



Chemical Constituents and Bioactive Properties in Some Parts of *Clerodendrum Paniculatum* from Hue City, Vietnam

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ABSTRACT

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Clerodendrum paniculatum, which is widely distributed across tropical regions, is recognized for its medicinal value in Vietnam and other countries. It has been traditionally used for its therapeutic properties, but scientific evidence regarding its chemical composition and biological activities remains limited. The purpose of this research was to profile the phytochemical constituents and biological properties of various plant parts (roots, stems, leaves, and flowers) of *Clerodendrum paniculatum* collected in Hue City, Vietnam. The chemical profiles of the plant parts were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). Antioxidant capacity was assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Cytotoxic activity against HepG2 (human hepatocarcinoma) liver cancer cells was evaluated using the Sulforhodamine B (SRB) assay. A total of 36 compounds were identified, with the highest number found in the roots (20), followed by the stems (15), flowers (10), and leaves (6). Antioxidant assays revealed that all extracts exhibited relatively weak activity, showing IC₅₀ levels in the range of 4.13–5.23 mg/mL. Among the four plant parts, the root extract had the lowest IC₅₀ value (4.13 mg/mL), followed by the flower (4.70 mg/mL) and stem extracts (4.83 mg/mL), whereas the leaf extract displayed the highest value (5.23 mg/mL). Cytotoxicity tests showed low inhibitory effects against HepG2 cells, with all extracts having IC₅₀ values exceeding 500 µg mL⁻¹. Overall, *C. paniculatum* contains several identifiable phytochemicals especially in its roots; however, it exhibits only modest antioxidant activity and minimal cytotoxic effects. These findings suggest limited biological activity under the tested conditions, and further studies are needed to clarify its potential pharmacological relevance.

Keywords: *Clerodendrum paniculatum*, phytochemistry, antioxidant activity, cytotoxicity, Gas Chromatography-Mass Spectrometry.

Introduction

The genus *Clerodendrum* belongs to the Lamiaceae family.¹ This genus exhibits significant diversity, comprising around 150 to 500 species found in tropical regions. *C. paniculatum*, *C. infortunatum*, *C. indicum*, *C. phlomidis*, *C. japonicum*, *C. trichotomum*, *C. petasites*, and *C. chinense* are well recognized in traditional medical practices for their roles in addressing a broad spectrum of human health problems.^{2,3} Previous studies have reported the diverse phytochemical composition and biological activities of *Clerodendrum* species. Over 280 chemical compounds, such as diterpenoids, flavonoids, phenylethanoid glycosides, and steroids—key substances with biological activity—have been identified in different *Clerodendrum* species.^{3,4} Research on *C. inerme* have identified a variety of chemical constituents in its fruits, leaves, flowers, and stems. The prominent compounds obtained from *C. inerme* include verbainoside, camneoside, melitoside, stigmasterol, 6-hydroxysalvinolone, and betulinic acid. Furthermore, *C. inerme* has triterpenoids, glycosides, phenols, alkaloids, and tannins.⁵

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Additionally, *C. phlomidis* has been found to contain phenolic compounds, terpenoids, and flavonoids, all of which have strong antibacterial and antioxidant qualities.⁶ Several studies have also demonstrated the pharmacological potential of *Clerodendrum* extracts, revealing anti-inflammatory, antioxidant, antimicrobial, and antihypertensive activities.³ Specifically, *C. inerme* has been investigated for its anti-inflammatory and hepatoprotective properties, while *C. phlomidis* has shown antidiabetic and hepatoprotective effects.⁷ Similarly, *C. colebrookianum* has been explored for a broad spectrum of pharmacological actions, including hypolipidemic, antihypertensive, antioxidant, anti-inflammatory, analgesic, hepatoprotective, anti-obesity, antihelminthic, central nervous system depressant, antimicrobial, antistress, and antipyretic activities, which are linked to its high phytochemical content.²

C. paniculatum, commonly known as the Pagoda Flower,⁴ is a medicinal plant distributed across various regions, including Vietnam.^{8,9} Traditionally, in Japan, China, Thailand, Indonesia, and India, it has attracted attention due to its anti-inflammatory activity and hepatoprotective effects. Also, it has long been beneficial for its antipyretic and anti-inflammatory qualities as well as for the treatment of wounds, rheumatism, neuralgia, inflammation, ulcers, and eye pain.^{4,10} Numerous bioactive compounds in the roots and leaves of *C. paniculatum* have been identified by standard phytochemical assays, chromatographic techniques, and spectrophotometric methods. These compounds comprise (24S)-ethylcholesta-5,22,25-triene-3β-ol, β-amyrin, β-sitosterol, along with sterols, coumarins, flavonoids, glycosides, phenols, phytosterols, saponins, terpenoids, tannins, phenolic acids, and alkaloids.^{11,12}

However, comprehensive research on the phytochemical profiles and functional characteristics of *C. paniculatum* collected in Hue City, Vietnam, remains limited, particularly regarding different plant parts such as the stems and flowers. Understanding its phytochemical profile

and bioactivities is essential for exploring its pharmacological potential and potential medicinal applications. Therefore, this study examines the phytochemical profiles and biofunctional properties of the roots, stems, leaves, and flowers collected from *C. paniculatum* in Hue City, aiming to provide scientific evidence that reinforces its medicinal value and guides future pharmacological investigation.

Materials and Methods

Plant samples

Four parts of *C. paniculatum* i.e. stems, leaves, flowers, roots - were gathered from the natural populations in Hue City, Vietnam (16°27'22.63" N; 107°32'3.68" E) during September 2023. The plant materials were subsequently authenticated at the Biology Department of Hue University of Education, and a voucher specimen was deposited under the code Cle.pa-9-2023. Healthy organs of comparable maturity were chosen, and were thoroughly cleaned, dried to a constant weight, finely powdered, and kept in an airtight containers with desiccant for subsequent analyses.¹³

Preparation of plant extracts

A total of 100 g of the dried powdered material of *C. paniculatum* was extracted by soaking it in 500 mL of 70% (v/v) methanol (Merck) for a 24-h period. The mixture was subsequently filtered via Whatman No. 41 filter paper to obtain the liquid phase. This maceration procedure was performed three consecutive times to maximize the recovery of bioactive constituents from each plant part including the stem, leaf, flower, and root. The resulting filtrates were pooled and evaporated under reduced pressure with a rotary evaporator (Hei-VAP Value, Heidolph, Germany) to yield the crude extracts.¹³

GC-MS method

After dissolving the crude extract sample (1 mg) in 1 mL of hexane, the solution was then clarified by centrifugation and passed through a Polytetrafluoroethylene membrane filter (PTFE). The supernatant was then subjected to GC-MS analysis using a TSQ 9000 Triple Quadrupole GC-MS/MS system (Thermo) equipped with a DB-5ms column (30 m × 0.25 mm, 0.25 µm). The analysis was performed at a flow rate of 1 mL/min with helium as the carrier gas. The oven temperature was programmed in a multi-step ramp: it started at 60°C with a 5-minute isothermal phase, then the temperature was raised at 10°C per minute up to 310°C, where it was maintained for a further 30 minutes.¹ The identified compounds were characterized by comparing their mass spectra with reference spectra available in the Wiley and NIST library. These databases were used to assign compound names and obtain the corresponding peak areas.⁹

Determination of Antioxidant Properties

The antioxidant potential of the extracts were based on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method.¹⁴ Solutions of the extracts and ascorbic acid were diluted in 70% methanol to prepare solutions with concentrations ranging from 1:50 to 1:1000 and 1 mg/mL. For each concentration, 1 mL of the sample was combined with 1 mL of 0.2 mM DPPH reagent, thoroughly mixed, and maintained in the dark condition for 30 minutes. The absorbance (Abs) was subsequently recorded at 517 nm by a UV-Vis spectrophotometer (G10S UV-Vis BIO, USA). Ascorbic acid served as the positive control under identical experimental conditions. The DPPH radical-scavenging percentage was measured following the formula below (equation 1):

$$SC\% = \frac{ODc - ODm}{ODc} \times 100 \quad \text{Equation 1}$$

Where: ODm represents the optical density of the experimental sample containing DPPH after blank correction (sample without DPPH), and ODc represents the optical density of the control containing DPPH without the test sample.

The percentage inhibition at different concentrations was applied to construct a calibration curve. The IC₅₀ value i.e. the concentration of extract or ascorbic acid required to achieve 50% DPPH scavenging was determined from the linear regression equation 2

$$y = ax + b \quad \text{Equation 2}$$

Where y is fixed at 50% and x corresponds to the IC₅₀, while, a and b represents the slope and intercept, respectively

Cytotoxic Assay

Cytotoxicity assessment followed the Sulforhodamine B (SRB) assay protocol described by Monk et al.¹⁵ In this procedure, the amount of cellular protein was quantified through optical density measurements of cells stained with SRB by a UV-Vis spectrophotometer (G10S UV-Vis BIO, USA). Stock solutions of the extracts were prepared by dissolving the test samples in 100% dimethyl sulfoxide (DMSO) at 20 mg/mL, after which they were diluted with serum-free medium in 96-well plates to generate four working concentrations. HepG2 cells, after trypsinization, were quantified and dispensed into 96-well plates at 190 µL per well (medium supplemented with 5% Fetal Bovine Serum (FBS)), followed by incubation for 18–20 h to stabilize. After this, 10 µL of each sample dilution was added to the wells; a day-0 control containing cells with 1% DMSO was fixed with 20% Trichloroacetic acid (TCA) after 1 h. Treated plates were incubated for 48 h, then fixed with cold 20% TCA for 1 h, rinsed, air-dried, and then exposed to 0.4% SRB solution for staining. Unbound dye was eliminated using 1% acetic acid, and the retained dye was later dissolved in 10 mM Tris base. Absorbance was recorded at 540 nm using an ELISA microplate reader (BioTek Instruments, Inc., Winooski, VT, USA).

The growth-inhibitory effect of each test sample was quantified according to equation 3:

$$\% \text{ Inhibition} = 100 - \frac{OD_{\text{sample}} - OD_{\text{(day - 0)}}}{OD_{\text{(DMSO)}} - OD_{\text{(day - 0)}}} \times 100 \quad \text{Equation 3}$$

The assay was performed in triplicate. Ellipticine at 10, 2, 0.4, and 0.08 µg/mL was employed as the reference drug, whereas 1% DMSO (final concentration in the wells: 0.05%) was used as the negative control.

The IC₅₀, defined as the extract concentration that suppresses 50% of cell proliferation, was obtained using TableCurve 2Dv4 software (Systat Software Inc., USA).

Statistical Analysis

All experiments were carried out in triplicate, and data were reported as mean ± standard error of the mean (SEM). Statistical differences among treatments were assessed by one-way ANOVA followed by Duncan's multiple range test, with significance set at p < 0.05. Data processing was performed using Microsoft Excel 2010 and IBM SPSS Statistics 20.

Results and Discussion

Compound composition in crude extract from different parts of the *C. paniculatum*

As summarized in Table 1, the GC-MS profiling revealed a decreasing trend in the diversity of constituents detected across the plant parts of *C. paniculatum*. Specifically, 20 compounds were identified in the root extract, followed by 15 in the stem extract, 10 in the flower extract, and 6 in the leaf extract. The major compounds in the methanol roots extract were 6-Octadecenoic acid (44.62%), 1-(+)-Ascorbic acid 2,6-dihexadecanoate (13.81%), 9,12-Octadecadienoic acid (Z,Z)-. methyl ester (8.91%), 9-Octadecenoic acid. methyl ester. (E)- (8.66%), Hexadecanoic acid. methyl ester (5.82%), and Palmitoleamide (4.52%). Methanolic stems extract was found to contain 15 bioactive compounds. The most abundant were 9-Octadecenoic acid. methyl ester. (E)- (38.50%), 9,12-Octadecadienoic acid (Z,Z)-. methyl ester (26.06%), Hexadecanoic acid. methyl ester (19.45%), Methyl stearate (4.14%), and 6-Octadecenoic acid (3.89%). Moreover methanolic flowers extract was found to contain 10 bioactive compounds. The most abundant were 6-Octadecenoic acid (69.43%), 1-(+)-Ascorbic acid 2,6-dihexadecanoate (16.39%), 9-Octadecenoic acid. methyl ester. (E)- (5.71%), 9,12-Octadecadienoic acid (Z,Z)-. methyl ester (3.73%), and Hexadecanoic acid. methyl ester (2.99%).

Table 1: Compounds identified in crude extract from different parts of *C. paniculatum* based on GC-MS analysis

No.	Synonyms	Molecular	Molecular	Retention	%Area			
		Formula	Weight	time (min)	Roots	Stems	Leaves	Flowers
1	Cycloheptasiloxane. tetradecamethyl-	C ₁₄ H ₄₂ O ₇ Si ₇	519.08	9.81	1.08	-	-	-
2	Hexadecanoic acid. methyl ester	C ₁₇ H ₃₄ O ₂	270.45	17.60	5.82	19.45	8.17	2.99
3	l-(+)-Ascorbic acid 2.6-dihexadecanoate	C ₃₈ H ₆₈ O ₈	652.49	18.34	13.81	1.47	10.65	16.39
4	9.12-Octadecadienoic acid (Z,Z)-. methyl ester	C ₁₉ H ₃₄ O ₂	294.47	20.70	8.91	26.06	-	3.73
5	9-Octadecenoic acid. methyl ester. (E)-	C ₁₉ H ₃₆ O ₂	296.49	20.81	8.66	38.50	-	5.71
6	Heptadecanoic acid. 15-methyl-. methyl ester	C ₁₉ H ₃₈ O ₂	298.50	21.25	1.06	1.38	0.48	0.27
7	6-Octadecenoic acid	C ₁₈ H ₃₄ O ₂	282.46	21.62	44.62	3.89	-	69.43
8	Trinexapac-ethyl. TMS derivative	C ₁₆ H ₂₄ O ₅ Si	324.14	23.95	0.36	-	-	-
9	Palmitoleamide	C ₁₆ H ₃₁ NO	253.24	25.24	4.52	-	-	-
10	Gibb-3-ene-1.10-dicarboxylic acid. 2.4a-dihydroxy-1-methyl-8-methylene-. 1.4a-lactone. 10-methyl ester.	C ₂₀ H ₂₄ O ₅	344.16	26.31	0.70	-	-	-
	(1α.2β.4α.4bβ.10β)-							
11	1.1.1.3.3.5.5.7.7.9.9.11.11.13.13.15.15.15-	C ₁₈ H ₅₄ O ₇ Si ₈	606.20	28.52	0.40	-	-	-
	octadecamethyloctasiloxane							
12	2-(7-Oxo-2.3-dihydrofuro[3.2-g]chromen-2-yl)propan-	C ₁₉ H ₂₀ O ₅	328.13	29.08	0.36	-	-	-
	2-yl (Z)-2-methylbut-2-enoate							
13	1.1.1.3.3.5.5.7.7.9.9.11.11.13.13.15.15.17.17.17-	C ₁₈ H ₅₄ O ₇ Si ₈	606.20	30.60	0.52	-	-	-
	icosamethylnonasiloxane							
14	cis-11-Eicosenamide	C ₂₀ H ₃₉ NO	309.30	31.51	0.24	-	-	-
	1.3.5.7.9.11-hexasiloxane. 1.1.3.3.5.5.7.7.9.9.11.11-	C ₁₆ H ₂₄ O ₅ Si	324.14	34.34	0.36	-	-	-
15	dodecamethyl-1.11-di(tert.butyl)-							
16	Rescinnamine	C ₃₅ H ₄₂ N ₂ O ₉	634.29	36.05	0.26	-	-	-
	Spirost-8-en-11-one. 3-hydroxy-. (3β.5α.14β.20β.22β.25R)-	C ₂₇ H ₄₀ O ₄	428.29	36.86	0.31	-	-	-
17								

18	Phytyl linoleate	C ₃₈ H ₇₀ O ₂	558.54	37.29	1.74	-	-	-
	Tricyclo[20.8.0.0(7.16)]triacontane. 1(22).7(16)-	C ₃₀ H ₅₂ O ₂	444.40	37.67	4.45	-	-	-
19	diepoxy-							
20	17-Epitestosterone. trimethylacetate	C ₂₄ H ₃₆ O ₃	372.27	40.20	1.82	-	-	-
21	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	228.37	14.51	-	-	-	0.50
22	cis-13-Eicosenoic acid	C ₂₀ H ₃₈ O ₂	310.51	17.92	-	-	-	0.63
	2-(Furan-3-yl)-7.8-dihydroxy-6a.7.10b-trimethyl-							
	2.4a.5.6.8.9.10.10a-octahydro-1H-benzo[f]isochromen-	C ₂₈ H ₃₅ F ₅ O ₃	514.25	37.34	-	-	-	0.14
23	4-one							
	2.2'-Methylenebis-(6-tert-butyl)-4-ethylphenol. O-	C ₂₈ H ₃₅ F ₅ O ₃	514.25	40.20	-	-	-	0.20
24	pentafluoropropionyl-							
25	Methyl 9-cis.11-trans-octadecadienoate	C ₁₉ H ₃₄ O ₂	294.26	20.72	-	-	6.67	
26	9.12.15-Octadecatrienoic acid. methyl ester. (Z.Z.Z)-	C ₁₉ H ₃₂ O ₂	292.24	20.90	-	-	25.23	-
27	Butyl 9.12.15-octadecatrienoate	C ₂₂ H ₃₈ O ₂	334.29	21.84	-	-	48.80	-
28	2-Propenoic acid. 3-phenyl-. methyl ester. (E)-	C ₁₀ H ₁₀ O ₂	162.07	8.34	-	0.26	-	-
29	Nonanedioic acid. dimethyl ester	C ₁₁ H ₂₀ O ₄	216.14	10.65	-	0.33	-	-
30	Methyl stearate	C ₁₉ H ₃₈ O ₂	298.29	21.29	-	4.14	-	-
31	Eicosanoic acid. methyl ester	C ₂₁ H ₄₂ O ₂	326.32	24.69	-	0.96	-	-
32	Methyl 22-hydroxydocosanoate	C ₂₂ H ₄₄ O ₂	340.33	26.31	-	0.59	-	-
33	Octadecanoic acid. 11-methyl-. methyl ester	C ₂₂ H ₄₄ O ₂	340.33	27.88	-	0.77	-	-
34	Methyl 21-methyldocosanoate	C ₂₂ H ₄₄ O ₂	340.33	29.39	-	0.35	-	-
35	Methyl 13-methyl-eicosanoate	C ₂₂ H ₄₄ O ₂	340.33	30.84	-	0.55	-	-
36	Urs-12-en-28-al. 3-(acetyloxy)-. (3β)-	C ₃₂ H ₅₀ O ₃	482.38	34.80	-	1.30	-	-

Note: “-”: Not detected; Peak areas (%) are calculated based on the number of compounds for each different type of crude extract.

In addition, 6 major phytochemicals were identified in leaves extracts with the most abundant compounds were Butyl 9,12,15-octadecatrienoate (48.80%), 9,12,15-Octadecatrienoic acid, methyl ester. (Z,Z,Z)- (25.23%), 1-(+)-Ascorbic acid 2,6-dihexadecanoate (10.65%), Hexadecanoic acid, methyl ester (8.17%), and Methyl 9-cis,11-trans-octadecadienoate (6.67%).

A total of 36 compounds were identified in the crude methanolic extract of *C. paniculatum*, but only 3 compounds (Hexadecanoic acid, methyl ester; Heptadecanoic acid, 15-methyl-, methyl ester, and 1-(+)-Ascorbic acid 2,6-dihexadecanoate) appeared in all parts of *C. paniculatum* and 3 compounds appeared only in the roots, stems and flowers (9,12-Octadecadienoic acid (Z,Z)-, methyl ester; 9-Octadecenoic acid, methyl ester. (E)-; 6-Octadecenoic acid), the remaining compounds were mostly distributed in the roots, stems and a few in the flowers (Table 1 and Figure 1). Similar studies on *C. paniculatum* leaf extracts identified compounds such as phytol, 22-tritetracontanone, and 6,9,12-octadecatrienoic acid phenyl methyl ester using GC-HRMS analysis.¹⁶ Another investigation into the flower extracts of *C. paniculatum* highlighted the presence of flavonoids and phenolic compounds, which were associated with notable hepatoprotective and antioxidant activities.¹⁰ Comparative GC-MS analyses of other plant species also show variation in chemical composition across different parts. For instance, a study on *Leptadenia reticulata* detected various bioactive phytochemicals in its leaves, stems, roots, and callus tissues, emphasizing the importance of tissue-specific phytochemical profiling.¹⁷ These findings underscore the significance of comprehensive phytochemical analyses across different plant parts to fully understand their potential medicinal properties.

However, it is essential to note that the present findings are restricted by the use of crude extracts and the intrinsic characteristics of GC-MS analysis. In particular, several siloxane-related signals detected in the chromatograms such as *Cycloheptasiloxane*, *tetradecamethyl-* (RT 9.81), *octadecamethyloctasiloxane* (RT 28.52), *icosamethylnonasiloxane* (RT 30.60), and various *hexasiloxane derivatives* (RT 34.34) are most likely attributable to instrument-related artifacts, including column bleeding or minor contamination from silicone-containing components, rather than genuine plant metabolites.¹⁸ Therefore, these peaks were not included in the interpretation of the phytochemical composition of *C. paniculatum* to avoid misleading conclusions.

GC-MS predominantly detects volatile and non-polar compounds; therefore, the identification of mainly fatty acids and their derivatives is expected.¹⁹ Consequently, the compound profile reported herein does not fully represent the complete phytochemical composition of *C. paniculatum*. Future studies employing fractionation, compound isolation, and complementary techniques such as LC-MS/MS or NMR are required to obtain a more comprehensive chemical profile and to clarify the contributions of polar constituents to the plant's biological activities.²⁰

Antioxidant activity analysis

The DPPH radical-scavenging assay was applied to evaluate the bioactivity of the condensed extracts obtained from various parts of *C. paniculatum*. The corresponding findings are presented in Tables 2 and 3, and are reported in terms of percentage scavenging capacity (%SC) and IC₅₀ values.

As presented in Table 2, the DPPH scavenging activity decreased as the dilution ratio increased from 1:50 to 1:1000, indicating a direct correlation between antioxidant capacity and extract concentration i.e. higher extract concentrations exhibited stronger antioxidant activity. The free radical scavenging activity across different plant parts ranged from 30.00% to 92.64%. Among these, the root demonstrated the highest scavenging capacity (35.41%-92.64%), while the leaves exhibited the lowest activity (30.00%-90.47%).

As reported in Table 3, the IC₅₀ results of *C. paniculatum* extracts varied from 4.13 mg mL⁻¹ to 5.23 mg mL⁻¹, which were significantly higher than that of ascorbic acid (81.89 µg mL⁻¹). The extract obtained from the root of *C. paniculatum* exhibited the strongest antioxidant potential (4.13 mg mL⁻¹), followed by the flower (4.70 mg mL⁻¹) and stem extracts (4.83 mg mL⁻¹). The leaf extract showed the lowest activity, requiring the highest concentration to scavenge 50% of free radicals

(5.23 mg mL⁻¹). Compared to ascorbic acid (0.08189 mg mL⁻¹ i.e. 81.89 µg mL⁻¹), all plant extracts demonstrated considerably lower antioxidant potency. These results indicate that, although the root shows relatively stronger activity than other parts, the overall antioxidant activity of *C. paniculatum* remains modest.

Moreover, our results align with previous studies on the *Clerodendrum* genus. Swargiary et al. reported that the antioxidant effect of *Clerodendrum viscosum* root extract was higher than that of its leaf extract, with an IC₅₀ value of 137.0 µg mL⁻¹.²¹ Similarly, Sumayya and Gopinathan in the year 2022, found that ethyl acetate extracts from *C. infortunatum* roots exhibited stronger antioxidant activity than aqueous leaf extracts, with corresponding IC₅₀ values of 217.9 µg mL⁻¹ and 223.6 µg mL⁻¹, respectively.²²

These findings indicate that although the root exhibits relatively stronger activity than the other plant parts, the overall antioxidant potential of *C. paniculatum* remains modest. This limited activity may be partly due to the fact that the present study was conducted using crude extracts, in which impurities have not been removed, and thus the activity may not fully reflect the true potential of the purified compounds. Therefore, the use of this species as a natural antioxidant source appears limited, and further investigations should be carried out, particularly at the level of compound isolation which is relevant prior to its consideration as a medicinal therapeutic.

Table 2: DPPH free radical scavenging activity of crude extract from different parts of the *C. paniculatum*

Parts of <i>C. paniculatum</i>	Dilution rate					
	1:50	1:100	1:200	1:400	1:800	1:1000
Roots	92.64 ^a ± 0.81	69.03 ^b ± 0.22	56.32 ^c ± 0.39	47.01 ^d ± 0.51	41.60 ^e ± 0.24	35.41 ^f ± 0.33
Stems	90.56 ^c ± 0.29	67.12 ^b ± 0.19	55.40 ^b ± 0.17	44.85 ^b ± 0.22	40.27 ^b ± 0.33	32.26 ^c ± 0.05
leaves	90.47 ^c ± 0.08	66.37 ^c ± 0.24	54.13 ^c ± 0.16	45.65 ^b ± 0.19	38.13 ^c ± 0.13	30.00 ^d ± 0.22
Flowers	91.30 ^b ± 0.22	66.06 ^c ± 0.24	54.46 ^c ± 0.11	46.76 ^a ± 0.32	40.51 ^b ± 0.24	33.62 ^b ± 0.35

Note: Different letters on the same column indicate a statistically significant difference of the sample mean with $p < 0.05$ based on the Duncan's test.

Table 3: IC₅₀ values of crude extract from different parts of *C. paniculatum* in the antioxidant assay

Parts of <i>C. paniculatum</i>	Unit	R ²	IC ₅₀
Roots		0.972	4.13 ^c
Stems	mg mL ⁻¹	0.961	4.83 ^b
Leaves		0.954	5.23 ^a
Flowers		0.971	4.70 ^b
Ascorbic Acid	µg mL ⁻¹	0.957	81.89

Note: Different letters on the same column indicate a statistically significant difference of the sample mean with $p < 0.05$ based on the Duncan's test.

Cytotoxic activity analysis

The results of the toxicity test of crude extracts obtained from different parts of *C. paniculatum* on the liver cancer cell line HepG2 are shown in Table 4. When tested at 500 µg/mL, the leaf extract exhibited the greatest inhibitory activity (40.10%), followed by the root and flower extracts, which showed 36.87% and 35.03% inhibition, respectively. Among all samples, the stem extract exhibited the weakest inhibitory activity (26.11%). These results demonstrate differential cytotoxic effects among extracts from different plant parts. Moreover, the inhibitory ability of each type of extract was still low, and the IC₅₀ value could not be calculated for this concentration range. According to the standards of the National Cancer Institute (NCI), extracts are regarded as having significant activity i.e. exhibit good activity when IC₅₀ ≤ 20 µg mL⁻¹, while pure compounds are classified as active when IC₅₀ ≤ 5 µM.²³ The positive control substance Ellipticine showed a better IC₅₀ value in the experiment (Table 4).

Scientific research on the anti-liver cancer activity of *C. paniculatum* is still limited, although this plant has for many years been utilized in folk medicine to support the management of liver-related conditions. Despite its traditional use, modern scientific evidence confirming its anticancer properties remains scarce.

This study provides a connection between long-standing ethnobotanical uses and contemporary pharmacological investigations, thereby supporting the validation of the medicinal potential of *C. paniculatum*. It provides preliminary but valuable evidence of the plant's anticancer potential, laying the foundation for more in-depth investigations. Future research focused on isolating bioactive compounds, optimizing extraction methods, and studying the mechanism of action could contribute to the establishment of new plant-based therapies for liver cancer therapy.

Table 4: Inhibitory effect of crude extract different parts collection of the *C. paniculatum* on cell line HepG2

Con. (µg/mL)	Inhibitory on cell line HepG2 (%)				Con. (µg mL ⁻¹)	Ellipticine
	Roots	Stems	Leaves	Flowers		
500	36.87 ^b ± 1.96	26.11 ^c ± 2.56	40.10 ^b ± 1.19	35.03 ^b ± 2.63	10	96.83 ^a ± 2.15
100	17.63 ^b ± 0.62	13.09 ^b ± 1.29	13.96 ^b ± 0.89	14.52 ^b ± 1.18	2	86.12 ^a ± 1.61
20	9.77 ^b ± 0.28	6.18 ^c ± 0.52	9.90 ^b ± 0.76	6.58 ^c ± 0.72	0.4	52.25 ^a ± 1.05
4	3.56 ^b ± 0.17	2.55 ^{bc} ± 0.23	3.61 ^b ± 0.28	1.63 ^c ± 0.18	0.08	22.37 ^a ± 1.59
IC₅₀	> 500	> 500	> 500	> 500	IC₅₀	0.32 ± 0.02

Note: SD: standard deviation; Con.: Concentration. Different letters on the same row indicate a statistically significant difference of the sample mean with $p < 0.05$ (Duncan's test).

Conclusion

Overall, the different parts of *C. paniculatum* collected from Hue City demonstrate a diverse phytochemical profile and measurable antioxidant and cytotoxic activities, although these biological effects remain generally modest. The GC-MS analysis of crude methanolic extracts primarily detected volatile and non-polar constituents. This study provides valuable baseline information on the phytochemical distribution and preliminary bioactivities of *C. paniculatum*, contributing important insight into a species that remains understudied. These findings highlight the need for further investigations, including fractionation, compound purification, and the integration of complementary analytical techniques, to comprehensively elucidate the therapeutic potential of this medicinal plant.

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.

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