



Antibacterial Screening of *Chromolaena odorata* Root Extracts and *in silico* Physico-chemical Properties of their Bioactive Compounds

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ABSTRACT

Bacterial infection constitutes global health challenges due to antimicrobial resistance prevalence, and the search for natural and potent antibacterial agents continues. This study aimed to evaluate the antibacterial activity *Chromolaena odorata* root aqueous and methanolic extracts, and to also predict *in silico* the physicochemical properties of their bioactive compounds. The plant roots were air-dried and ground into fine powder using mortar and pestle. The aqueous and methanolic extracts were prepared using the *C. odorata* root powder. The chemical composition of the two extracts was analyzed using gas chromatography-mass spectrometry (GC-MS) to identify their bioactive compounds. The physicochemical properties of the identified compounds were then predicted using the SwissADME server. Additionally, the antibacterial properties of the extracts were evaluated by testing their inhibitory effects on the growth of four bacterial species: *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella spp.*, and *Salmonella spp.* The aqueous and methanolic extracts of *C. odorata* roots showed 20 compounds having 10 peaks, and 19 compounds having 10 peaks respectively. This study's findings highlight two compounds from *C. odorata* roots with promising physicochemical properties, making them potential drug candidates: 1-dioxide-4-methyl-3-(4-methyl-1-piperziny) from the methanolic extract and 2,3-dihydroxypropylleaidate from the aqueous extract. The methanolic extract demonstrated superior antibacterial activity against *S. aureus*, *E. coli*, and *Salmonella spp.*, while the aqueous extract showed greater efficacy against *Klebsiella spp.* Although the targeted bacteria may develop resistance to these extracts, our results indicate that both extracts possess antibiotic properties, warranting further investigation.

Keywords: Antibacterial, *Chromolaena odorata*, Characterization, Bioactive, Physicochemical

Introduction

Bacterial infection diseases (BIDs) pose a significant global threat, exacerbated by escalating antibiotic resistance (Imarenezor *et al.*, 2020). Bacteria like *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella spp.*, and *Salmonella*

manifest various infections, ranging from meningitis to urinary tract infections, contributing to the burden of BIDs (Mahajan *et al.*, 2015; Akpaka *et al.*, 2018; Li *et al.*, 2023). Despite the development of antibiotics targeting specific bacterial pathogens, the emergence of antibiotic resistance and associated side effects have

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prompted the exploration of more potent and natural alternatives (Fasugba *et al.*, 2015; Maka and Popowska, 2016; McGuinness *et al.*, 2017; Hartanyo *et al.*, 2020).

Chromolaena odorata, known as "ewe Akintola" in Western Nigeria, has been recognized for its diverse pharmacological properties, including antibacterial activity (Sukanya *et al.*, 2011). However, while its ecological and medicinal aspects have been extensively studied, comprehensive chemical characterization of its root aqueous extract and *in silico* evaluation of the physicochemical properties of its bioactive compounds remain unexplored (Bamisaye *et al.*, 2014). As the knowledge of physicochemical properties of drug candidates is crucial in drug discovery and development (O'Shea and Moser, 2008), this study aims to investigate the antibacterial potential of the aqueous extract of *C. odorata* root (COR). Additionally, it seeks to characterize the bioactive constituents of the extract and predict their physicochemical properties, shedding light on the therapeutic potential of *C. odorata* as a natural antibacterial agent and contributing to the advancement of drug discovery efforts in combating BIDs.

Materials and Methods

Chromolaena odorata roots; Distilled water; SWISSADME web server; Mentholated spirit; Ethanol and Nutrient agar; Measuring cylinder; Water bath; Beaker; Air tight bottle; weighing balance.

Collection of Plant Sample

A large amount of *Chromolaena odorata* was obtained from the garden of Kwara State Polytechnic, Ilorin. The plant was identified and authenticated at the Plant Biology Herbarium at the University of Ilorin with the voucher number UILH/001/1282/2024. The *C. odorata* roots were removed, cleaned, rinsed, and air-dried for 21 days.

Preparation and Extraction of Plant Sample

The extraction of *C. odorata* roots was carried out using the method described by Owoyele *et al.* (2005).

GC-MS Analysis of Extracted Root and Leaves of Siam Weed and in silico Prediction of their Physicochemical Properties

The GC-MS of *C. odorata* was carried out using the method described by Lawal *et al.* (2015), while the method of Jaiswal *et al.* (2021) was used for physicochemical predictions.

Antibacterial Analysis

Preparation of Culture Media and Antibacterial Screening

With slight modification, the method reported by Sulaiman (2018) was used for the preparation of nutrient agar, which was used as the media for antibacterial screening of the extracts. The extracts were screened for antibacterial activity against Gram-positive bacteria, *Staphylococcus aureus*, and Gram-negative bacteria; *Escherichia coli*, *Klebsiella spp.*, and *Salmonella*. The zone of inhibition was measured (triplicates) in mm using a ruler, which described how effective the extract was on the microorganisms. For the positive controls, tetracycline was used for all the bacteria. Negative controls used were the bacteria with neither the addition of known antibacterial agent nor the extracts. The inhibition zones were used to determine the bacteria susceptible to both the antibacterial drugs and the extracts according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (Böhm *et al.*, 2021). The Susceptible, intermediate and resistance inhibition zones for tetracycline against the bacteria is ≥ 19 mm, 15-18 mm and ≤ 14 mm (Böhm *et al.*, 2021).

Statistical Analysis

Data was presented as the mean \pm standard error of the mean (SEM) of three replicates. All data was statistically evaluated with SPSS version 25

(IBM Corporation, 2017). $P < 0.05$ was considered statistically significant in all analyses.

Result

GC-MS Analysis

The aqueous and methanolic extracts of *C. odorata* roots contained 21 compounds having 10 peaks, and 24 compounds having 10 peaks respectively (tables 1 and 2).

Physicochemical Properties of Characterized Compounds

As predicted *in silico*, out of the compounds characterized from aqueous extract of *C. odorata* roots, (1H-Pyrrol-3-yl) acetic acid had the highest water solubility (ESOL) value (table 3), and decanoic acid had the lowest ESOL value (-8.55). A compound characterized from methanolic extract of *C. odorata* roots, 1-dioxide-4-methyl-3-(4-methyl-1-piperziny), had highest ESOL value (table 4).

Table 1: The Compounds Characterized from Aqueous Extract of COR

S/N	Peak	Compound name	Retenti on time	Peak Area
1	1	1-Methyl-1H-pyrrole	3.412	1.17
2	1	(1H-Pyrrol-3-yl) acetic acid	3.412	1.17
3	2	3-Oxatricyclo[3.2.1.0(2,4)]octane	4.623	0.09
4	2	Bi-2-cyclohexen-1-yl	4.623	0.09
5	2	2,4-Heptadiene, (E,E)-	4.623	0.09
6	3	Hexanoic acid	5.384	0.79
7	3	Heptanoic acid	5.384	0.79
8	4	Dodecanoic acid	7.806	0.00
9	4	Nonanoic acid	7.806	0.00
10	5	Docosanoic acid	10.032	-0.04
11	5	Oleic Acid	10.032	-0.04
12	6	Undecanoic acid	10.454	0.02
13	7	n-Hexadecanoic acid	10.708	0.00
14	7	Pentadecanoic acid	10.708	0.00
15	8	2-octyl- Cyclopropaneoctanal	11.356	0.07
16	8	9-Hexadecenoic acid	11.356	0.07
17	8	2,3-Dihydroxypropyl elaidate	11.356	0.07
18	9	Dodecanoic acid, 1,2,3-propanetriyl ester	12.792	0.59
19	9	1-aminomethyl- Cyclododecanol	12.792	0.59
20	10	Lauric anhydride	13.581	8.52

Table 2: The compounds Characterized from Methanolic Extract of COR

S/N	Peak	Compound Name	Retention Time	Peak Area
1	1	1-methyl-1H-pyrrole,	3.412	0.88
2	1	N-(2-Cyanoethyl)-pyrrole	3.412	0.88
3	2	Fenchol,	4.285	0.00
4	3	Dodecanoic acid	8.623	0.00
5	4	2-Tridecenal	8.905	0.10
6	4	(E)-5-Ethyl-1-nonene	8.905	0.10
7	4	7-chloro 1,2,4-Benzothiadiazine	8.905	0.10
8	4	1-dioxide-4-methyl-3-(4-methyl-1-piperziny)	8.905	0.10
9	5	n-Decanoic acid	10.003	-0.04
10	6	Octadecanoic acid	10.201	0.00
11	7	n-Hexadecanoic acid	10.595	0.02
12	7	Tridecanoic acid	10.595	0.02
13	8	Cis-vaccenic acid	11.553	0.10
14	8	15-Tetracosenoic acid	11.553	0.10
15	8	Methyl ester	11.553	0.10
16	8	(z)-oleyl alcohol	11.553	0.10
17	8	Heptafluorobutyrate	11.553	0.10
18	10	Lauric anhydride	13.591	8.36
19	10	Carazolol	13.591	8.36

Antibacterial Analysis

Table 5 shows the zone of inhibition (diameter) of microbial growth by *C. odorata* aqueous and methanolic extracts in *S. aureus*, *E. coli*, *Klebsiella spp.* and *Salmonella spp.* There was no inhibition zone in the negative control for all the bacteria, while tetracycline used for positive control, showed higher inhibition zones than the two extracts in all of the bacteria. However, it had inhibition zones lower than 15 mm (the breakpoint for intermediate susceptibility). The mean of inhibition zone of methanolic extract (12.17 ± 0.60) was significantly higher ($p=0.001$) than that of aqueous extract (9.50 ± 0.29) in inhibiting the growth of *S. aureus*, while the inhibition of *E. coli* by methanolic extract (14.50 ± 0.29) was higher ($p=0.001$) than that of the aqueous extract (10.17 ± 0.73). *Klebsiella spp.* growth inhibition by aqueous extract (7.50 ± 0.29) was significantly higher ($p=0.001$) than that of the methanolic extract (8.50 ± 0.50). There was no significant ($p=0.001$) difference between the mean of methanolic (8.33 ± 0.33) and aqueous

(7.00±0.29) extracts in *Salmonella spp.* growth inhibition.

Table 3: Physicochemical Properties and Drug likeness of Bioactive Components of Aqueous Extract of *C. odorata* Roots

S/N	Compounds	Water solubility (ESOL)	Solubility Class	Lipophilicity (ILOGP)	PAINS	Lead Likeness	Lipinski Drug likeness
1	1-Methyl-1H-pyrrole	-1.72	Very soluble	1.58	-	-	0 violation
2	(1H-Pyrrol-3-yl) acetic acid	-1.00	Very soluble	0.69	-	-	0 violation
3	3-oxatricyclo[3.2.1.0(2,4)]octane	-1.52	Very soluble	1.92	-	-	0 violation
4	Bi-2-Cyclohexene-1-yl	-3.48	Soluble	2.86	-	-	0 violation
5	2,4-Heptadiene(E,E)	-2.03	Soluble	2.43	-	-	0 violation
6	Hexanoic acid	-1.51	Very soluble	1.57	-	-	0 violation
7	Heptanoic acid	-1.84	Very soluble	1.79	-	-	0 violation
8	Dodecanoic acid	-3.07	Soluble	2.70	-	-	0 violation
9	Nonanoic acid	-2.51	Soluble	2.30	-	-	0 violation
10	Docosanoic acid	-7.16	Poorly soluble	5.26	-	-	1 violation
11	Oleic acid	-5.41	Moderately soluble	4.27	-	-	1 violation
12	Undecanoic acid	-3.19	Soluble	2.74	-	-	0 violation
13	n-Hexadecanoic acid	-5.02	Moderately soluble	3.85	-	-	1 violation
14	Pentadecanoic acid	-4.66	Moderately soluble	3.66	-	-	0 violation
15	2-Octyl-cyclopropanoic acid	-3.91	Soluble	3.37	-	-	1 violation
16	9-Hexadecenoic acid	-4.70	Moderately soluble	3.58	-	-	0 violation
17	2,3-Dihydroxypropyl laurate	-0.25	Very soluble	1.40	-	-	0 violation
18	Decanoic acid, 1,2,3-tripropyl ester	-8.55	Poorly Soluble	7.02	-	-	2 violation
19	1-aminomethyl-cyclododecanol	-3.29	Soluble	2.64	-	-	0 violation
20	Lauric anhydride	-7.20	Poorly soluble	5.67	-	-	1 violation

P: PAINS: Pan-assay Interference compounds; LL: Lead Likeness.

Table 4: Physicochemical Properties and Drug likeness of Bioactive Components of Methanolic Extract of *C. odorata* Roots

S/N	Compounds	Water solubility (ESOL)	Solubility Class	Lipophilicity (ILOGP)	PAINS	Lead Likeness	Lipinski Drug likeness
1	1-Methyl-1H-pyrrole	-1.72	Very soluble	1.58	-	-	0 violation
2	N-(2-Cyanoethyl)-pyrrole	-1.05	Very soluble	1.47	-	-	0 violation
3	Fenchol	-2.79	Soluble	2.42	-	-	0 violation
4	Dodecanoic acid	-3.07	Soluble	2.70	-	-	0 violation
5	2-Tridecenal	-3.74	Soluble	3.35	-	-	0 violation
6	(E)-5-Ethyl-1-nonene	-3.72	Soluble	3.34	-	-	1 violation
7	7-Chloro-1,2,4-Benzothiadiazine	-2.34	Soluble	1.50	-	-	0 violation
8	1-dioxide-4-methyl-3-(4-methyl-1-piperziny)	-0.41	Very Soluble	1.78	-	-	0 violation
9	n-Decanoic acid	-2.96	Soluble	2.50	-	-	0 violation
10	Octadecanoic acid	-5.73	Very soluble	4.30	-	-	1 violation
11	n-Hexadecanoic acid	-5.02	Moderately soluble	3.85	-	-	1 violation
12	Tridecanoic acid	-3.95	Soluble	3.21	-	-	0 violation
13	Cis-vaccenic acid	-5.41	Moderately soluble	4.12	-	-	1 violation
14	15-Tetracosanoic acid	-7.59	Poorly soluble	5.54	-	-	1 violation
15	Methyl ester	-2.66	Soluble	2.03	-	-	0 violation
16	(Z)-Oleyl alcohol	-5.21	Moderately Soluble	4.73	-	-	1 violation
17	Heptafluorobutyrate	-2.77	Soluble	1.99	-	-	0 violation
18	Lauric anhydride	-7.20	Poorly soluble	5.67	-	-	0 violation
19	Carazolol	-3.99	Soluble	2.23	-	-	0 violation

P: PAINS: Pan-assay Interference compounds; LL: Lead Likeness.

Table 5: Zone of inhibition (diameter) of Microbial Growth by *C. odorata* Aqueous and Methanolic Extracts in Selected Microbes

Bacteria	Negative Control (mm)	Positive Control (mm)	Aqueous Extract (mm)	Methanolic Extract (mm)
<i>S. aureus</i>	0	14.86±0.31 ^c	9.50±0.29 ^a	12.17±0.60 ^b
<i>Escherichia coli</i>	0	14.01±0.45 ^c	10.17±0.73 ^a	14.50±0.29 ^b
<i>Klebsiella spp.</i>	0	13.99±0.10 ^c	8.50±0.50 ^b	7.50±0.29 ^a
<i>Salmonella spp.</i>	0	12.95±0.23 ^c	7.00±0.29 ^a	8.33±0.33 ^b

Values were expressed as mean ± standard error of mean (SEM) of three replicates. Means with different superscripts along the rows are statistically different (p < 0.05)

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Discussion

C. odorata extracts have been documented to contain phytochemicals such as steroids, triterpenes, flavonoids, and phenolic compounds, with reported applications in wound dressing and combating skin infections (Sukanya *et al.*, 2011). This study investigated the antibacterial activity of aqueous and methanolic extracts of *C. odorata* roots, alongside their *in silico* physicochemical properties. Consideration of parameters like solubility, Lipinski's drug-likeness, lead-likeness, lipophilicity, hydrophilicity, pan-assay interference compound, and synthetic accessibility is crucial for evaluating drug properties, impacting drug discovery success rates (Jia *et al.*, 2020). Drug solubility is vital for oral absorption and bioavailability (Meanwell, 2018). Compounds like (1H-Pyrrol-3-yl) acetic acid, with high solubility, may enhance absorption, while those with poor water solubility, like decanoic acid and Lauric anhydride, might require prodrug design or nanotechnology for improved solubility (Joshi *et al.*, 2019). Lipophilicity facilitates drug permeability through biological membranes, affecting absorption, as seen with decanoic acid, 1,2,3-propanetriylester's potential as an antibacterial agent due to its high lipophilicity (Zhang *et al.*, 2019). Lipinski's rule serves as a guide for drug development, but violations observed in certain compounds indicate challenges that may necessitate further optimization (Lipinski *et al.*, 1997). Compounds

like 1-dioxide-4-methyl-3-(4-methyl-1-piperziny) and 2,3-dihydroxypropylalaidate from methanolic and aqueous extracts, respectively, exhibit promising antibacterial properties without violating Lipinski's rule. This is the first study that combines *in silico* and *in vitro* analyses to predict *C. odorata* root bioactive compounds' properties and investigate their antibacterial activity, respectively.

The methanolic extract demonstrated greater efficacy against *S. aureus* and *E. coli* compared to the aqueous extract, consistent with previous studies (Imarenezor *et al.*, 2020). However, this is contrary to the reports of Hanphakphoom *et al.* (2016). Interestingly, the aqueous extract showed more efficacy against *Klebsiella spp.*, indicating specific compounds or mechanisms at play. Although there was bacterial growth inhibition in all bacterial, it is worthy of notice that the bacteria showed resistance to the test extracts, since their inhibition zones were lower than those of the positive control against which the bacteria have also shown resistance. This implies that the test extracts fall under the breakpoints of resistance with respect to the target bacteria. Similar inhibition zones against *Salmonella spp.* suggest comparable efficacy, regardless of the extraction solvent, highlighting consistent antibacterial constituents (Sukanya *et al.*, 2011). Variations in extract efficacy against different bacterial strains imply non-universal antibacterial activity, possibly due to differences in cell walls or other bacterial differences.

Conclusion

This research work has revealed compounds from *C. odorata* roots that are good drug candidates, as suggested by their in silico physicochemical properties. Specifically, 1-dioxide-4-methyl-3-(4-methyl-1-piperziny) and 2,3-dihydroxyl propylelaidate, which were characterized from methanolic and aqueous extracts, respectively, have shown good physicochemical properties. Also, the root extracts inhibited the proliferation of *S. aureus*, *E. coli*, *Klebsiella spp.*, and *Salmonella spp.* Thus, further study is recommended to investigate the susceptibility of the bacteria to the extracts, and to also examine the promising bioactive components of *C. odorata* root, as its extracts have shown to be a potential natural source for antibiotic drug discovery and development.

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Conflicts of Interest

The authors declare no conflict of interest.

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