

Phytochemical screening study in different parts of *Chromolaena odorata* by LC MS method and related parameters

G. Bhavani Devi ¹, K.S. Ramya ², Dappula Sandhya Sri ¹, P. Josthna ³ and C.V. Naidu ^{1,*}

¹ Department of Biotechnology, Dravidian University, Kuppam-517426, A.P., India.

² Department of Biotechnology, Shri Gnanambica Degree College, Madanapalli-517325, A.P. India.

³ Department of Biotechnology, Sri Padmavathi Mahila Visvavidyalayam (Women's University), Tirupati- 517502, A.P. India.

Abstract

Chromolaena odorata (Syn: *Eupatorium odoratum* L.) (Asteraceae) is a perennial herb consisting of biologically potent chemical. It is mainly found in the humid tropics and sub-tropics in worldwide. *C. odorata* displayed allelopathic effects and have been reported to cause livestock death. *C. odorata* is used against dysentery, malaria, diarrhea, wound healing, toothache and headache in traditional medicine. In the present study, we investigated the assays tested for the presence of several phytochemicals (alkaloids, terpenoids, flavonoids, saponins, phenols, cardiac glycosides, resins and anthraquinones) in different extracts which included ethanol, methanol, petroleum ether and aqueous. All the extracts were tested against Bacteria. Furthermore, we studied phytochemical screening by QTOF-MS analysis it represents various acids, flavonoids etc. Phytochemical profiling revealed the presence of approximately 20 compounds each from the leaf and stem extracts whereas root consisted of 10 compounds. Among these, the main compounds were Vanillic acid, Chlorogenic acid, Benzoic acid, Genkwanin, Naringenin and Luteolin. The ethanol and methanol extracts were found active alongside the tested bacteria as they showed potential phytochemical constituents. The bacteria, *Escherichia coli* and *Bacillus subtilis* showed promising activity against ethanol and methanol leaf, stem and root extracts which proved that the plant extracts are potential candidates for antibiotic resistance against such bacteria. In addition, the Chlorogenic acid present in leaf, stem and root extracts of the plant helps process blood sugar in a way that helps prevent and reduce the number of blood sugar spikes. It also helps boost metabolism, helping people with diabetes maintain a healthy weight.

Keywords: QTOF-MS; *Chromolaena odorata*; Acids and phenolic compounds Phytochemical screening; Various extracts (Ethanol; Methanol; Petroleum ether and Aqueous); Antibacterial activity

1. Introduction

Chromolaena odorata is the family of Asteraceae, is a weed of 13 plantation harvests and fallows of 23 countries and the species *Chromolaena* includes small shrubs, herb, distributed chiefly in the Americas, a few in Asia, Europe, and tropical Africa [1]. *C. odorata*, and closely associated *Eupatorium* spp., have been revealed in France, Thailand, China and Indo-China [2]. To Compare with other large families, such as Leguminosae, the number of significant products derived from the family Compositae is low [3]. *C. odorata* is a longwinded and scrambling perennial shrub that grows to a height of 3-7 m in the open [4]. It is a productive weed that flourishes in the majority of soil types, is found in abundance on open wasteland and along roadsides, and stops the formation of other vegetation [5]. *C. odorata* is a toxic plant that holds

* Corresponding author: CV Naidu

Department of Biotechnology, Dravidian University, Kuppam-517426, A.P., India.

remarkably high levels of nitrates in young plants, at 5-6 times superior than the toxic wildlife [6]. It is considered a hazard owing it affects estates and other ecosystems due to its destructive nature [7].

C. odorata fresh leaves has been used conventionally in numerous tropical countries as a treatment for tissue wounds, leech bite, skin infections, and burns [8]. The leaf extract of *C. odorata* is also known to prevent the growth of bacterial strains [8-12]. *C. odorata* extracts have been described to show potent activity against *Mycobacterium tuberculosis* [13], anti-inflammatory activity [14], invitro antioxidant activity [15]. Earlier examinations of the leaves, stems and flowers of *C. odorata* stated that it possesses of steroids [16], essential oils [16-18], triterpenes [19], flavonoids [20], fats [21] and alkaloids [22]. The present study was planned to study the phytochemical screening of *C. odorata* by QTOF-MS analysis and various biological properties, up to our knowledge there are no reports for the combine study (QTOF-MS and Bioactivity) of the *C. odorata*

2. Material and methods

2.1. Collection of plant material

The whole plant (leaves, stems and roots) of *C. odorata* was collected from the herbal garden at Dravidian University campus, Chittor district, Andhra Pradesh. Botanical evidence for plant material was established and well documented using data available in previous literature.

2.2. Chemicals

HPLC grade methanol, formic acid, and ammonium formate solvents were brought from Hi media. Mobile phase was prepared using double-distilled water.

2.3. Extraction of plant material

Plant samples taken from the field were first rinsed with distilled water to remove soil from the plants, dried on paper towels in the shade for 1 week, and placed in an airtight container at room temperature (23 °C). The dried leaves were coarsely ground in a blender before extraction.

Soxhlet extractor thimbles were packed separately with 50 g of dried leaf, stem and root powder and extracted with various polar and non-polar solvents such as ethyl alcohol, methanol, chloroform, ethyl acetate, hexane and diethyl Ether separately.

Hexane and chloroform extracts were prepared at temperatures below 600 °C, i.e., 30 °C to 400 °C, and methanol and ethyl acetate extracts were prepared at temperatures between 60 °C and 800 °C for approximately 12 to 16 hours. The extract was concentrated under vacuum to a dry mass following the procedure of Harbourne (1973) [23].

2.4. Qualitative and Quantitative Phytochemical Analysis

Phytochemical screening of reconstituted extracts obtained after Soxhlet extraction according to the method of the Indian Pharmacopoeia [24], phytochemical analysis of all solvent extracts was conducted. This assay tested for the presence of several phytochemicals (alkaloids, terpenoids, flavonoids, saponins, phenols, cardiac glycosides, resins and anthraquinones). Depending on the colour intensity, the concentrations of detected metabolites are absent (-), low (+), moderate (++) , high (+++) and very high (++++).

Mayer's test became implied for trying out alkaloids. Presence of cream coloured precipitate shows the presence of alkaloids [25]. For terpenoids, a 0.5 ml extract + 2 ml chloroform + three ml conc. Sulphuric acid, reddish brown coloration of the interface indicated the presence of terpenoids [26]. For flavonoids, a 2 ml filtrate + conc. HCl + magnesium ribbon purple tomato colour formation indicated the presence of flavonoids [27]. Formation of solid continual froth indicated the presence of saponins [28] while combined vigorously with distilled water. For phenols, 1 ml of every solvent extract dissolved in alcohol and when treated with few ml of neutral ferric chloride, colour change was observed which indicated the presence of phenols [29]. For cardiac glycosides, a brown ring shows a deoxy sugar feature of cardenolides [30]. For resins, to at least one ml of extract 10 ml of distilled water was added and for 15 min ultra-sonicated at 30 °C and the presence of turbidity indicates the presence of resins [31]. For anthroquinones, extract is blended with benzene and 10% ammonium solution, purple satiation formation withinside the ammonical segment shows the presence of anthroquinones [32]. Antioxidants have been measured with the aid of using DPPH (2, 2 diphenyl 1 picrylhydrazyl) free radical scavenging activity and the proportion became measured with the aid of using the formula $[(A_0 - A_1)/A_0] \times 100$ in which A₀ is absorbance of the control and A₁ is absorbance of the extracts [33].

2.5. LC-MS analysis

Identification of the phytochemical constituents present in leaf, stem, and root extracts of *C. odorata* was performed using an Agilent 6530, equipped with an LC-QTOF system (Inertial ODS-4, 150 x 2.1 mm, 3 µm) using stationary column phase. The mobile phases were 80: 20, 5 mM ammonium formate in water-methanol and 0.1% formic acid (mobile phase A) and 10: 90 5 mM ammonium formate in water-methanol and 0.1% formic acid (mobile phase B). It consisted of Gradient elution started at 90% A/10% B 0-2 min, 5% A/95 B 25-30 min, 90% A/10% B 32-55 min, increasing the temperature to 45 °C with a flow rate of 0.4 ml. /min set. The injection volume was 5 µl, the total run time was 35 minutes, and mass spectrometry was performed on a model number FSLs (AS)-RES-QTOF-MS-01. Acquisition modems was used to record mass spectral data over the mass range from m/z 50 to 3000. Gas temperature (°C): 300, Gas flow (L/min): 10, Nebulizer (psi): 45, Shield gas temperature (°C): 350, Shield gas flow (L/min): 11, Compounds were analysed using V Cap (Volt) in: 3500, V Charging: 500, Fragmentor: 135, Skimmer: 65, Octopole RF peak: 750.

2.6. Antibacterial activity

2.6.1. Preparation of bacterial inoculum

Gram-negative bacteria *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and Gram-positive bacteria *Bacillus subtilis* were cultured in broth medium and cell densities were measured spectrophotometrically.

2.6.2. Antibacterial assay

Antibacterial activity was tested using the previously used agar disk method [34] with slight modifications [35]. A nutrient agar medium was prepared, sterilized and allowed to cool to solidify the medium. Immediately prior to solidification, 0.1 ml of a diluted inoculum of test organism (10⁵ CFU/ml) was added to the medium and poured into sterile petri dishes under aseptic conditions. These plates were then cooled and 6 mm filter paper discs were impregnated with known concentrations of antimicrobial compounds on agar plates (50 µl of 500 µg/ml plant extract). Plates were incubated at 37 °C for 18 hours. Antimicrobial activity was assessed by measuring the zone of inhibition (in millimetres) against test organisms. The antibiotic ciprofloxacin at a concentration of 500 µg/ml was used in the test system as a positive control and DMSO as a negative control.

2.7. Statistical analysis

All experimental results were carried out in six-fold and were expressed as average of six analyses ± SD (Standard deviation).

3. Results and discussion

3.1. Phytochemical screening in *Chromolaena odorata* extracts

The results obtained from *C. odorata* leaves, stems, and roots for phytochemical analysis indicated the presence of alkaloids, terpenoids, flavonoids, saponins, glycosides, resins, anthraquinones, and phenols in four different solvent extractions i.e., ethanol, methanol, petroleum ether and aqueous extractions (Table 1).

Moderate activity of Steroids was observed with aqueous and ethanol extract of leaf while weak activity was observed with other extracts except PE. Only traces of Terpenoids were observed in all extracts except PE with which no activity was revealed. Very strong activity of Flavonoids was exhibited by aqueous extract and strong activity by ethanol extract of leaf. Moderate activity was found with methanol extract of leaf while weak activity with other extracts except PE extract of root and stem. Leaf extract with water showed higher concentration of Alkaloids while with methanol and ethanol revealed moderate concentration. Other extracts indicated only weak activity. Saponins were found in moderate concentration in leaf and stem with all solvents except PE extracts of all three parts and methanol extract of leaf as well as root extracts with the four solvents, which showed only traces of saponins. Glycosides were found in higher concentration in ethanol extracts of leaf and stem along with aqueous extract of leaf. Aqueous extracts of root and stem as well as methanol extract of leaf exhibited moderate activity while the remaining extracts showed only weak activity. Only aqueous and ethanol extracts revealed moderate concentrations of Phenols while the other extracts showed traces of Phenols. Traces of Resins and Anthraquinones were observed only in leaf and stem while they were not found in root.

Table 1 Phytochemical screening of *C. odorata* leaf, stem and root extracts

Phytochemicals	Extracts	Plant parts of <i>Chromolaena odorata</i>		
		Leaf	Stem	Root
Alkaloids	Aqueous	+++	+	+
	Ethanol	++	+	++
	Methanol	+++	+	+
	Petroleum Ether	+	+	--
Terpenoids	Aqueous	+	++	+
	Ethanol	+	+	+
	Methanol	++	+	+
	Petroleum Ether	--	--	--
Flavonoids	Aqueous	+++	+	++
	Ethanol	+++	+	+
	Methanol	+++	++	+
	Petroleum Ether	+	+	+
Saponins	Aqueous	++	++	++
	Ethanol	++	++	+
	Methanol	+	+	+
	Petroleum Ether	+	--	+
Phenols	Aqueous	++	+	++
	Ethanol	++	+	++
	Methanol	++	++	+
	Petroleum Ether	+	+	+
Cardiac glycosides	Aqueous	+++	++	+
	Ethanol	+++	+++	+
	Methanol	++	+	+
	Petroleum Ether	+	--	+
Resins	Aqueous	+	+	--
	Ethanol	+	--	--
	Methanol	+	--	--
	Petroleum Ether	+	--	--
Anthraquinones	Aqueous	+	--	--
	Ethanol	+	--	--
	Methanol	+	--	--
	Petroleum Ether	--	+	--

Absent (-), less (+), moderate (++) , high (+++) and very high (++++)

3.2. Total phenols

The total phenol content of different plant parts of *C. odorata* plant in different extracts were represented in table 2. The aqueous extract of leaf showed high amount of total phenolics (16.11 ± 0.80 mg/gm) followed by the methanolic leaf extract (15.01 ± 0.20 mg/gm). The least amount was noted in the petroleum ether extract of stem (4.01 ± 0.68 mg/gm). Phenolic compounds are a class of widely distributed secondary metabolites in plants, consisting of thousands of compounds with different structures, from simple phenolic acids to complex macromolecules. Differences in total phenolic compounds recorded in different parts of the plant may be due to contributions to the formation of phenolic profiles upon metabolism of photosynthetic mesophilic tissue or typical phloem exudates.[34]

Table 2 Total phenol content (mg/gm) of different plant parts of *C. odorata* plant in different extracts

Plant part	Different extracts (mg/gm)			
	Aqueous	Ethanol	Methanol	Petroleum Ether
Leaf	16.11 ± 0.80	14.14 ± 0.24	15.01 ± 0.20	5.26 ± 0.40
Stem	4.81 ± 0.50	6.02 ± 0.40	13.15 ± 0.11	4.01 ± 0.68
Root	11.12 ± 0.13	10.81 ± 0.56	6.32 ± 0.35	7.81 ± 0.31

3.3. DPPH free radical scavenging assay

The plant parts contain free radical scavengers like polyphenols, flavonoids and phenolic compounds. The antioxidant activity of aqueous extract in 5 concentrations (100 µg, 200 µg, 300 µg, 400 µg and 500 µg) of various parts of *C. odorata* was examined by comparing it to the activity of standard antioxidant Ascorbic acid (Table -3).

Table 3 DPPH free radical scavenging assay of different plant parts of *C. odorata* plant in aqueous extract

	Concentration of the sample				
	100 µg	200 µg	300 µg	400 µg	500 µg
Ascorbic acid (Standard)	47.21 ± 0.41	56.73 ± 0.35	65.54 ± 0.97	68.43 ± 0.35	74.20 ± 0.98
Leaf	30.24 ± 0.91	38.54 ± 0.51	48.36 ± 1.01	57.76 ± 1.15	62.31 ± 0.37
Stem	20.17 ± 0.61	29.02 ± 0.60	38.35 ± 0.97	46.66 ± 0.90	51.71 ± 0.90
Root	15.05 ± 0.80	24.93 ± 0.65	31.32 ± 0.72	40.01 ± 0.70	46.17 ± 0.35

Values are mean $\pm$ S.E. of six independent determinations.

The aqueous leaf extract exhibited the highest percentage of antioxidant activity at a concentration of 500 µg, followed by the aqueous root and stem extracts. The DPPH activity of aqueous extracts of leaves, stems and roots of *C. odorata* was lower than that of standard ascorbic acid. This may be because the balance between production and removal of reactive oxygen species is disturbed under some stressful conditions such as salinity, drought, high light, and toxicity due to metals and pathogens. The natural antioxidants present in plant parts are responsible for inhibiting the toxic consequences of oxidative stress [35].

3.4. Phytochemical screening in *Chromolaena odorata* leaf extract

The results obtained from *Chromolaena odorata* leaves, stem, and roots for finding the phytochemical compounds in various extracts. The data indicates the presence of monoterpenes, sesquiterpenes hydrocarbons, triterpenes/steroids, alkaloids and flavonoids.

The QTOF-MS study of the *C. odorata*. Leaf extract consisting of 20 compounds which were presented in Table 4. The compound peak at m/z 269.03, 315.04, and 329.06 ($C_{15}H_{10}O_5$) $[M-H]^-$, $[M+HCOO]^-$, and $[M+CH_3COO]^-$ with retention time 16.52 min which indicates Apigenin [36]. *C. odorata* leaf have been found to be a rich source of flavonoids such as quercetin peak at m/z 303.04 ($C_{15}H_{10}O_7$) $[M+H]^+$ with retention time 8.57. It also consists of phenolic acids such as (Protocatechuic acid, Benzoic acid and P-Coumaric acid) with their relative abundance. A peak at m/z 273.07, 294.97 ($C_{15}H_{12}O_5$) $[M+H]^+$, $[M+Na]^+$ with the retention time of 16.43 min which indicates Naringenin. Compound was eluted with retention time 14.19 min that showed a peak at m/z 287.05, 309.03 ($C_{15}H_{10}O_6$) $[M+H]^+$, $[M+Na]^+$ is referred as

Luteolin. The compound which exhibits the peak at m/z 355.10, 377.08 ($C_{16}H_{18}O_9$) $[M+H]^+$, $[M+Na]^+$ at retention time 2.80 min corresponds to chlorogenic acid. While the compound that shows peak at m/z 611.15, 633.13 in 8.57 min refers to Rutin ($C_{27}H_{30}O_{16}$) $[M+H]^+$, $[M+Na]^+$. All the obtained results are match with literature reports [37]. In this methanolic extract we have find 11 new compounds such as Genkwanin, Protocatechuic acid Hexaside, Cirsiliol, Carnosol, Catechin, Chrysoeriol, Rosmarinic acid, Luteolin 3' glucoside, Hyperoside and Luteolin-7-O-rutinoside were identified in QTOF-MS analysis. In this Genkwanin (5-hydroxy-2-(4-hydroxyphenyl)-7-methoxy-4H-chromen-4-one) is found in all ionic positions. The compounds retention time, mass value, chemical formula and ionic nature is presented in Table 4.

Table 4 QTOF -MS profile of methanol extracts of *Chromolaena odorata* leaf

S. No	Tentative compound	RT (min)	m/z	Formula	Ion
1	Apigenin	16.52	269.03	$C_{15}H_{10}O_5$	$[M-H]^-$
			315.04	$C_{15}H_{10}O_5$	$[M+HCOO]^-$
			329.06	$C_{15}H_{10}O_5$	$[M+CH_3COO]^-$
2	Genkwanin	17.20	283.05	$C_{16}H_{12}O_5$	$[M-H]^-$
			286.07	$C_{16}H_{12}O_5$	$[M+H]^+$
			307.05	$C_{16}H_{12}O_5$	$[M+Na]^+$
			329.06	$C_{16}H_{12}O_5$	$[M+HCOO]^-$
3	Protocatechuic acid Hexaside	17.02	315.07	$C_{13}H_{16}O_9$	$[M-H]^-$
			361.16	$C_{13}H_{16}O_9$	$[M+HCOO]^-$
			339.06	$C_{13}H_{16}O_9$	$[M+Na]^+$
4	Cirsiliol	17.20	329.06	$C_{17}H_{14}O_7$	$[M-H]^-$
			331.08	$C_{17}H_{14}O_7$	$[M+H]^+$
			353.06	$C_{17}H_{14}O_7$	$[M+Na]^+$
5	Carnosol	22.48	329.17	$C_{20}H_{26}O_4$	$[M-H]^-$
			375.18	$C_{20}H_{26}O_4$	$[M+HCOO]^-$
			391.15	$C_{20}H_{26}O_4$	$[M+CH_3COO]^-$
6	Benzoic acid	5.39	123.04	$C_7H_6O_2$	$[M+H]^+$
7	P-Coumaric acid	13.42	165.05	$C_9H_8O_3$	$[M+H]^+$
			187.06	$C_9H_8O_3$	$[M+Na]^+$
8	Apigenin	14.66	271.05	$C_{15}H_{10}O_5$	$[M+H]^+$
9	Naringenin	16.43	273.07	$C_{15}H_{12}O_5$	$[M+H]^+$
			294.97	$C_{15}H_{12}O_5$	$[M+Na]^+$
10	Luteolin	14.19	287.05	$C_{15}H_{10}O_6$	$[M+H]^+$
			309.03	$C_{15}H_{10}O_6$	$[M+Na]^+$
11	Catechin	18.22	313.06	$C_{15}H_{14}O_6$	$[M+Na]^+$
12	Chrysoeriol	18.77	301.07	$C_{16}H_{12}O_6$	$[M+H]^+$
			323.04	$C_{16}H_{12}O_6$	$[M+Na]^+$
13	Quercetin	8.57	303.04	$C_{15}H_{10}O_7$	$[M+H]^+$
14	Hesperidin	13.86	303.08	$C_{16}H_{14}O_6$	$[M+H]^+$
			326.07	$C_{16}H_{14}O_6$	$[M+Na]^+$

15	Chlorogenic acid	2.80	355.10	C ₁₆ H ₁₈ O ₉	[M+H] ⁺
			377.08	C ₁₆ H ₁₈ O ₉	[M+ Na] ⁺
16	Rosmarinic acid	16.77	361.01	C ₁₈ H ₁₆ O ₈	[M+H] ⁺
			383.07	C ₁₈ H ₁₆ O ₈	[M+ Na] ⁺
17	Luteolin 3' glucoside	8.56	449.10	C ₂₁ H ₂₀ O ₁₁	[M+H] ⁺
18	Hyperoside	8.22	465.09	C ₂₁ H ₂₀ O ₁₂	[M+H] ⁺
		9.91	487.08	C ₂₁ H ₂₀ O ₁₂	[M+ Na] ⁺
19	Luteolin-7-O-rutinoside	4.53	595.16	C ₂₇ H ₃₀ O ₁₅	[M+H] ⁺
			617.14	C ₂₇ H ₃₀ O ₁₅	[M+ Na] ⁺
20	Rutin	8.57	611.15	C ₂₇ H ₃₀ O ₁₆	[M+H] ⁺
			633.13	C ₂₇ H ₃₀ O ₁₆	[M+ Na] ⁺

3.5. Phytochemical screening in *Chromolaena odorata* stem extract

The methanolic extracts of *Chromolaena odorata* stem was studied by using QTOF -MS method. According to literature reports tentatively identified 27 compounds which were presented in Table 5. The Ferulic acid, P-hydroxybenzoic acid, P-Coumaric acid, Vanillic acid, Gallic acid and Ferulic acids are belongs to phenolic acid derivatives are present in stem extract which are similar to the earlier reports [38]. Benzoic acid, Naringenin, Genkwanin, Luteolin, Chrysoeriol, Quercetin, Hesperidin, Protocatechuic acid Hexaside, Cirsiliol, Luteolin-7-O-rutinoside, Rutin, P-Coumaric acid, and Apigenin these compounds are exactly match with the methanolic extract of *C. odorata* leaf. On the other hand, Verbenacinine, 3,5-Di-caffeoylquinic acid, Trans-Cinnamic acid, Rosmarinic acid, Methyl arsonate, Caffeic acid and Hesperidin were separately identified in stem extracts, i.e., based on the obtained results the stem consisting of very good bioactive compounds compared to the leaf and the corresponding data are represented in Table 5.

Table 5 QTOF -MS profile of methanol extracts of *Chromolaena odorata* stem

Sl. No	Tentative compound	RT (min)	m/z	Formula	Ion
1	Benzoic acid	22.64	121.02	C ₇ H ₆ O ₂	[M-H] ⁻
			123.04	C ₇ H ₆ O ₂	[M+H] ⁺
			145.07	C ₇ H ₆ O ₂	[M+ Na] ⁺
			167.03	C ₇ H ₆ O ₂	[M+HCOO] ⁻
			181.04	C ₇ H ₆ O ₂	[M+CH ₃ COO] ⁻
2	Trans-Cinnamic acid	19.39	149.05	C ₉ H ₈ O ₂	[M+H] ⁺
			193.04	C ₉ H ₈ O ₂	[M+HCOO] ⁻
			207.05	C ₉ H ₈ O ₂	[M+CH ₃ COO] ⁻
3	Ferulic acid	12.42	195.06	C ₁₀ H ₁₀ O ₄	[M+H] ⁺
			217.05	C ₁₀ H ₁₀ O ₄	[M+ Na] ⁺
4	Naringenin	16.42	273.07	C ₁₅ H ₁₂ O ₅	[M+H] ⁺
			271.05	C ₁₅ H ₁₂ O ₅	[M-H] ⁻
			317.06	C ₁₅ H ₁₂ O ₅	[M+HCOO] ⁻
			331.09	C ₁₅ H ₁₂ O ₅	[M+CH ₃ COO] ⁻
5	Genkwanin	18.86	285.07	C ₁₆ H ₁₂ O ₅	[M+H] ⁺
			283.02	C ₁₆ H ₁₂ O ₅	[M-H] ⁻

			307.05	C ₁₆ H ₁₂ O ₅	[M+ Na] ⁺
			329.06	C ₁₆ H ₁₂ O ₅	[M+HCOO] ⁻
			343.08	C ₁₆ H ₁₂ O ₅	[M+CH ₃ COO] ⁻
6	Luteolin	14.34	287.05	C ₁₅ H ₁₀ O ₆	[M+H] ⁺
			285.03	C ₁₅ H ₁₀ O ₆	[M-H] ⁻
			345.07	C ₁₅ H ₁₀ O ₆	[M+CH ₃ COO] ⁻
7	Chrysoeriol	18.86	301.07	C ₁₆ H ₁₂ O ₆	[M+H] ⁺
			323.05	C ₁₆ H ₁₂ O ₆	[M+ Na] ⁺
8	Quercetin	8.55	303.05	C ₁₅ H ₁₀ O ₇	[M+H] ⁺
			302.03	C ₁₅ H ₁₀ O ₇	[M-H] ⁻
9	Hesperidin	13.84	303.08	C ₁₆ H ₁₄ O ₆	[M+H] ⁺
			301.07	C ₁₆ H ₁₄ O ₆	[M-H] ⁻
			325.06	C ₁₆ H ₁₄ O ₆	[M+ Na] ⁺
			347.09	C ₁₆ H ₁₄ O ₆	[M+HCOO] ⁻
			361.06	C ₁₆ H ₁₄ O ₆	[M+CH ₃ COO] ⁻
10	Protocatechuic acid Hexaside	14.76	315.07	C ₁₃ H ₁₆ O ₉	[M+H] ⁺
			317.08	C ₁₃ H ₁₆ O ₉	[M+ Na] ⁺
11	Cirsiliol	18.94	331.08	C ₁₇ H ₁₄ O ₇	[M+H] ⁺
			329.06	C ₁₇ H ₁₄ O ₇	[M-H] ⁻
			353.06	C ₁₇ H ₁₄ O ₇	[M+ Na] ⁺
12	Rosmarinic acid	18.92	361.09	C ₁₈ H ₁₆ O ₈	[M+H] ⁺
13	Luteolin-7-O-rutinoside	9.94	595.16	C ₂₇ H ₃₀ O ₁₅	[M+H] ⁺
			617.14	C ₂₇ H ₃₀ O ₁₅	[M+ Na] ⁺
14	Rutin	8.31	611.15	C ₂₇ H ₃₀ O ₁₆	[M+H] ⁺
			609.14	C ₂₇ H ₃₀ O ₁₆	[M-H] ⁻
			634.14	C ₂₇ H ₃₀ O ₁₆	[M+ Na] ⁺
			655.12	C ₂₇ H ₃₀ O ₁₆	[M+HCOO] ⁻
			669.23	C ₂₇ H ₃₀ O ₁₆	[M+CH ₃ COO] ⁻
15	Methyl arsonate	2.00	135.03	C ₃ H ₆ O ₃	[M+HCOO] ⁻
			149.04	C ₃ H ₆ O ₃	[M+CH ₃ COO] ⁻
16	P-hydroxybenzoic acid	6.42	137.02	C ₇ H ₆ O ₃	[M-H] ⁻
			183.06	C ₇ H ₆ O ₃	[M+HCOO] ⁻
			197.05	C ₇ H ₆ O ₃	[M+CH ₃ COO] ⁻
17	Protocatechuic acid	2.08	153.01	C ₇ H ₆ O ₄	[M-H] ⁻
		4.66	213.06	C ₇ H ₆ O ₄	[M+CH ₃ COO] ⁻
18	P-Coumaric acid	5.72	163.03	C ₉ H ₈ O ₃	[M-H] ⁻
			209.04	C ₉ H ₈ O ₃	[M+HCOO] ⁻
19	Vanillic acid	5.57	167.03	C ₈ H ₈ O ₄	[M-H] ⁻

			212.98	C ₈ H ₈ O ₄	[M+HCOO] ⁻
			227.04	C ₈ H ₈ O ₄	[M+CH ₃ COO] ⁻
20	Gallic acid	1.60	169.01	C ₇ H ₆ O ₅	[M-H] ⁻
			215.03	C ₇ H ₆ O ₅	[M+HCOO] ⁻
			229.03	C ₇ H ₆ O ₅	[M+CH ₃ COO] ⁻
21	Caffeic acid	3.92	179.03	C ₉ H ₈ O ₄	[M-H] ⁻
			225.07	C ₉ H ₈ O ₄	[M+HCOO] ⁻
			239.07	C ₉ H ₈ O ₄	[M+CH ₃ COO] ⁻
22	Ferulic acid	8.60	193.04	C ₁₀ H ₁₀ O ₄	[M-H] ⁻
			239.06	C ₁₀ H ₁₀ O ₄	[M+HCOO] ⁻
			255.11	C ₁₀ H ₁₀ O ₄	[M+CH ₃ COO] ⁻
23	Apigenin	14.87	269.04	C ₁₅ H ₁₀ O ₅	[M-H] ⁻
			315.05	C ₁₅ H ₁₀ O ₅	[M+HCOO] ⁻
			329.07	C ₁₅ H ₁₀ O ₅	[M+CH ₃ COO] ⁻
24	Verbenacinine	26.14	377.23	C ₂₀ H ₃₀ O ₃	[M+CH ₃ COO] ⁻
25	3,5-Di-caffeoylquinic acid	8.78	515.12	C ₂₅ H ₂₄ O ₁₂	[M-H] ⁻
26	Luteolin-7-O-rutinoside	9.99	595.16	C ₂₇ H ₃₀ O ₁₅	[M+H] ⁺
			593.14	C ₂₇ H ₃₀ O ₁₅	[M-H] ⁻
			617.09	C ₂₇ H ₃₀ O ₁₅	[M+ Na] ⁺
			653.16	C ₂₇ H ₃₀ O ₁₅	[M+CH ₃ COO] ⁻
27	Hesperidin	8.77	669.20	C ₂₀ H ₃₄ O ₁₅	[M+CH ₃ COO] ⁻

3.6. QTOF -MS profile of methanol extracts of *Chromolaena odorata* root

The QTOF -MS study of methanol extracts of *Chromolaena odorata* root is consisting of 10 compounds. Leaf, Stem, Root, among these three stem is consisting of no of phytochemicals which were mainly used in various applications such as wound healing, antimicrobial, and anticancer and so on. The three parts (L, S, R) commonly consisting of Benzoic acid, Genkwanin, Chrysoberyl and Quercetin compounds and the obtained compounds some are matching in leaf and stem. In root Carnosic acid was identified which is not present in Leaf and Stem. The methanolic extracts of *C. odorata* obtained results are completely match with the previous reports of ethanolic extracts of *C. odorata*. The chemical compounds molecular mass, chemical formula and their relative ions of *C. odorata* are present in Table 6.

Table 6 LC-MS profile of methanol extracts of *Chromolaena odorata* root

Sl. No	Tentative compound	RT (min)	m/z	Formula	Ion
1	Benzoic acid	5.39	121.03	C ₇ H ₆ O ₂	[M-H] ⁻
			167.03	C ₇ H ₆ O ₂	[M+HCOO] ⁻
			181.04	C ₇ H ₆ O ₂	[M+CH ₃ COO] ⁻
2	P-hydroxybenzoic acid	6.421	137.02	C ₇ H ₆ O ₃	[M-H] ⁻
			197.04	C ₇ H ₆ O ₃	[M+CH ₃ COO] ⁻
3	Trans-Cinnamic acid	11.43	147.04	C ₉ H ₈ O ₂	[M-H] ⁺
			193.05	C ₉ H ₈ O ₂	[M+HCOO] ⁻
			207.06	C ₉ H ₈ O ₂	[M+CH ₃ COO] ⁻
4	Genkwanin	18.90	283.06	C ₁₆ H ₁₂ O ₅	[M-H] ⁻
			285.07	C ₁₆ H ₁₂ O ₅	[M+H] ⁺
			329.08	C ₁₆ H ₁₂ O ₅	[M+HCOO] ⁻
			343.17	C ₁₆ H ₁₂ O ₅	[M+CH ₃ COO] ⁻
5	Chrysoeriol	18.89	299.08	C ₁₆ H ₁₂ O ₆	[M-H] ⁻
			301.07	C ₁₆ H ₁₂ O ₆	[M+H] ⁺
6	Carnosic acid	22.27	291.20	C ₂₀ H ₂₈	[M+ Na] ⁺
7	Quercetin	31.78	303.05	C ₁₅ H ₁₀ O ₇	[M+H] ⁺
8	Verbenacinine	23.44	319.22	C ₂₀ H ₃₀ O ₃	[M+H] ⁺
9	Chlorogenic acid	4.35	355.10	C ₁₆ H ₁₈ O ₉	[M+H] ⁺
			377.08	C ₁₆ H ₁₈ O ₉	[M+ Na] ⁺
10	Luteolin 3' glucoside	24.66	449.11	C ₂₁ H ₂₀ O ₁₁	[M+H] ⁺
			471.05	C ₂₁ H ₂₀ O ₁₁	[M+ Na] ⁺

3.7. Antibacterial activity

Antibacterial activity with ethanolic and methanolic extracts showed good activity against various microorganisms tested at several levels. Initial phytochemical testing indicated that alkaloids, terpenoids, flavonoids, phenols, cardiac glycosides and saponins were the main components of ethanol and aqueous extracts of leaves, stems and roots. Thus, one can decide which phytochemicals are the main antibiotic agents of this plant. The results on the antibacterial activity of different plant part extracts are presented in Table 7. Based on the results, the ethanolic and methanolic extracts showed activity better than petroleum ether and aqueous extracts.

The methanolic root extract showed significant inhibitory activity against methicillin-resistant bacteria such as *Staphylococcus aureus*, possibly due to the presence of Quercetin which have some ability to help prevent diseases like cancer and heart disease and reduce choroiditis. Research shows it does this by preventing harmful cells from accumulating in the body. In general, the majority of quercetin studies have been conducted in animals or in cell cultures [39]. Early research shows that luteolin might reduce the brain's production of inflammatory cytokines. This effect may help maintain recall the age. Luteolin exhibits antioxidant activity that is particularly helpful for muscle and nerve cells [40]. Similarly, Sundar and Habibur [41] reported phytochemicals and antioxidants from peels, leaves, and fruits. This study showed that extracts from *C. odorata* can be used as a medicinal extract and also illustrates application for its antioxidant and antimicrobial activities.

Table 7 Antibacterial activity of leaf, stem and root extracts of *Chromolaena odorata* with standard antibiotic ciprofloxacin

Type of extract	Zone of inhibition (or) antibacterial activity (in mm)			
	<i>Escherichia coli</i>	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Klebsiella pneumoniae</i>
Leaf				
Ethanol	25.23	20.32	25.84	17.12
Methanol	35.34	22.51	37.96	25.55
Petroleum Ether	15.56	10.73	15.57	11.27
Aqueous	--	--	10.29	--
Stem				
Ethanol	24.31	25.34	24.30	35.13
Methanol	22.45	19.25	19.21	23.24
Petroleum Ether	13.13	11.18	15.13	--
Aqueous	--	--	--	--
Root				
Ethanol	20.30	15.57	20.22	20.36
Methanol	24.42	20.26	23.34	25.43
Petroleum Ether	12.20	15.43	--	--
Aqueous	--	--	--	--
Antibiotic Ciprofloxacin	27.36	29.72	28.58	28.36

4. Conclusion

C. odorata is the best medicinal plant and is of utmost importance because of its valuable alkaloids, terpenoids, flavonoids, phenols and secondary metabolites (glycosides and saponins). Plant extracts that inhibit the growth of pathogenic bacteria without harming the host may have potential applications as therapeutic agents. Therefore, the present study attempted to evaluate the antibacterial activity of the crude, leaf, stem and root extracts of *C. odorata* using selected pathogenic bacterial strains. Ethanol, methanol, petroleum ether, and subsequent aqueous extracts of the leaf, stem, and root extracts of *C. odorata* were analyzed for their phytochemical components. Most of the chemical components were found in aqueous extracts, ethanol and methanol in the three test samples. The ethanol and methanol extracts showed good activity against the tested pathogenic bacteria because they showed potential phytochemical constituents. Of all the bacteria tested *B. subtilis* and *E. coli* showed the highest activity in ethanol and methanol extracts. Therefore, extracts from the leaves, stems and roots of *C. odorata* contain important antibacterial compounds.

Compliance with ethical standards

Acknowledgment

The authors are grateful to UGC, New Delhi for sanctioned Mid Carrer Award to Prof. C. Varadarajulu Naidu to carry out the present research work.

Disclosure of conflict of interest

The authors declare no conflict of interest.

References

- [1] Phan TT, Wang L, See P, Grayer RJ, Chan SY and Lee ST: Phenolic compounds of *Chromolaena odorata* protect cultured skin cells from oxidative damage: Implication for cutaneous wound healing. *Biol Pharm Bull* 24: 1373-1379, 2001.
- [2] Phan TT, Hughes MA and Cherry GW: Effects of an aqueous extract from the leaves of *Chromolaena odorata* (Eupolin) on the proliferation of human keratinocytes and on their migration in an in vitro model of epithelialization. *Wound Repair Regen* 9:305-313, 2001.
- [3] Heywood VH, Harborne JB and Turner BL: *Biology and Chemistry of the Compositae*. Vol. 1. Academic Press, London: 1977.
- [4] Ambika SR and Jayachandra: Influence of light on seed germination in *Eupatorium odoratum* L. *Ind Forester* 106: 637-640, 1980.
- [5] Warea O: *Chromolaena* (Siam) weed. Pest Advisory Leaflet no. 43, Secretariat of the Pacific Community, 2004.
- [6] Sajise PE, Palis RK, Norcio NV and Lales JS: The Biology *C. odorata* L. King and Robinson. Flowering behavior, pattern of growth and nitrate metabolism. *Phil Weed Sci Bull* 1: 17-24, 1974.
- [7] Muniappan R and Bamba J: Biological control of *Chromolaena odorata*: Successes and Failures. *Proceedings of the X International Symposium on Biological Control of Weeds* 81, 1999, Montana State University, Bozeman, Montana, USA. Neal R. Spencer [ed.]. 2000, 81-85.
- [8] Le TT. (1995) The use of Eupolin prepared from *Eupatorium odoratum* to treat soft tissue wounds. The 5th European tissue repair society annual meeting, Padova, Italy (Abst.30).
- [9] Akash PA. (1990) Anti-microbial effects of crude *Chromolaena odorata* leaf extract. *International Journal of Crude Drug Research*, 28, 233-235.
- [10] Bamba D, Bessiere JM, Marion C, Pelissier Y, Fourate I. (1993) Essential oil of *Eupatorium odoratum*. *Planta Medica*, 59, 184-185.
- [11] Irobi ON. (1992) Activities of *Chromolaena odorata* (Compositae) leaf extract against *Pseudomonas aeruginosa* and *Streptococcus faecalis*. *Journal of Ethnopharmacology*, 37, 81-83.
- [12] Cáceres A, Menéndez H, Méndez E, Cohobón E, Samayoa BE, Jauregui E, Peralta E, Carrillo G. (1995) Anti-gonorrheal activity of plants used for the treatment of sexually transmitted diseases. *Journal of Ethnopharmacology*, 48, 85-88.
- [13] Collins LA, Franzblau SG. (1997) Microplate Alomar blue assay versus BACTEC 460 system for high-throughput screening of compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium*. *Antimicrobial Agents and Chemotherapy*. 41, 1004–1009.
- [14] Owoyele, BV; Adedeji, JO and Soladoye, AO (2005), "Anti-inflammatory activity of aqueous leaf extract of *Chromolaena odorata*", *Inflammopharmacology*, Vol. 13, 479–484.
- [15] A, Balakrishna; P, Josthna and CV, Naidu (2014), "Evaluation of in vitro antioxidant activity of root bark of *Chromolaena odorata* - an important antidiabetic medicinal plant", *Pharmacophore*, Vol. 5 (1), 49-57.
- [16] Inya-Agha SI, Oguntimein BO, Sofowora A, Benjamin TV. (1987) Phytochemical and antibacterial studies on the essential oil of *Eupatorium odoratum*. *International Journal of Crude Drug Research*, 25, 49-52.
- [17] Lamaty G, Menut C, Amvam Zollo PH, Kuiate JR, Bessiere JM, Quamba JM, Silou T. (1992) Aromatic plants of tropical central Africa, IV. Essential oils of *Eupatorium odoratum* from Cameroon and Congo. *Journal of Essential Oil Research*, 4, 101-105.
- [18] Chowdhury AR. (2002) Essential oils of the leaves of *Eupatorium odoratum* from Shillong (N.E). *Journal of Essential Oil Bearing Plants*, 5, 14- 18.
- [19] Ahmad M, Nabi MN. (1967) Chemical investigations on the leaves of *Eupatorium odoratum*. *Scientific Research*, Dacca, Pakistan, 4, 154-157.
- [20] Talapatra SK, Bhar DS, Talapatra B. (1974) Flavonoid and terpenoid constituents of *Eupatorium odoratum*. *Phytochemistry*, 13, 284-285.
- [21] Talapatra SK, Bhar DS, Talapatra B. (1977) Terpenoids and related compounds; Part XIII. *Indian Journal of Chemistry*, 15B, 806-807.

- [22] Baruah RN, Pathak MG. (1993) Fatty acid compositions of *Chromolaena odorata* flower fat. Indian Journal of Natural Products, 9, 17-18.
- [23] Biller A, Boppre M, Witte L, Hartmann T. (1994) Pyrrolizidine alkaloids in *Chromolaena odorata*. Chemical and chemo ecological aspects. Phytochemistry, 35, 615-619.
- [24] Harborne JB. Phytochemical methods: a guide to modern techniques of plant analysis. Chapman and Hall Ltd, London, 1984.
- [25] Zakaria Zainul Amiruddin, Somchit MN, Zaiton Hala, Jais AM, Sulaiman Mohd Roslan et al., The in vitro Antibacterial Activity of *Corchorus olitorius* Extracts. Int J Pharmacol, 2006; 2: 10.3923/ijp.2006.213.215.
- [26] R Udayakumar, K Velmurugan, D Srinivasan, Raghu Ram Krishna. Phytochemical and antimicrobial studies of extracts of *Solanum xanthocarpum*. Anc Sci Life, 2003; 23: 90.
- [27] Kaufman PB, Cseke LJ, Warber S, Duke JA, Briellmann HL. Natural Products from Plants. Phytomedicines: Back to the future. 2nd ed CRC Press, 1999; 1589-1592.
- [28] Wink M. Function of plant secondary metabolites and their exploitation in biotechnology. Sheffield Academic Press and CRC Press, Annual Plant Reviews, 1999; 3: 362.
- [29] Humayun S, Ibrar M, Barkatullah, Ahmad I. Comparison of three extracts of *Fumaria indica* for the evaluation of cytotoxic and phytotoxic activities. Int J Biosci, 2012; 2(12): 112-119.
- [30] Ajiboye BO, Ibukun EO, Edobor G, Ojo AO, Onikanni SA. Qualitative and quantitative analysis of phytochemicals in *Senecio bialfrae* leaf. Int J Invent Pharm Sci., 2013; 1(5): 428-432.
- [31] Sheel R, Nisha K, Kumar J. Preliminary phytochemical screening of methanolic extract of *Clerodendron infortunatum*. IOSR J Appl Chem 2014; 7(1): 10-13.
- [32] Djaafar Z, Ridha OM. Phytochemical study of selected medicinal plant, *Solanum nigrum*, the Algerian Dessert. Int Let Chem Phys Astron, 2014; 1: 25-30.
- [33] Raaman N. Phytochemical techniques. New India Publishing, New Delhi, 2006.
- [34] Jaradat N, Hussien F, Ali AA. Preliminary phytochemical screening, quantitative estimation of total flavonoids, total phenols and antioxidant activity of *Ephedra alata Decne*. J Mater Environ Sci., 2015; 6(6): 1771-1778.
- [35] Mir SA, Mishra AK, Reshi ZA, Sharma MP. Preliminary phytochemical screening of some Pteridophytes from District Shopian (J & K). Int J Pharm Sci., 2013; 5(4): 632-637.
- [36] Perez C, Pauli M and Bazerque P. An Antibiotic Assay by Agar Well Diffusion Method. Acta Biologiae et Medicinae Experimentalis, 1990; 15: 113-115.
- [37] Ahmad I and Beg AZ. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. J Ethnopharmacology, 2001; 74(2): 113-23. Doi: 10.1016/s0378-8741(00)00335-4. PMID: 11167029.
- [38] Kirby WMM, Yoshihara GM, Sundsted KS and Warren JH. Clinical usefulness of a single disc method for antibiotic sensitivity testing. Antibiotic Annu, 1957; 892.
- [39] Dar KB, Bhat AH, Amin S, Zargar MA, Masood A, et al. Evaluation of Antibacterial, Antifungal and Phytochemical Screening of *Solanum nigrum*. Biochem Anal Biochem, 2017; 6: 309.
- [40] 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 (Basel), 2019; 8(7): 237. doi:10.3390/antiox8070237
- [41] Pallavi Sharma, Ambuj Bhushan Jha, Rama Shanker Dubey, Mohammad Pessarakli. Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. J Bot, 2012; 12: 26. pages <https://doi.org/10.1155/2012/217037>