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Antioxidant and antibacterial potential of *Chromolaena odorata* (L.) medicinal plant extracts

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Abstract

Chromolaena odorata, or Siam weed, is an invasive plant species that inhibits crop growth. Ethanolic extracts of *C. odorata* were assessed across various plant parts (roots, stems, leaves, and flowers) to compare their phenolic and flavonoid contents as well as their antioxidant and antibacterial activities. Results revealed that all samples, with particular emphasis on the leaf extracts, contained significant amounts of phenolic and flavonoid secondary metabolites. The ethanolic extracts of *C. odorata* exhibited strong antioxidant and antibacterial properties against gram-positive and gram-negative bacterial strains. This comprehensive methodology provides an alternative strategy for inhibiting pathogenic bacteria and indicates potential medicinal applications utilizing plant-based natural remedies.

Introduction

Siam weed, *Chromolaena odorata* (L.) R.M. King & H. Rob or *Eupatorium odoratum* is classified in the sunflower weed family (Asteraceae). This climbing perennial shrub is native to the tropics and subtropical Americas and also widely spread in many parts of the world including Sub-Saharan Africa, Asia, and Oceania.[1,2] *C. odorata* can grow in various environments and has a high reproductive rate, with high food absorption and inhibitory allelopathic effects on other plants and the ability to grow underground and in variable weather conditions.[3] *C. odorata* is a widely used medicinal plant in Asia and Africa with pharmacological benefits including treatment for malaria, wounds, diarrhea, skin infections, toothache, dysentery, stomach pain, sore throat, convulsions, coughing, and fever.[4] Various parts of this herb have antioxidant, anticancer, antidiabetic, anti-inflammatory, and antimicrobial properties.[5] The phenolic and flavonoid compounds in the leaves of *C. odorata* protected cultured skin cells from oxidative damage.[5] An *in vitro* study on *C. odorata* leaf extracts showed increase in the growth of fibroblasts, endothelial cells, and keratinocytes. The extracts also stimulated keratinocyte growth in a wound-healing assay, promoted the production of extracellular matrix proteins and basement membrane components by keratinocytes, and reduced collagen lattice contraction in fibroblasts.[6]

C. odorata is widely recognized for its potent antioxidant properties, attributed to its diverse phytochemical profile.[7] Extracts derived from plants have shown significant antifungal and antiprotozoal activities, effectively inhibiting the growth of various pathogens.[8] These extracts display anti-lipid effects, which are crucial for modulating lipid profiles in the bloodstream and supporting overall cardiovascular health.[8] The stems of *C. odorata* contain biologically active compounds including flavonoids that are recognized for their anti-inflammatory and antioxidant properties, triterpenoids, which are believed to play roles in cellular signaling and apoptosis, alkaloids that exhibit a wide range of pharmacological activities, and essential oils known for their antimicrobial effects.[2] Collectively, these constituents contribute to the plant's effectiveness in addressing various pathological conditions, making it a valuable subject for advanced pharmacological research and a promising candidate for clinical applications.[9] Different plant parts of *C. odorata* extracted with various solvents contain single substances or mixtures of biologically active substances that work together to stimulate physiological responses.[2] This research evaluated the crude extracts of *C. odorata* sourced from Thailand, focusing on their phenolic and flavonoid concentrations as well as their antioxidant and antibacterial properties.

Materials and Methods

Plant materials

Chromolaena odorata L. R.M. King & H. Rob. plants were collected from natural locations in Thailand including Surin (14°44'51.2"N 103°53'52.1"E), Pathum Thani (14°10'36.7"N 100°44'06.5"E), and Kalasin (16°15'15.7"N 103°42'27.6"E) Provinces. The samples were identified and deposited at the Sireeruckhachati Nature Learning Park, Mahidol University, Nakhon Pathom, Thailand under voucher specimen number PBM 006425. Table 1 shows the number of *C. odorata* collections. The plant samples were separated into leaves, stems, roots, and flowers, washed with clean water, and dried using a hot air oven dryer (Heraeus Heating and Drying Ovens, Thermo Scientific, Dreieich, Germany) at 60°C for 4-5 h or until the moisture content was lower than 10%. The oven-dried samples were milled to powder and placed in a bottle which was tightly closed and stored in the dark at 25°C until further extraction.

Chromolaena odorata L. extraction

The roots, stems, leaves, and flowers of oven-dried *C. odorata* were extracted with 99.9% ethyl alcohol (Duksan, Ansan-si Gyeonggi-do, South Korea) at a biomass to solvent ratio of 1:10 (w/v). The plant parts were placed in a shaker (Innova 4230, New Brunswick Scientific, NJ, USA) and shaken at 120 rpm for 24 h in the dark. The crude extract was separated from the residue by filtration through Whatman filter paper No. 41. The ethanolic extracts were evaporated using a rotary evaporator (Rotavapor® R-300, Buchi, Flawil, Switzerland). All experiments were performed in triplicate. The evaporated extracts were dissolved in absolute ethanol at a final concentration of 10 mg/mL for further analysis.

Determination of total phenolic and total flavonoid contents

The crude extracts were assessed for their total phenolic and flavonoid contents. The Folin-Ciocalteu colorimetric method was used to determine the total phenolic content (TPC) of the crude extracts.[10] Briefly, a 20 µL sample of the extracts or standard solution was mixed with 100 µL of 0.2 N Folin-Ciocalteu solution (Sisco Research Laboratories Pvt. Ltd., Mumbai, Maharashtra, India) and then added with 80 µL of 0.7 M sodium carbonate solution. The mixture was left to stand at room temperature for 8 min before adding 50 µL of distilled water and incubating at 40°C for 30 min. The absorbance was measured at 750 nm by a M965+ microplate reader (Metertech, Taipei, Taiwan). Gallic acid was used as the standard, with results expressed as mg gallic acid equivalent (mg GAE/g extract).

The Total Flavonoid Content (TFC) was analyzed using the aluminum chloride colorimetric method with slight modifications.[11] A 100 µL aliquot of the extracts or standard solution was mixed with 100 µL of 2% aluminum chloride. The mixture was incubated at room temperature for 10 min and the absorbance was measured at 405 nm by a M965+ microplate reader (Metertech, Taipei, Taiwan). Quercetin was used as the standard, with results expressed as mg quercetin equivalent (mg QE/g extract).

Determination of the antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

Sample extracts were evaluated for radical scavenging properties using the DPPH antioxidant assay (2, 2-diphenyl-1-picryhydrazyl) with slight modifications.[12] Briefly, 100 µL of each extract or standard solution was mixed with 100 µL of 200 µM DPPH solution. The mixtures were incubated at room temperature for 30 min, and the absorbance was measured at 517 nm by a M965+ microplate reader (Metertech, Taipei, Taiwan). Ascorbic acid was used as the standard, with antioxidant properties expressed as mg ascorbic acid equivalent (mg AAE/g extract).

Determination of the antioxidant activity using the 2, 2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) assay

Sample extracts were evaluated for radical scavenging properties using the ABTS assay (2, 2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt).[13] The reaction between 7 mM

Table 1. Yield of crude extraction (%) from different parts of *Chromolaena odorata* (L.) R.M. King & H. Rob. plants.

Part	*N*	Provinces	Extraction yield (%)
Roots	*N*=2	Surin Pathum Thani	$11.09^{b}\pm6.29$
Stems	*N*=2	Surin Pathum Thani	$19.68^{ab}\pm6.06$
Leaves	*N*=2	Surin Pathum Thani	$26.46^{a}\pm4.35$
Flowers	*N*=1	Kalasin	$12.22^{b}\pm0.44$

N, number of Siam weed locations. Data in the same column with different superscripts are significantly different (p <0.05).

ABTS and 245 mM ammonium persulfate at 505.05 and 5.05 µL produced the ABTS radical solution. The ABTS solution was kept at room temperature in the dark for 16 h, and diluted with distilled water to achieve a final optical density of 0.7 at 750 nm. A 10 µL aliquot of the crude extracts or standard solution was combined with 190 µL of ABTS solution. The mixture was incubated for 5 min in the dark, and the absorbance was measured at 750 nm by a M965+ microplate reader (Metertech, Taipei, Taiwan). Ascorbic acid was used as the standard, with antioxidant properties expressed as mg ascorbic acid equivalent (mg AAE/g extract).

Determination of the antioxidant activity using the ferric ion reducing antioxidant power (FRAP) assay

Sample extracts were evaluated for radical scavenging properties using the FRAP (ferric ion reducing antioxidant power) assay with slight modifications.[14] In brief, the FRAP reagent was prepared from 300 mM sodium acetate (pH 3.6), 10 mM TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) in 40 mM HCl, and 20 mM ferric chloride at 25, 2.5, and 2.5 mL, respectively. A 10 µL aliquot of the crude extracts or standard solution was then combined with FRAP reagent (190 µL) and incubated in the dark for 30 min. The absorbance was measured at 593 nm by a M965+ microplate reader (Metertech, Taipei, Taiwan). Ascorbic acid was used as the standard, with antioxidant properties expressed as mg ascorbic acid equivalent (mg AAE/g extract).

Antibacterial properties of the crude extract from different plant parts

The antibacterial activities of the ethanolic extractions of different plant parts were tested against two strains of bacteria: gram-negative *Escherichia coli* DMST 4212 and gram-positive *Staphylococcus aureus* DMST 8840 obtained from the Food Quality Assurance Service Centre (FQA), Institute of Food Research and Product Development, Kasetsart University, Bangkok, Thailand. The bacterial strains were cultured in nutrient broth (NB, Merck, Darmstadt, Germany) and incubated at 37°C for 18 h. The broth microdilution method was used to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the samples with slight modifications.[15,16] Briefly, 100 µL of sample extracts were prepared with two-fold dilutions in nutrient broth in 96-well microplates. A 100 µL aliquot of each bacterial suspension was diluted 1:100 in culture media to obtain approximately 10^5 Colony Forming Units (CFU)/mL in a total volume of 200 µL. The final ethanolic crude extract concentration ranged from 0.019 mg/mL to 10 mg/mL. The mixtures were covered with sterile lids and incubated at 37°C for 24 h. The lowest concentration of extracts that inhibited the growth of each bacterium was determined as the MIC, while the MBC was determined by subculturing the broth of clear wells onto nutrient agar.

Statistical analysis

Total phenolic and flavonoid contents and antioxidant capacities were statistically evaluated by One-Way Analysis Of Variance (ANOVA) using SPSS. Multiple comparisons of all parameters were conducted using Duncan's Multiple Range Test (DMST) with significance set at $p < 0.05$. Data were shown as mean values ± standard deviation (SD).

Results

Figure 1 shows the appearances of the roots, stems, leaves, and flowers of *Chromolaena odorata*. The yield of crude extracts from different plant parts of *C. odorata* ranged from 11.1 to 26.5% (Table 1). Leaves of *C. odorata* gave the highest extraction yield,

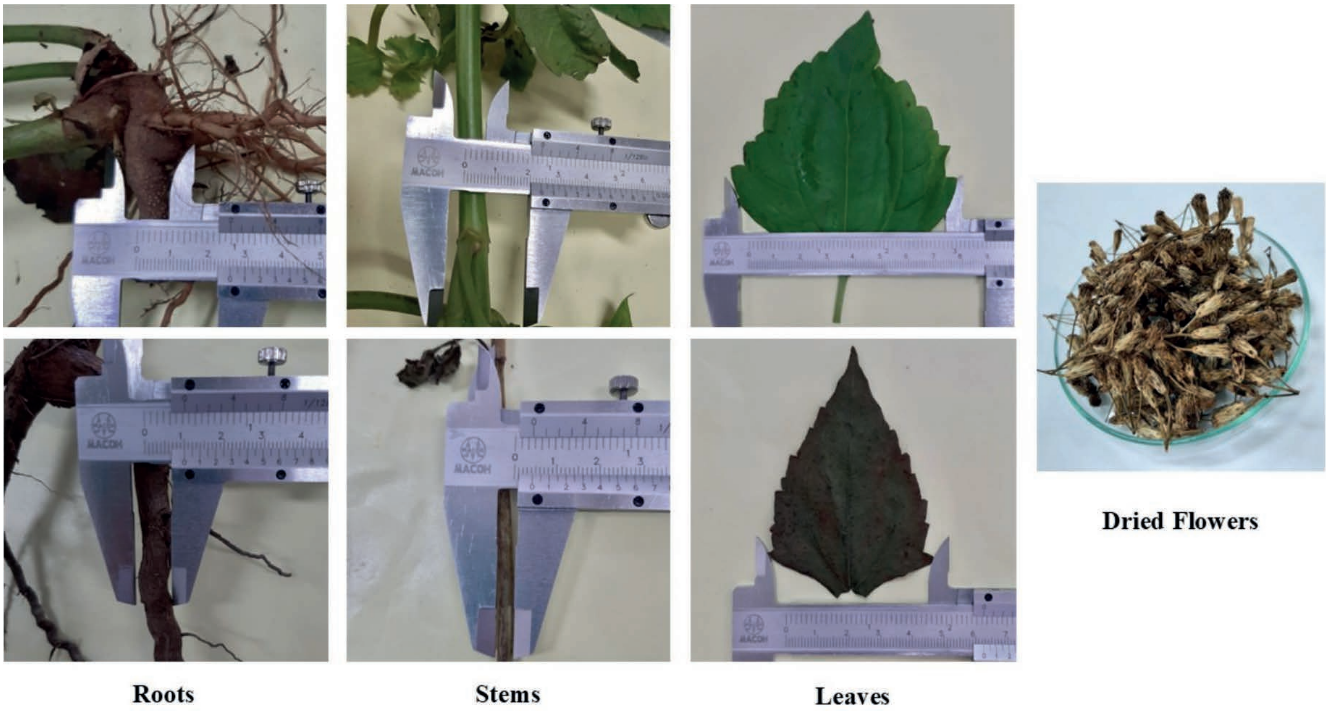

Figure 1. Appearances of the roots, stems, leaves and flowers of *Chromolaena odorata* (L.) R.M. King & H. Rob.

with non-significant difference from the stems, while the roots and flowers showed lower extraction yields.

The total phenolic and flavonoid contents of the crude extracts from various parts of *C. odorata* are shown in Table 2. The total phenolic content ranged from 0.48 to 0.73 mg GAE/g across the different plant parts, indicating a significant concentration of these bioactive compounds. The leaves of *C. odorata* had the highest total phenolic content but differences among the leaves, roots, stems, and flowers were statistically non-significant, suggesting a relatively uniform distribution of phenolic compounds throughout the plant. The total flavonoid content ranged from 11.18 to 49.63 mg QE/g. The leaves again exhibited the highest accumulation of flavonoids, reinforcing their value as a rich source of these beneficial compounds. By contrast, the roots, stems, and flowers presented comparable, albeit lower, flavonoid levels.

The antioxidant activities of the crude extracts from *C. odorata* were determined using the DPPH, ABTS, and FRAP assays, with results shown in Table 3. The antioxidant activities were quantified through these three distinct assays, each providing valuable insights into the sample's capabilities. The DPPH assay yielded antioxidant activity values ranging from 2.13 to 21.29 mg AAE/g, indicating a modest level of free radical scavenging potential. By comparison, the ABTS assay revealed a more comprehensive range of antioxidant activities, ranging from 25.73 to 128.80 mg AAE/g, suggesting a stronger capacity for neutralizing reactive oxygen species. Lastly, the FRAP assay demonstrated antioxidant activity levels between 25.04 and 84.05 mg AAE/g, further highlighting the variability in antioxidant effectiveness depending on the assay employed. The root extract from *C. odorata* gave higher scavenging activity using the DPPH assay than the other plant parts. The antioxidant activities measured by the ABTS and FRAP assays were similar, with the highest recording by the leaf extracts at 128.80 and 84.05 mg AAE/g, respectively.

Table 4 presents the antimicrobial properties of the crude extracts from *C. odorata* against two bacterial strains. All the crude extracts demonstrated inhibition of *E. coli* DMST 4212 at a MIC of 1.25 mg/mL, with MBC values ranging from 2.50 to 5.00 mg/mL. By contrast, the extracts exhibited varying degrees of efficacy against *S. aureus* DMST 8840, with MIC values between 0.63 and 2.50 mg/mL and MBC values ranging from 1.25 to 5.00 mg/mL. Notably, the leaf extract of *C. odorata* displayed the most substantial antibacterial properties, achieving MIC of 0.63 mg/mL and MBC of 1.25 mg/mL.

Table 2. Total phenolic and flavonoid contents of various *Chromolaena odorata* (L.) R.M. King & H. Rob crude extracts.

Crude extract	TPC (mg GAE/g extract)	TFC (mg QE/g extract)
Roots	0.48^{a}±0.08	11.18^{b}±5.57
Stems	0.48^{a}±0.06	12.67^{b}±0.86
Leaves	0.73^{a}±0.12	49.63^{a}±9.92
Flowers	0.64^{a}±0.13	11.25^{b}±0.33

Data in the same column with different superscripts are significantly different (p <0.05). TPC, total phenolic content; TFC, total flavonoid content; GAE, gallic acid equivalent; QE, quercetin equivalent.

Table 3. Antioxidant activities of various *Chromolaena odorata* (L.) R.M. King & H. Rob crude extracts.

Crude extract	Antioxidant (mg AAE/g extract)		
	DPPH	ABTS	FRAP
Roots	21.29^{a}±2.68	25.73^{b}±5.46	25.04^{b}±1.53
Stems	16.02^{b}±1.92	30.14^{b}±2.84	38.90^{b}±6.69
Leaves	15.48^{b}±2.08	128.80^{a}±9.86	84.05^{a}±6.23
Flowers	2.13^{c}±0.23	59.82^{b}±1.26	43.78^{b}±3.46

Data in the same column with different superscripts are significantly different (p <0.05). AAE, ascorbic acid equivalent; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2, 2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, FRAP, ferric ion reducing antioxidant power.

Table 4. Antibacterial properties of various *Chromolaena odorata* (L.) R.M. King & H. Rob crude extracts

Crude extract	MIC (mg/mL)	MBC (mg/mL)
E. coli DMST 4212		
Roots	1.25	5.00
Stems	1.25	2.50 – 5.00
Leaves	1.25	2.50 – 5.00
Flowers	1.25	2.50
S. aureus DMST 8840		
Roots	1.25 - 2.50	2.50
Stems	1.25	2.50 – 5.00
Leaves	0.63 – 1.25	1.25
Flowers	1.25	5.00

MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration.

Discussion

Chromolaena odorata, also known as citronella grass, is a common plant that grows in various locations and has recently gained interest due to its biological potential.[17] Plants utilized in traditional medicine have been studied to assess their biological activities. This research evaluated the antioxidant and antibacterial properties of extracts from various plant parts and the extraction yields varied significantly. Plant growth is influenced by environmental factors including nutrition, light, temperature, water, and humidity.[18] Therefore, the location of plant harvesting affects growth and secondary metabolites.[19] A previous investigation of the phytochemical profile of *C. odorata* identified several key metabolites including alkaloids, free anthracene, coumarins, flavonoids, mucilage, tannins, reducing agents, saponins, quinone derivatives, and steroids with significant variations in phytochemical contents across different geographical locations.[20]

The average extraction yield from experiments varied from 11.09 to 26.46%. Another study found that *C. odorata* ethanolic leaf extract had a yield of 8.42%, while extraction yields obtained with water, methanol, and hexane were 12.16%, 10.45%, and 2.37%, respectively.[21] Plant extracts containing antioxidants mainly contain polyphenols (such as phenolic acids, flavonoids, anthocyanins, lignin and stilbenes), carotenoids and vitamins.[22] Polyphenols are natural antioxidants that offer a wide range of biological effects and are considered one of the most important factors in evaluating the health benefits of plants.[23] Polyphenols are natural chemical substances found in foods and medicinal plants that exhibit biological effects and possess antioxidant activity.[24] Results revealed that the leaf extracts had the highest concentrations of phenolic and flavonoid compounds, concurring with previous research that focused on methanolic extracts of *C. odorata* derived from various plant parts including flowers, stems, and leaves.[7] The methanolic extracts of the leaves and flowers of *C. odorata* showcased elevated levels, with phenolic contents of 182.26±1.99 mg GAE/g for the leaves and 172.65±0.48 mg GAE/g for the flowers. The flavonoid contents were 128.57±7.62 mg QE/g for leaves and 121.74±7.06 mg QE/g for flowers.[7] A previous study on the ethanolic extract of *C. odorata* leaves identified a range of phenolic and flavonoid secondary metabolites. Notable phenolic acids included protocatechuic acid, ferulic acid, and vanillic acid. The extract also contained various flavonoid aglycones such as sinensetin, rhamnetin, tamarixetin, and kaempferide.[5] Previous studies indicated that essential oils derived from plant roots contain several bioactive compounds, notably himachalol (a sesquiterpene), 7-isopropyl-1,4-dimethyl-2-azulenol, androencecalinol, and 2-methoxy-6-(1-methoxy-2-propenyl) naphthalene. A dichloromethane extract of the flowers yielded various flavonoids and chalcones including acacetin, luteolin, isosakuranetin, persicogenin, 5,6,7,4'-tetramethoxyflavanone, and 4'-hydroxy-5,6,7-trimethoxyflavanone.[25,26] The ethanolic extract from the leaves of *Annona muricata* L. revealed 24 distinct types of secondary metabolites including the three most notable, dodecanoic acid, 1,2,3-propanetriyl esters, and glycerol trilaurate, while the water extract exhibited a diverse range of metabolites such as phenolic compounds, tannins, flavonoids, alkaloids, and steroids and demonstrated superior antioxidant activity in comparison to the ethanol-extracted samples.[27] Our ethanolic extracts from *C. odorata* contained phenolics and flavonoids, similar to previous reports of secondary metabolites.[27]

Plants possess an inherent capability to produce various non-enzymatic antioxidants that reduce oxidative damage caused by Reactive Oxygen Species (ROS). Numerous *in vitro* techniques have been utilized to evaluate the antioxidant potential of plants. Most of these methods have revealed their strong antioxidant activity.[28] Our study confirmed the high antioxidant potential of all the *C. odorata* extracts, consistent with previous findings of the effectiveness of *C. odorata* leaves in terms of antioxidant capacity from isolated flavonoids.[29] Previous studies also reported the presence of antioxidant properties in *C. odorata* extracts[1,4] using DPPH and ABTS free-radical scavenging activities and the Ferric Reducing Antioxidant Potential (FRAP) assay.[30] Polysaccharides extracted from *C. odorata* leaves recorded significant DPPH and ABTS radical scavenging activities.[30] Our results indicated that the extracts of *C. odorata* showed antibacterial activity, with the highest inhibition observed in leaf extracts against *S. aureus*. However, all the plant part extracts (roots, stems, leaves, and flowers) were effective against *E. coli* and *S. aureus*. Previous studies concurred with our results. The ethanolic extract inhibited the growth of *S. aureus*, while the aqueous extract inhibited the growth of *E. coli*.[31] Antibacterial activity against wound-isolated *E. coli*, *S. aureus*, *Pseudomonas aeruginosa*, and *Klebsiella* species was recorded by crude extracts of *C. odorata* leaves, with MIC values ranging from 25% to 50%.[32] Inhibition by leaf extracts from *C. odorata* against *S. aureus*, *S. pyogenes*, *E. coli*, and *K. pneumoniae* demonstrated MIC values of 3.125, 0.781, 12.5, and 0.781 mg/mL, respectively.[33] The leaf extracts of *C. odorata* showed significant antibacterial properties, with effectiveness observed from 0.156 mg/mL to 1.25 mg/mL.[34] The application of silver nanoparticles synthesized from the leaf extract of *C. odorata* in acetone, ethanol, methanol, butanol, diethyl ether, n-hexane, and distilled water exhibited significant antibacterial and antifungal activities, with the exception of the butanol solvent.[33] Antibacterial activity was reported from *C. odorata*, with antifungal activity also confirmed from ethanol and methanol leaf extracts.[35] The acetone extract of *C. odorata* demonstrated significant antibacterial activity against gram-negative bacteria isolated from wounds. This extract was characterized by its rich composition of bioactive compounds including phenols, saponins, tannins, glycosides, steroids, terpenoids, and flavonoids which may contribute to its therapeutic potential.[36] Therefore, *C. odorata* showed promise as a potential source of polyphenolic secondary metabolites with excellent antimicrobial properties.[37] These findings suggest that *C. odorata* can inhibit bacterial growth at relatively low concentrations, underscoring their potential application as a natural antimicrobial agent in various fields including pharmacology and agriculture. Further investigations into the specific compounds responsible for antimicrobial activity and their mechanisms of action will provide valuable insights to develop novel antibacterial therapies.

Conclusions

Chromolaena odorata showed notable potential regarding its phenolic and flavonoid content, along with robust antioxidant and antibacterial properties. Our results indicated that ethanolic extracts, particularly from the leaves, can serve as effective natural agents against pathogenic bacteria. This research highlights the intricate role of *C. odorata* within the ecosystem and also paves the way for its medicinal applications, offering a sustainable alternative in the battle against bacterial infections. Further exploration and development will enhance our understanding and utilization of this plant in therapeutic contexts.

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