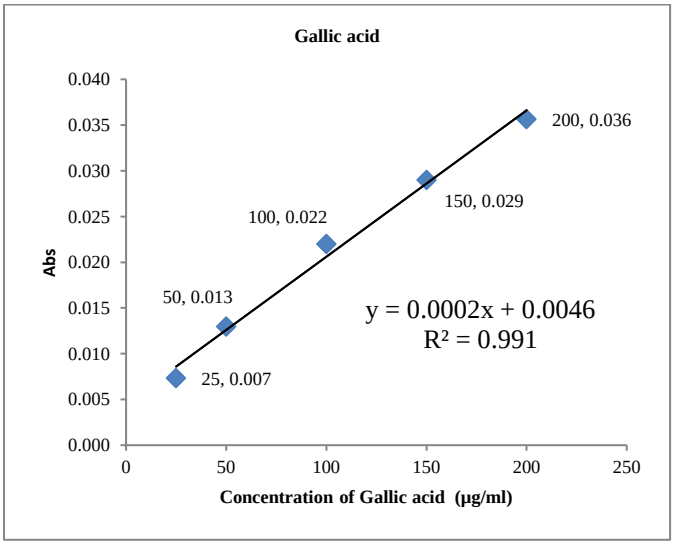

Figure 2: Gallic acid standard calibration curve

Conclusion

Proximate analysis of the powdered samples of the leaf and flower of *C. paniculatum* shows that the samples possess good qualitative and quantitative parameters which favour longer shelf storage and extraction of bioactive phytochemicals. The extracts of both plant parts showed potent concentration-dependent antibacterial properties against *E. coli* and *B. subtilis*, which may have been due to the presence of copious amounts of flavonoids and polyphenols in the plant extracts. The extracts also exhibited potent antioxidant activities validating their ethnomedicinal uses in the treatment of various diseases (inflammations, cancer, itching, skin infections etc.). This study's findings demonstrate the medicinal potential of *C. paniculatum* distributed in Thua Thien Hue and provide a scientific basis for further research to exploit and apply this medicinal plant in Thua Thien Hue province. The results will help reaffirm the plant's potential, enhance local medicinal resources, and provide foundational data for future research endeavours.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

This research is funded by the University of Education, Hue University under grant number "T.24.TN.106.04"

References

1. Hafiz I, Rosidah SJ. Antioxidant and anti-inflammatory activity of pagoda leaves (*Clerodendrum paniculatum* L.) ethanolic extract in white male rats (*Rattus novergicus*). Int. J. Pharmtech Res**.** 2016; 9(5):165 – 170.
2. Kekuda TP, Sudharshan SJ. Ethnobotanical uses, phytochemistry and biological activities of *Clerodendrum paniculatum* L. (Lamiaceae): A comprehensive review. J. Drug Deliv. Ther. 2018; 8(5-s):28 – 34. DOI: ttp://dx.doi.org/10.22270/jddt.v8i5-s.1930.
3. Joseph J, Joseph L, George M, Bindhu AR. Phytochemical Screening And Antioxidant Activity Of Various Extracts Of *Clerodendru M Paniculatum* Linn. World J. Pharm. Res. 2018; 7(13):555 – 568.
4. Hebbar DR, Devika M, Rashmi G, Nalini M. Phytochemicals and antioxidant activity of C*lerodendrum paniculatum* (L.) Leaf and flower extracts. Asian J. Pharm. Clin. Res. 2022; 15(1):31 – 34.
5. Praveen M, Radha K, Kumar RH, Padmaja V, Mathew A, Kumar PA. Preliminary phytochemical, antimicrobial and toxicity studies on *Clerodendrum paniculatum* Linn. leaves. Hygeia. J.D.Med. 2012; 4(1):41 – 50.
6. Yasir B, Astuti AD, Raihan M, Natzir R, Subehan, Rohman A, Alam G. Optimization of Pagoda (*Clerodendrum paniculatum* L.) Extraction Based By Analytical Factorial Design Approach, Its Phytochemical Compound, and Cytotoxicity Activity. Egypt. J. Chem. 2022; (65)9: 421 – 430.
7. Yamin Y, Sabarudin S, Zubaydah WO, Sahumena MH, Arba M, Elnawati E, Andriani R, Suryani S. Determination of Antiradical Activity, Total Phenolic and Flavonoid Contents of Kamena-mena (*Clerodendrum paniculatum*. L) Leaves. Trop J Nat Prod Res. 2021; 5(2):287 – 293. doi. org/10.26538/tjnpr/v5i2. 12.
8. Katisart T, Butkhup L, Sumalee A, Taepongsorat L, Konsue A. Acute Toxicity of Aqueous Extract of a Diabetic Folklore Recipe Thai Traditional Medicine in Rats. Trop. J. Nat. Prod. Re. 2023; 7(6):3168 – 3176. Doi: 10.26538/tjnpr/v7i6.16.
9. Shruthi G, Joyappa MP, Chandrashekhar S, Kollur SP, Prasad MNN, Prasad A, Shivamallu C. Bactericidal property of *Clerodendrum paniculatum* and *Saraca asoka* against multidrug-resistant bacteria, restoring the faith in herbal medicine. Int J Pharm Sci Res. 2017; 8(9):3863 – 3871.
10. Siddik ASNU, Alam S, Borgohain R, Chutia P. Antioxidant properties of *Clerodendrum* species found in Northeast India: A review. J. Pharmacogn. Phytochem. 2021; 10(4):390 – 394.
11. Phontree K, Sichaem J, Siripong P, Tip-pyang S. Chemical constituents of the roots of *Clerodendrum paniculatum*. Chem Nat Compd. 2014; 50:950 – 951.
12. Selvin S, Rakhi A. Evaluation of hypolipidemic effect of various extracts of whole plant of *Clerodendrum paniculatum* in rat fed with high-fat diet. Eur J Biomed Pharm Sci. 2014; 1(1):87 – 96.
13. Mohan J, Narkilli SN, Prashobh, Surabhi GS, Kurup SB, Rupitha NS. Review on Pharmacological Activities of *Clerodendrum Paniculatum* Linn. Int. J. Pharm. Res. Appl. 2021; 6(6):693 – 700.
14. Joseph J, Bindhu AR, Aleykutty NA. Antimicrobial activity of *Clerodendrum paniculatum* Linn leaves. Int. J. Res. Ayurveda Pharm. 2011; 2(3):1003 – 1004.
15. Trang NTQ, Ngoc NQT, Long DT, Thanh P. Antioxidant and Antibacterial activity of Lotus tea in Thua Thien Hue province from Vietnam. Res. J. Biotech. 2023; 18(12):48 – 54.
16. Hong PTA. Biochemical engineering. 2003.
17. Long DT, Le VTM, Quang HT, Han HTN, Hue TT, Trang NTQ. Bioactive Components of *Helicteres hirsuta* Collected in Thua Thien Hue. Indian J. Sci. Technol. 2023; 16(44):4114 – 4122.
18. Thanh LD, Thu TNT, Thi TC, Tien LN. Identification of Nutritional Components of Different Varieties of Peanut Oil by Chromatographic Analysis. Trop J Nat Prod Res. 2023; 7(10):4894 – 4901. http://www.doi.org/10.26538/tjnpr/v7i10.26.
19. Kumar CC, Mythily R, Venkatachalapathy R, Chandraju S. Bio-mimic conversion of Maida (Polysaccharides) to reducing sugars by acid hydrolysis and its estimation using standard methods. Int. Food Res. J. 2014; 12(2):523 – 526.
20. López-Bascón M, De Castro ML, Soxhlet extraction. In: Liquid-phase extraction. Elsevier. 2020;327 – 354.
21. Chen Q, Cheng M, Wang Y, Yao M, Chen Y, Gao Y, Ding W. A simple method of catalase purification for the undergraduate experimental course. Mol. Med. Rep. 2014; 11:1340 – 1343.
22. Arya S, Mahajan M, Jain P., Non-spectrophotometric methods for the determination of Vitamin C. Anal. Chim. Acta. 2000; 417(1):1 – 14.
23. Ribarova F, Atanassova M, Marinova D, Ribarova F., Atanassova M. Total phenolics and flavonoids in Bulgarian fruits and vegetables. JU Chem. Metal. 2005; 40(3):255 – 260.
24. Feduraev P, Chupakhina G, Maslennikov P, Tacenko N, Skrypnik L. Variation in phenolic compounds content and antioxidant activity of different plant organs from Rumex crispus L. and Rumex obtusifolius L. at different growth stages. Antioxidants; 2019; 8(7): 237.
25. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 1966; 45(4):493 – 496.
26. Hai NTT, Bui TQ, Quy PT, Phu NV, Thuy NTT, Nguyen TVA, Triet NT, Pham DT, Tran VD, Nhung NTA. Assessment of Antifungal Potentials of Ethanol Extract of *Cleistocalyx operculatus* Roxb Against *Cercospora nicotianae* for Pesticidal Applicability: *In Vitro* and *In Silico* Screening. Trop J Nat Prod Res. 2024; 8(8):7947 – 7955. https://doi.org/10.26538/tjnpr/v8i8.3.
27. Kopilakkal R, Chanda K, Balamurali M.M. Hepatoprotective and Antioxidant Capacity of *Clerodendrum paniculatum* Flower Extracts against Carbon Tetrachloride-Induced Hepatotoxicity in Rats. ACS Omega. 2021; 6(40):26489 – 26498. Doi: 10.1021/acsomega.1c03722
28. Sumayya TK, Gopinathan M. Correlation of antioxidant, antibacterial and phytochemicals of leaves and roots extracts of *Clerodendrum infortunatum*. Int. J. Pharm. Sci. Res. 2022; 13(5):2144 – 2153.
29. Dong LNT, Anh NTV, Biet HV, Ly TTP. Optimization of the extraction of Polyphenols from Red Glorybower (*Clerodendrum Japonicum*) Leaf by Response Surface Methodology. National Biotechnology Conference. 2022;1 – 6.
30. Singh M, Thrimawithana T, Shukla R, Adhikari B. Extraction and characterization of polyphenolic compounds and potassium hydroxycitrate from *Hibiscus sabdariff*. Future Foods. 2021; 4:2 – 10.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

31. Saboonchian F, Jamei R, Hosseini Sarghein S. Phenolic and flavonoid content of *Elaeagnus angustifolia* L. (leaf and flower). Avicenna J Phytomed. 2014; 4(4):231 – 238.