Original Article

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Phytochemical Components and Antimicrobial Activity of Ethanolic Extracts from Different Parts of *Calotropis gigantea* (L.) Dryand.

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Abstract:

Objective: The present study aimed to determine the antimicrobial activity and phytochemicals of different parts of *C. gigantea*.

Material and Methods: The flower, leaf, stem bark, and latex of *C. gigantea* were extracted using 50%, 70%, and 95% ethanol. Phenolic and flavonoid content were measured using colorimetric assays. The disc diffusion method was used to evaluate antimicrobial activity against a panel of microorganisms.

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Results: The ethanolic extracts of *C. gigantea* (2 mg/disc) from the tested parts contained total phenolic and flavonoid contents ranging from 13–38 mg GAE/ g extract and 1–175 mg RTE/ g extract. Extracts from the flower, leaf, and stem bark showed similar inhibitory effects against *Pseudomonas aeruginosa* TISTR 1467. Additionally, extracts from the flower and leaf demonstrated inhibition zones against *Candida tropicalis* TISTR 5136. No inhibitory activity was observed against *Staphylococcus aureus* ATCC 6538, *Acinetobacter baumannii* ATCC 19606, *Candida albicans* TISTR 5554, and *Candida krusei* TISTR 5351 from any of the tested samples.

Conclusion: The ethanolic extracts from various parts of *C. gigantea* contained a high amount of phenolics and flavonoids, and exhibited antimicrobial activity, particularly against *P. aeruginosa* and *C. tropicalis*. These findings suggest that *C. gigantea* may be a promising natural source for further isolation and purification, which could enable the discovery of new antimicrobial agents.

Keywords: *Calotropis gigantea*, ethanolic extracts, phytochemicals, phenolic, flavonoid, antimicrobial activity

Introduction

Calotropis gigantea (L.) Dryand. (*C. gigantea*) is known as giant milkweed, which belongs to the Apocynaceae family. It contains various phytochemical compounds, including flavonoids, alkaloids, triterpenoids, saponins, steroids, and glycosides[1,2]. In Ayurvedic medicine, *C. gigantea* was used as traditional medicine to treat epilepsy, leprosy, snake bites, and cancer[2]. The Indian folks used various parts of this plant to cure multiple symptoms, such as using fresh leaves for the treatment of convulsions[3]. Its latex was used to treat skin infections[3], while its bark was used for diaphoretic remedies[4]. Additionally, several biological activities of *C. gigantea* have been reported, including anti-inflammatory, cytotoxic activity against cancer cells, antimicrobial properties[1], and anti-H1N1 influenza virus activity[5].

The World Health Organization (WHO) has identified the rising prevalence of multidrug-resistant (MDR) pathogens as one of the top ten critical issues for international healthcare[6]. Therefore, it is crucial to discover effective agents to combat those pathogens. Natural products are promising sources for the discovery of new and effective antimicrobial agents to combat resistant microbes. *C. gigantea* has recently been recognized as a possible source of antimicrobial compounds. Owing to the phytochemicals inside the Apocynaceae family plants, they represent a significant inhibitory effect on microbial growth[7].

Extraction is a process used to achieve secondary metabolites from plants using selective solvents. Utilizing a proper solvent is necessary to obtain extracts with the required pharmacological properties. The ability of a solvent to extract a desired compound depends on the solvent's polarity[8,9]. Ethanol is frequently used as a universal solvent for plant extraction due to its safety. Both absolute ethanol and aqueous ethanol have been effectively used to isolate bioactive compounds from various plants, yielding great results[10].

Therefore, this research aimed to evaluate the antimicrobial activity and quantify the phytochemicals in ethanolic extracts from different parts of *C. gigantea* using 50%, 70%, and 95% ethanol in water. The goal was to obtain fundamental information for further research on discovering new antimicrobial remedies from *C. gigantea*.

Material and Methods

Collection of plants

The flower, leaf, stem bark, and latex of *C. gigantea* used in this experiment were collected from the Thoen District, Lampang Province, Thailand, in April 2018. Its voucher specimen (number 5194) was authenticated in our previous study[11] and was deposited at the PNU Herbarium, Department of Biology, Faculty of Science, Naresuan University, Phitsanulok, Thailand.

Preparation of ethanolic crude extracts

The flower, leaf, and stem bark were dried at 50 °C in a hot-air oven (Memmert, Germany) for two days and finely ground using a blender (Sharp, Thailand). The latex was collected in a 50 mL sterile conical centrifuge tube by cutting the aerial part of *C. gigantea*. The collected latex was frozen (-20 °C, Hitachi, Thailand) until use. Thirty grams of dried powder (flower, leaf and stem bark) and latex of *C. gigantea* were extracted by 180 mL (repeated three times) of 50% (v/v), 70% (v/v) and 95% (v/v) ethanol (Liquor distillery organization, Thailand) by ultrasonic assistance with a sonicator (40 kHz, Shenzhen Jie Tai, China) for 1 hour at ambient temperature (30±2 °C). After filtration with Whatman No. 1 filter paper, the ethanolic layers from each part were evaporated using a rotary evaporator (Buchi, Switzerland) at 50 °C until they were completely dried. All ethanolic crude extracts were stored in a refrigerator (4±2 °C) until use. The percent yields (% w/w) of each ethanolic crude extract were calculated from their weight (g) compared to the weight of dried plants (100 g).

Total phenolic content (TPC)

The total phenolic content of *C. gigantea* ethanolic crude extracts was quantified using a modified colorimetric assay, with slight modifications to the previous method[12]. Sample solutions (2 mg/mL) or standard gallic acid (Sigma-Aldrich, USA) in methanol (25 µL) were mixed with 100 µL of 25% Folin–Ciocalteu reagent (Merck, Germany) in a 96-well plate. After shaking, 75 µL of 6% sodium hydrogen carbonate was added, followed by a 2-hour incubation at 27±2 °C in the dark. Absorbance was measured at 765 nm. The phenolic content was calculated using a gallic acid calibration curve and expressed as milligrams of gallic acid equivalent per gram of extract. Results were averaged from three independent experiments with standard deviation (S.D.).

Total flavonoid content (TFC)

The total flavonoid content of the ethanolic crude extracts from four parts of *C. gigantea* was measured according to the method described by Chatatikun and Chiabchalard[13], with slight modifications. Briefly, 50 µL of sample solution or standard rutin (Sigma-Aldrich, USA) solution (0–50 µg/mL) in 95% v/v ethanol was mixed with 10 µL of 10% aluminum chloride (AR grade, KemAus, Australia) solution, followed by the addition of 150 µL of 95% v/v ethanol. The reaction mixtures were kept for 40 minutes in the dark at room temperature (27±2 °C) before measuring their absorbance at 415 nm using a microplate reader. Total flavonoid content was calculated from a rutin calibration curve and presented as mg rutin equivalents (RTE) per g extract.

Thin-layer chromatography (TLC)

Lupeol (1 mg/mL, Tokyo Chemical Industry Co., Ltd., Japan) and 2 mg/mL of calactin[14] standards, along with *C. gigantea* ethanolic extracts (10 mg/mL), were dissolved in methanol (RCI Labscan, Thailand). Two µL of standards and 5 µL of samples were spotted on a TLC silica gel 60 F254 sheet (Merck, Germany) using a Limonat 5 applicator (0.8 mm band, 150 nL/s, Camag, Switzerland). A 95:5 dichloromethane–methanol mixture was used as the developing solvent. TLC fingerprints were observed under visible light, UV (254/366 nm), and after spraying with 10% sulfuric acid in ethanol, followed by heating at 110 °C.

High-performance liquid chromatography (HPLC)

HPLC analysis was conducted to separate and identify the secondary metabolites in each part of *C. gigantea* extracts using different solvent extractions. The extracts were dissolved in methanol (HPLC grade, RCI Labscan, Thailand) at a concentration of 15 mg/mL and injected into the HPLC system, equipped with a C18 column (150 mm × 4.6 mm), at an injection volume of 20 µL. A gradient mobile phase consisting of acetonitrile (HPLC grade, RCI Labscan, Thailand) and water with 0.1% formic acid was utilized, ranging from 0-95% acetonitrile to water. The extracts were eluted at a flow rate of 0.8 mL/min for 45 minutes, with the column temperature maintained at 25 °C. Detection was conducted using a UV/visible detector at wavelengths of 222 nm.

Disc diffusion method

Selected microorganism strains, including gram-positive bacteria, *Staphylococcus aureus* ATCC 6538, twogram-negative bacteria, *Pseudomonas aeruginosa* TISTR 1467 and *Acinetobacter baumannii* ATCC 19606, and three fungi, *Candida albicans* TISTR 5554, *Candida tropicalis* TISTR 5136, and *Candida krusei* TISTR 5351, were selected to evaluate the inhibitory activity of the extracts. The tested microbes were obtained from the Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University. The biosafety approval number was NUIBC MI 66-02-04/ 66-15. A biosafety cabinet Class II AC2-4S8-NS (Esco, Thailand) was used throughout the experiments involving microorganisms.

Stock solutions (100 mg/mL) of the extracts were prepared in dimethyl sulfoxide (DMSO, Ameresco, Canada). Sterile paper discs (diameter 6 mm) were loaded with 20 µL of the sample solutions and placed on the surface of Mueller-Hinton agar plates (Himedia, India). After 24 hours of incubation at 37 °C, the data were reported by measuring the inhibition zones (mm) around the paper discs. Each microbe was evaluated for the diameter of inhibition zones in triplicate, then expressed as an average value ± S.D. DMSO was used as the negative control, while vancomycin (Phytotechlab, UK), streptomycin (Bio Basic Canada Inc., Canada), colistin (Sigma-Aldrich, China), and nystatin (Calbiochem, Czech Republic) were used as positive controls for *S. aureus*, *P. aeruginosa*, *A. baumannii*, and *Candida spp.*, respectively.

Statistical analysis

The results of % yield, total phenolic content, total flavonoid content, and the diameter of the inhibition zone were expressed as mean ± S.D., based on three independent experiments. Significant differences (p-value<0.05) were analyzed using one-way ANOVA, with a post hoc Bonferroni correction applied using MS Excel 2019.

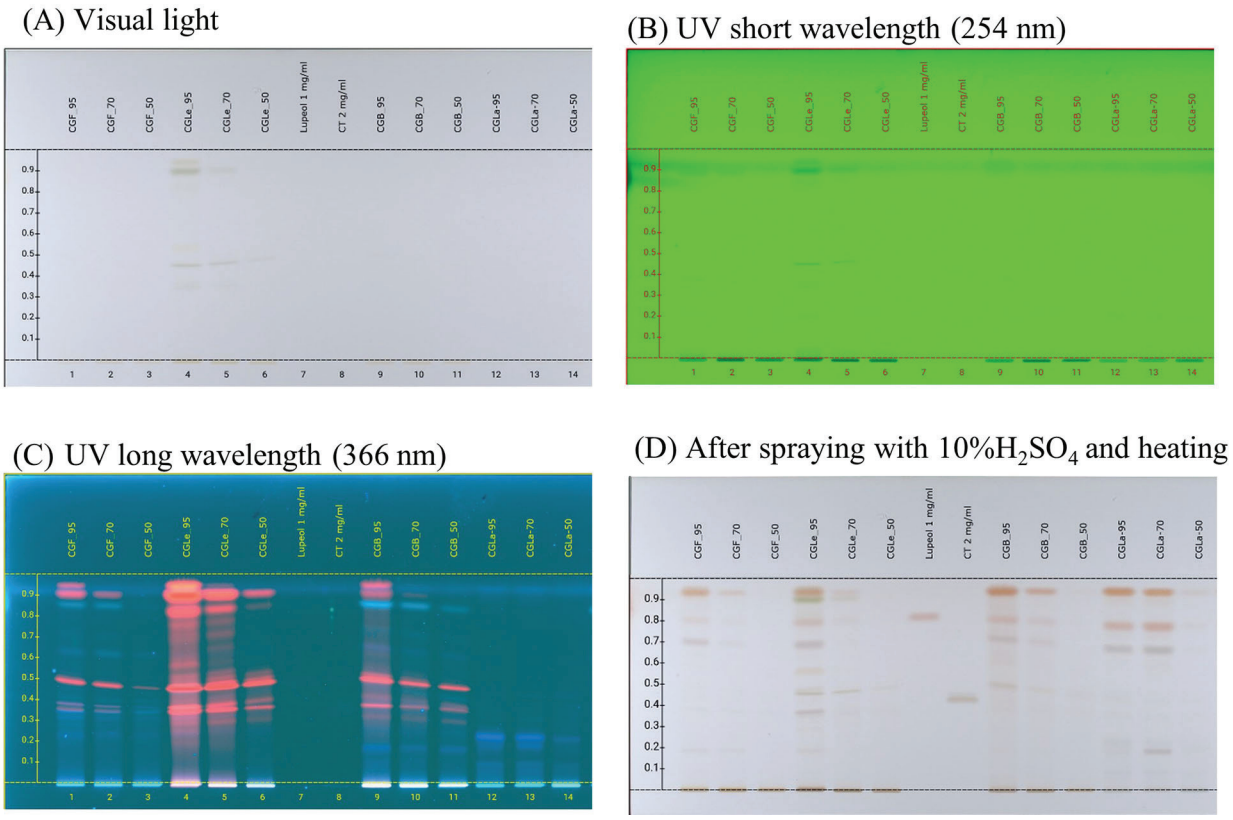
Results

Effect of solvent extraction

The flower, leaf, stem bark, and latex of *C. gigantea* were extracted using various concentrations (50%, 70%, and 95%) of ethanol, which yielded different percentages of ethanolic extracts: 7.92-25.57%, 9.77-24.72%, 6.34-14.30%, and 6.58-9.27%, respectively. The results in Table 1 revealed that 50% ethanol produced the highest yields of ethanolic extracts from *C. gigantea* flower (25.57±2.20%), leaf (24.72±0.60%), and stem bark (14.30±2.49%), which were statistically significantly higher than those obtained with 70% and 95% ethanol (p-value<0.05). Conversely, for latex, 95% ethanol (9.27±0.50%) achieved a significantly higher yield (p-value<0.05) compared to 50% and 70% ethanol.

Total phenolics and flavonoids contents

The total phenolic and flavonoid contents of the different parts of *C. gigantea* extracts are presented in Table 1. Interestingly, all parts of *C. gigantea* contained phenolic compounds (13.05–38.34 mg GAE/g extract) and flavonoid compounds (1.06–175.61 mg RTE/g extract) in varying amounts. The highest total phenolic content was found in extracts prepared using 70% and 95% ethanol, particularly in the leaf extracted with 95% ethanol (38.34±2.27 mg GAE/g extract). Meanwhile, the stem bark extract from 50% ethanol had the lowest phenolic content (13.05±0.88 mg GAE/g extract). The highest total flavonoid content (175.61±29.63 mg RTE/g extract) was observed in the extract from *C. gigantea* leaf using 95% ethanol, which was significantly higher than in the other parts. The lowest flavonoid content (1.06–2.48 mg RTE/g extract) was observed in the latex extracts across all ethanol concentrations.



CGF=flower, CGLe=leaf, CGB=stem bark, CGLa=latex of *C. gigantea*. The numbers 50, 70, and 95 represented extraction with 50% ethanol, 70% ethanol, and 95% ethanol, respectively

Figure 1 Thin–layer chromatographic fingerprints of the ethanolic crude extracts from various parts of *C. gigantea* (A) Visual light; (B) UV short wavelength (254 nm); (C) UV long wavelength (366 nm); and (D) After spraying with 10% H_2SO_4 and heating.

TLC fingerprints

Figure 1A–D presents TLC fingerprints of *C. gigantea* extracts observed under visible light and UV (254/366 nm), along with results following spraying with 10% H_2SO_4 and heating. The TLC bands indicate various phytochemicals, with lupeol (Rf 0.8) and calactin (Rf 0.45) serving as references. The 95% ethanolic extracts exhibited the highest number of bands (5–8, Rf 0.2–0.95), followed by 70% (3–4, Rf 0.2–0.95) and 50% (1–2, Rf 0.2–0.5), indicating a greater phytochemical diversity as the ethanol concentration increased. Leaf extracts revealed the highest band count (5–8, Rf 0.2–0.95), followed by stem bark (3–5, Rf 0.4–0.95), flower (2–6, Rf 0.4–0.95), and latex (1–4, Rf 0.2–0.95).

HPLC analysis

The HPLC chromatograms of the ethanolic crude extracts (15 mg/mL) from each part of *C. gigantea*, including the flower (Figure 2A), leaf (Figure 2B), stem bark (Figure 2C), and latex (Figure 2D), were extracted with 50%, 70%, and 95% ethanol, along with the vehicle control (blank, methanol) and the standard control (rutin solution at 1 mg/mL). At least 18 peaks were observed in the chromatograms of extracts from different parts of *C. gigantea*. Nearly all plant parts, except for the latex, displayed peaks corresponding to rutin at approximately 13.2 minutes. While the peak intensities varied, similar chromatographic patterns were identified for the same plant parts, regardless of ethanol concentration. Different compound patterns were found in the extracts from the various parts of *C. gigantea*: compounds 4, 6, and 18 were abundant in the flower; compounds 1, 4, and 16 in the leaf; compounds 7, 8, and 14 in the stem bark; and compounds 5, 10, 11, 15, and 16 in the latex. Increasing ethanol concentrations generally improved the extraction of specific compounds, such as compound 10 in the flower extract, compound 4 in the leaf extract, compounds 12 and 14 in the stem bark extract, and compound 5 in the latex extract. However, the optimal ethanol concentration varied by compound, and in some cases, higher ethanol concentrations did not correlate with increased compound levels—for example, with compounds 1 and 15.

Antimicrobial activity

The antimicrobial activity of ethanolic extracts from different parts of *C. gigantea* against one species of gram-positive bacteria (Figure 3), two species of gram-negative bacteria (Figures 4A–4B), and three species of fungi (Figures 5A–5C) was established using the disc diffusion method. The results indicated that extracts from various parts of *C. gigantea* exhibit antimicrobial activity against the gram-negative bacteria *P. aeruginosa* and one species of *Candida*, *C. tropicalis*, as demonstrated by the summary of the inhibition zones in Table 2.

Figure 3 demonstrates that the ethanolic extracts (2 mg/disc) from all parts of *C. gigantea* showed no inhibition zones against *S. aureus*. The differing results may be due to variations in the extraction protocol. Almost all parts of the ethanolic extracts from *C. gigantea*, except for latex, exhibited inhibition zones against *P. aeruginosa* (Figure 4A, with diameters ranging from 7.0 to 8.0 mm). At the same time, no activity was observed against *A. baumannii* (Figure 4B). Figure 5A indicates that no inhibition zone was seen against *C. albicans*. In contrast, Figure 5B illustrates the inhibition zones (with diameters ranging from 7.3 to 8.8 mm) produced by the ethanolic extracts from the flower (50% ethanol) and leaf (50% and 70% ethanol) against *C. tropicalis*. Similarly, no inhibition was observed against *C. krusei* (Figure 5C).

Discussion

Various plant extraction techniques, such as microwave extraction, Soxhlet apparatus, supercritical fluid extraction, and ultrasonic-assisted methods, influence yield and phytochemical diversity. However, extraction efficiency also depends on the type of solvent and its

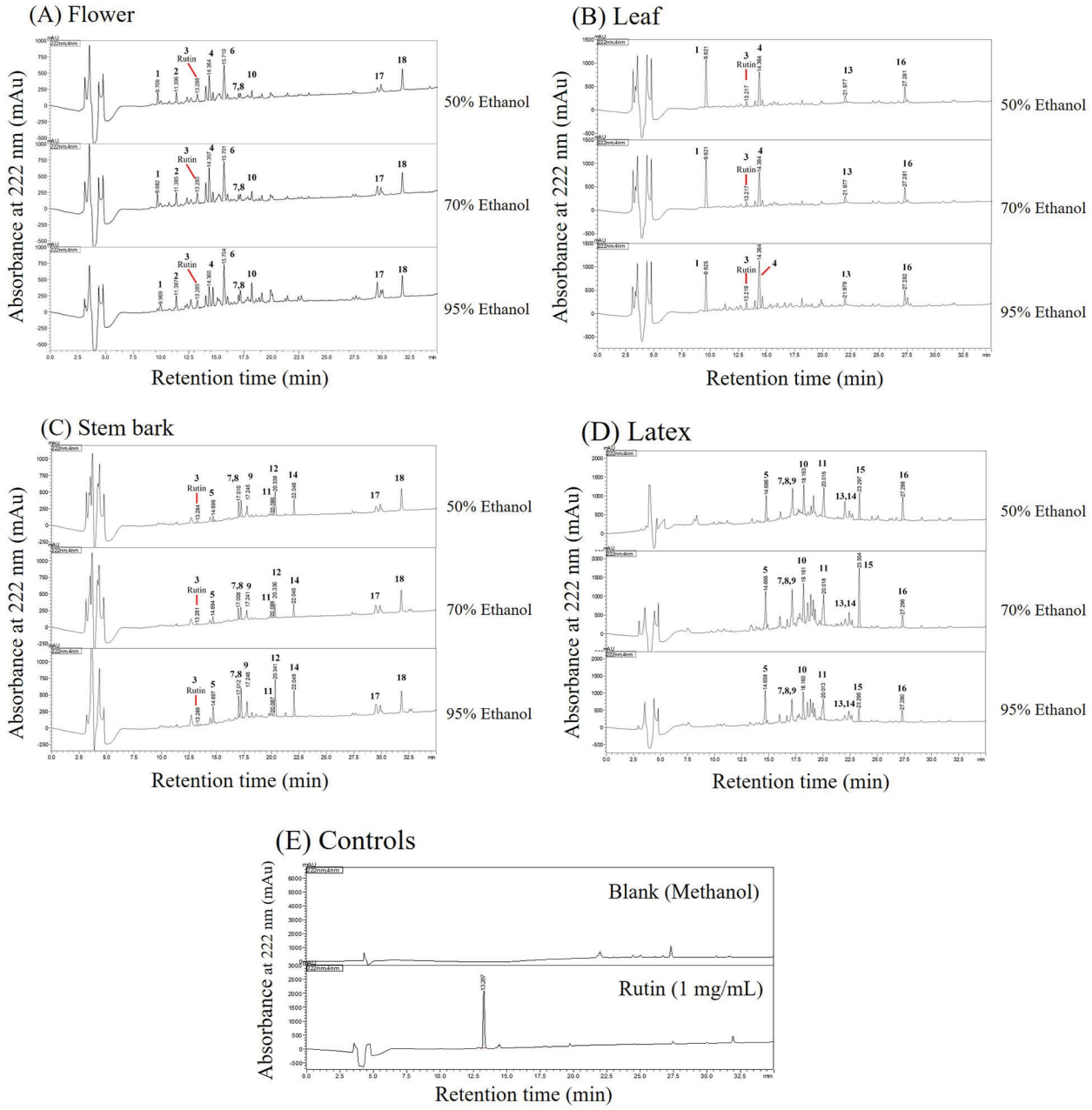
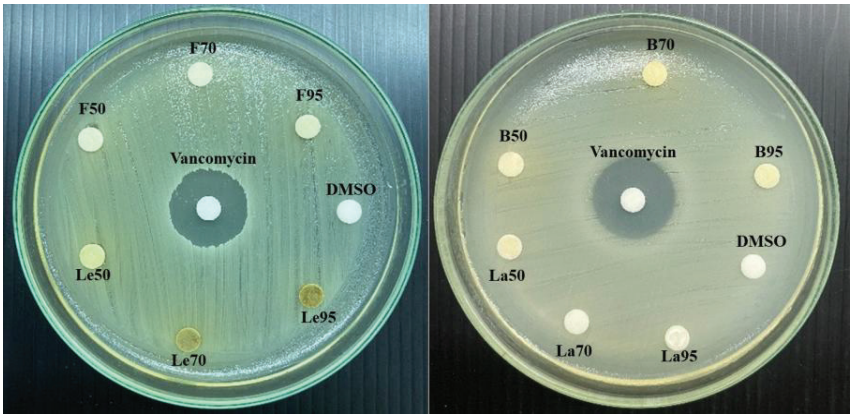


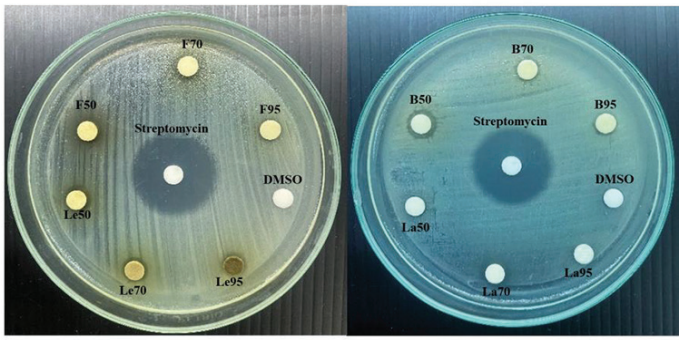
Figure 2 HPLC chromatograms of the 15 mg/mL *C. gigantea* ethanolic crude extracts from each part, including flower (A), leaf (B), stem bark (C), and latex (D), which were extracted with 50%, 70%, and 95% ethanol. The controls (E) include a blank (methanol) and a rutin standard solution (1 mg/mL).



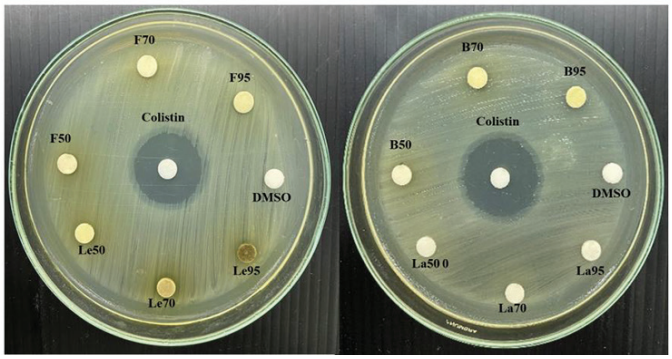
F, Le, B, and La represented flower, leaf, stem bark, and latex of *C. gigantea*, while 50, 70, and 95 were the ethanolic crude extracts from 50% v/v, 70% v/v, and 95% v/v ethanol, respectively. DMSO was used as a negative control, while vancomycin was a positive control.

Figure 3 Antimicrobial activity against gram-positive bacteria, *S. aureus*, of the ethanolic crude extracts from various parts of *C. gigantea*

(A) *P. aeruginosa*



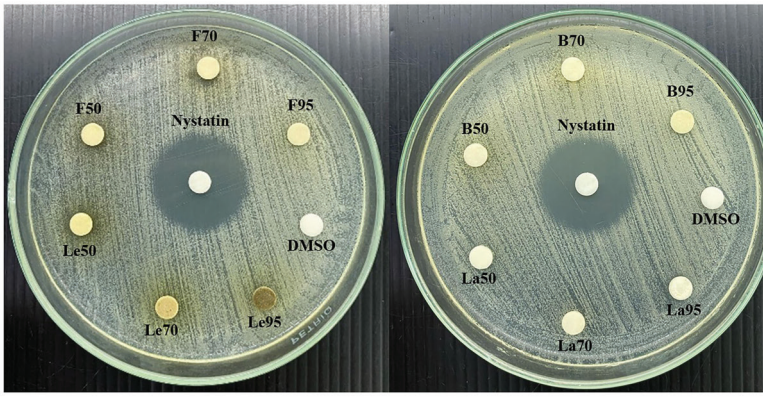
(B) *A. baumannii*



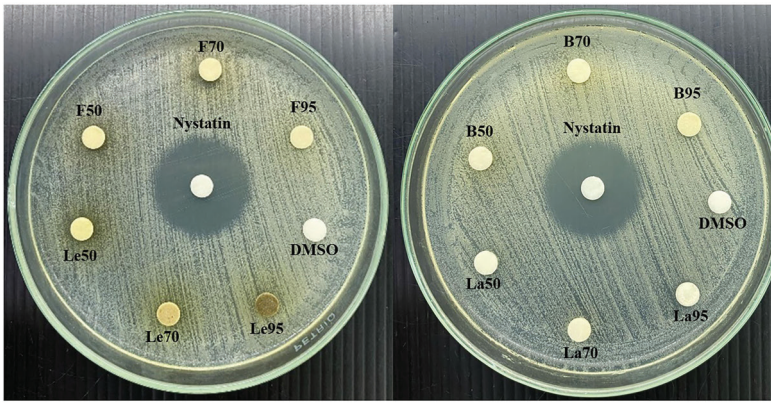
F, Le, B, and La represented flower, leaf, stem bark, and latex of *C. gigantea*, while 50, 70, and 95 were the ethanolic crude extracts from 50% v/v, 70% v/v, and 95% v/v ethanol, respectively. DMSO was used as a negative control; streptomycin and colistin were positive controls.

Figure 4 Antimicrobial activity against gram-negative bacteria (A) *P. aeruginosa* and (B) *A. baumannii* of the ethanolic crude extracts from various parts of *C. gigantea*

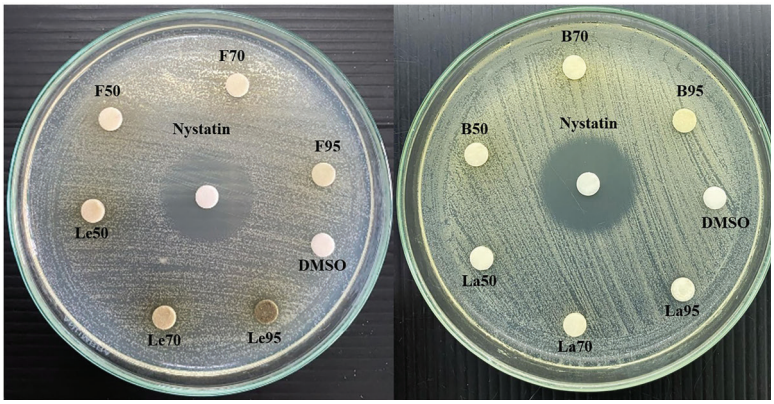
(A) *C. albicans*



(B) *C. tropicalis*



(C) *C. krusei*



F, Le, B, and La represented flower, leaf, stem bark, and latex of *C. gigantea*, while 50, 70, and 95 were the ethanolic crude extracts from 50% v/v, 70% v/v, and 95% v/v ethanol, respectively. DMSO was used as a negative control, and nystatin was a positive control.

Figure 5 Antimicrobial activity against fungi (A) *C. albicans*, (B) *C. tropicalis*, and (C) *C. krusei* of the ethanolic crude extracts from various parts of *C. gigantea*

concentration. In this study, the percentage yield of extracts from flower, leaf, and stem bark decreased with higher ethanol concentrations. Similar findings were reported by Dirar et al.[9] and Hikmawanti et al.[15], where 50% ethanol yielded the highest extract percentage. This may be due to its ability to dissolve a broader range of polar compounds, including proteins and carbohydrates[16]. Conversely, 95% ethanol effectively dissolves both polar and non-polar compounds, extracting a wider range of organic substances. *C. gigantea* latex, rich in hydrocarbons, sterols, and fatty acids, is primarily nonpolar, making it more soluble in high-concentration ethanol[17]. Lower ethanol concentrations (50% and 70%) may not efficiently dissolve these compounds, leading to lower extraction yields from the latex.

The ethanolic crude extracts from various parts of *C. gigantea* in this study contained high amounts of total phenolic and flavonoid content (Table 1), which corresponded with the previous works of Patel et al.[18], who reported the high total phenolics content found in the methanolic extract from the leaves of *C. gigantea*, and Kumar et al.[1], who demonstrated that an aerial part of *C. gigantea* contained several flavanols, such as isorhamnetin-3-*O*-rutinoside, taraxasteryl acetate, and isorhamnetin-3-*O*-Glucopyranoside. Additionally, a number of phytochemicals have been found in *C. gigantea*, including cardenolides, alkaloids, terpenes, phenolics, flavonoids, sterols, cyanogenic glycosides, and tannins[1,11]. The various concentrations of ethanol used to extract each part of the plant demonstrated different numbers of bands in the TLC fingerprint. The variation in Rf values of the phytochemicals in the TLC fingerprint is essential for understanding their polarity, which helps in choosing a suitable solvent system for the separation, isolation, and purification of pure compounds from the plant[19]. These findings suggested that every part of *C. gigantea* is a promising material for further isolation and purification of a variety of secondary metabolites.

Table 1 Percent yields and phytochemicals of ethanolic crude extracts of different parts of *C. gigantea* from different concentrations of ethanol

Parts	%EtOH	%Yields	Total phenolic content (mg GAE/ g extract)	Total flavonoid content (mg RTE/ g extract)
Flower	50%	$25.57\pm2.20^{b,c}$	32.36 ± 2.37^{c}	$8.65\pm0.24^{b,c}$
	70%	$17.21\pm0.69^{a,c}$	29.12 ± 2.00	$11.19\pm0.18^{a,c}$
	95%	$7.92\pm1.63^{a,b}$	21.17 ± 3.87^{a}	$17.47\pm1.00^{a,b}$
Leaf	50%	$24.72\pm0.60^{b,c}$	21.74 ± 1.18^{c}	$22.11\pm4.66^{b,c}$
	70%	$21.96\pm1.26^{a,c}$	29.80 ± 3.51^{c}	$61.72\pm3.30^{a,c}$
	95%	$9.77\pm0.74^{a,b}$	$38.34\pm2.27^{a,b}$	$175.61\pm29.63^{a,b}$
Stem bark	50%	14.30 ± 2.49^{c}	$13.05\pm0.88^{b,c}$	3.59 ± 0.72^{c}
	70%	14.00 ± 2.25^{c}	25.69 ± 0.72^{a}	5.83 ± 1.15^{c}
	95%	$6.34\pm1.08^{a,b}$	22.16 ± 1.84^{a}	$8.85\pm1.13^{a,b}$
Latex	50%	6.58 ± 0.93^{c}	24.38 ± 1.50^{c}	2.02 ± 0.80
	70%	7.88 ± 0.61^{c}	30.78 ± 3.32	1.06 ± 0.25
	95%	$9.27\pm0.50^{a,b}$	34.68 ± 1.01^{a}	2.48 ± 1.41

GAE=gallic acid equivalent, RTE=rutin equivalent. All data are presented from triplicate determinations as mean±S.D. The symbols of a, b and c represented a statistically significant difference (p-value<0.05) from 50% (v/v), 70% (v/v), and 95% (v/v) ethanol, respectively

The HPLC analysis revealed that different parts of *C. gigantea* possess distinct phytochemical profiles, as evidenced by varied compound patterns and peak intensities. Most plant parts showed the presence of rutin, and the distribution of compounds varied among the flower, leaf, stem bark, and latex extracts. The extraction efficiency of the compounds was influenced by ethanol concentration; however, higher ethanol levels did not always yield greater amounts. Similarly, Lim et al.[20] demonstrated that aqueous ethanol mixtures maximized phytochemical contents compared to ethanol alone. Meanwhile, the highest gallic acid compound was the 95% ethanol extract of *Blepharis linariifolia* Pers[9]. These findings highlight the importance of selecting suitable ethanol concentrations to maximize phytochemical extraction. The chemical diversity among plant parts may help explain why each part has been used for different purposes in traditional medicine.

C. gigantea is a well-known Ayurvedic medicinal herb; various parts of this plant have been recognized as antimicrobial agents for many years. For example, extracts from the flower have been shown to inhibit the growth of *S. aureus* and *E. coli*[21]. The ethanolic leaf extracts have demonstrated significant efficacy against several microbes, including *C. tropicalis* and *P. aeruginosa*[22], which corresponds to our findings that the ethanolic leaf extracts using 50% and 70% ethanol exhibited an inhibitory effect against *P. aeruginosa* and *C. tropicalis* (Table 2, Figures 4A and 5B). None of the *C. gigantea* extracts at any ethanol concentration used in this study exhibited inhibition zones against *S. aureus*, aligning with previous reports of

Table 2 Antimicrobial activity of ethanolic crude extracts from various parts of *C. gigantea*

Parts	%Ethanol (v/v)	Inhibition zone (diameter, mm) ± S.D.					
		Gram-positive bacteria	Gram-negative bacteria		Yeast		
		S. aureus	*P. aeruginosa*	*A. baumannii*	*C. albicans*	*C. tropicalis*	*C. krusei*
Flower	50%	-	7.0±0.0	-	-	8.8±0.29	-
	70%	-	7.0±0.0	-	-	-	-
	95%	-	7.0±0.0	-	-	-	-
Leaf	50%	-	7.0±0.0	-	-	8.2±0.75	-
	70%	-	7.0±0.0	-	-	7.3±0.51	-
	95%	-	7.0±0.0	-	-	-	-
Stem bark	50%	-	8.0±0.29	-	-	-	-
	70%	-	7.0±0.0	-	-	-	-
	95%	-	7.0±0.0	-	-	-	-
Latex	50%	-	-	-	-	-	-
	70%	-	-	-	-	-	-
	95%	-	-	-	-	-	-
Streptomycin		N/A	21.0±1.0	N/A	N/A	N/A	N/A
Colistin		N/A	N/A	20.0±0.50	N/A	N/A	N/A
Vancomycin		17.7±0.58	N/A	N/A	N/A	N/A	N/A
Nystatin		N/A	N/A	N/A	22.5±0.87	25.8±0.76	22.3±0.76
DMSO		-	-	-	-	-	-

Data are expressed as mean (n=3) ± S.D., and "-" represented "no inhibition zone"

resistance in some bacterial strains, including *S. aureus*, to the methanolic extract of *C. gigantea* stem[23]. The ethanolic extracts of latex in this study also showed no activity against either *S. aureus* or *P. aeruginosa* (Table 2). Similar results were found for the latex aqueous extract against *P. aeruginosa*, though different results were reported for *S. aureus*[24]. These variations may be due to differences in the phytochemical composition of the ethanolic and aqueous extracts of the latex.

The findings from the current research revealed a range of antimicrobial properties in *C. gigantea*, supporting some of the traditional uses of this plant[25]. This discovery demonstrates the potential of various parts of *C. gigantea* for further research, particularly in identifying, isolating, and purifying bioactive compounds in their ethanolic extracts that exhibit antimicrobial activity.

Conclusion

The percentage yield of ethanolic extracts from the flower, leaf and stem bark of *C. gigantea* increased with the polarity of aqueous ethanol. In contrast, the latex extract did not follow this trend. The ethanolic crude extracts from various parts of *C. gigantea* contained a high amount of phenolics and flavonoids, and exhibited antimicrobial activity, particularly against *P. aeruginosa* and *C. tropicalis*. These findings highlight the potential of the flower, leaf, and stem bark of *C. gigantea* as promising natural sources for further isolation and purification to discover new antimicrobial agents.

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Conflict of interest

All authors have no conflict of interest.

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