

Uncovering the Healing Properties of *Plumbago auriculata* L.

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Abstract

Background:

Plumbago auriculata L. is a medicinal plant widely used in traditional healing practices.

Aim:

This study aimed to identify the phytochemical constituents present in leaf extracts of *P. auriculata* and to evaluate their antibacterial activity.

Setting:

This experimental laboratory study used *P. auriculata* leaves collected in Durban, South Africa, in May 2023, followed by laboratory-based phytochemical and antibacterial analyses.

Methods:

Qualitative phytochemical screening and gas chromatography–mass spectrometry (GC–MS) were used to characterise bioactive constituents of the leaf extracts. Antibacterial activity was assessed against selected Gram-positive and Gram-negative bacteria using agar-based screening, with bacterial suspensions standardised to 0.5 McFarland.

Results:

GC–MS analysis of the methanolic leaf extract identified 50 compounds, with 13-docosenamide, hexadecanoic acid 2-hydroxy-1-(hydroxymethyl) ethyl ester, n-tetracosanol-1, 1,10-cycloicosanedione, and 5,7-dihydroxy-4-methylcoumarin among the major constituents. Qualitative screening showed multiple classes of secondary metabolites across the solvent extracts. The methanolic leaf extract showed antibacterial activity against the tested Gram-positive and Gram-negative bacteria.

Conclusion:

P. auriculata leaves contain diverse phytochemical constituents and demonstrate antibacterial potential, supporting further investigation of this species as a source of bioactive compounds.

Recommendation:

Future studies should include bioassay-guided isolation, cytotoxicity testing, mechanistic studies, and in vivo safety and efficacy assessments before therapeutic applications are considered.

Keywords: *Plumbago auriculata*, plant phytochemistry, Cape Leadwort, *Plumbago Capensis*, phytocompounds

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Introduction

Gas chromatography–mass spectrometry (GC–MS) is an analytical technique used to separate volatile components of complex mixtures and record the mass spectrum of each component (Mtunzi et al., 2013). GC–MS is widely used in food science, toxicology, forensics, environmental science, and animal and plant research. Medicinal plants are rich

bioresources for traditional and complementary medicine (Kalimuthu and Prabakaran, 2015). Plants are also extensively used in the cosmetic and pharmaceutical industries, food supplements, drug development, and nutraceuticals (Coopoosamy et al., 2022). According to the World Health Organization, a substantial proportion of the global population uses traditional or complementary medicine (Singh et al., 2018; Coopoosamy et al., 2022).

Qualitative and quantitative estimation of phytochemical constituents is therefore an important step in medicinal plant research (Goge et al., 2022). Ethnobotanical knowledge and pharmacological studies of the aerial parts and roots of *Plumbago auriculata* Lam. suggest a wide range of medicinal and pharmacological applications (Ittiyavirah and Paul, 2016; Singh et al., 2018).

Plumbago auriculata, also known as *Plumbago capensis*, belongs to the family Plumbaginaceae and is a bushy evergreen shrub native to South Africa. The species is widely distributed and is also cultivated in warm tropical regions elsewhere in the world (Foden and Potter, 2015; Singh et al., 2018). The plant bears trusses of pale sky-blue flowers, although deep-blue and white-flowered forms also occur (Singh et al., 2018). The leaves and calyces possess specialised secretory structures, including salt glands on the leaf surface and trichomes on the calyx (Panicker and Haridasan, 2016; Singh et al., 2019).

Different parts of *P. auriculata* contain a range of phytochemicals, with plumbagin being a prominent compound associated with several reported pharmacological activities, including antifungal, antimalarial, antimicrobial, anticancer, antioxidant, antifertility, anti-inflammatory, and cardiogenic effects (Singh et al., 2018). Previous phytochemical screening of methanolic root extracts reported tannins, flavonoids, phenols, alkaloids, saponins, proteins, and carbohydrates, with phenolic compounds among the abundant constituents (Lakshmanan et al., 2016; Singh et al., 2019). Previous studies have also reported antimicrobial activity in *P. auriculata* and related plumbagin-containing preparations (Padhye et al., 2010; Singh et al., 2018). Accordingly, the present study aimed to identify phytochemical constituents in *P. auriculata* leaf extracts and evaluate their antibacterial activity against selected bacterial strains.

Materials and Methods

Study design

This study employed an experimental laboratory design to characterise the phytochemical constituents of *P. auriculata* leaf extracts and assess their antibacterial activity.

Study setting and period

Plant material was collected in Durban, KwaZulu-Natal, South Africa, in May 2023. Phytochemical, GC-MS, and antibacterial analyses were conducted at the research laboratories at the University of KwaZulu-Natal.

Plant collection

Leaf samples of *P. auriculata* were collected in Durban, South Africa, during May 2023. The plant material was identified and authenticated by comparison with herbarium specimens held at the Durban Herbarium.

Preparation of extracts

Leaves of *P. auriculata* were air-dried for three months at room temperature and then powdered using a pestle and mortar. The powdered plant material (50 g) was exhaustively extracted with methanol using a Soxhlet apparatus for 9 h. The resultant extract was filtered and concentrated under reduced pressure using a rotary evaporator. Ten millilitres of the concentrated extract were retained for phytochemical screening, while the remaining extract was evaporated to dryness and stored at room temperature (24 °C) for further analysis.

Phytochemical screening

One millilitre of crude leaf extract was used for preliminary phytochemical screening. The phytochemical constituents of the different solvent extracts were screened using the methods described by Harborne (1973), as summarised in Table 1.

Gas chromatography–mass spectrometry (GC-MS)

GC-MS analysis was carried out on a Shimadzu GCMS-QP 2010SE instrument. The instrument was fitted with a Shimadzu HP-5MS capillary column (0.25 µm film thickness) having a dimension of 30 m (length) × 0.25 µm (I.D.). Samples of the methanol extract of the leaf were subjected to GC-MS analysis. Helium was used as the carrier gas at 7.5 kPa pressure with oven temperature programmed from 60 °C (for 2 min) to 270 °C (for 30 min) at a ramping rate of 4 °C per min. A 2 µL sample was manually injected at an injection temperature of 250 °C with a split ratio of 1:5. For the GCMS detection, a sample ionization energy of 70 eV was used. The system software was driven by Shimadzu GCMS solution workstation software (Japan). The compounds of interest were identified from the leaf chromatograms by comparing the retention time of each peak and their respective fragmentation patterns with those of the reference samples. The relative amount of each compound in the extracts as a percentage was then computed by comparing the area under a compound's peak to the total area.

Antibacterial activity

Preliminary antibacterial screening of the leaf extract was carried out against 2 Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923 and *Staphylococcus aureus* Rosenbach ATCC BAA-1683 (methicillin-resistant *S. aureus*, MRSA) and 4 Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 31488, *Escherichia coli* ATCC 25922 and *Salmonella typhimurium*). The bacteria were grown overnight in Nutrient Broth (Biolab, South Africa) at 37°C in a shaking incubator (100rpm). The bacterial

concentration was adjusted to 0.5 McFarland's Standard with sterile distilled water using a DEN-1B McFarland densitometer (Latvia). Mueller-Hinton Agar plates (MHA) (Biolab, South Africa) were inoculated with the prepared bacterial suspensions using a sterile throat swab, and 5 µl (17000 µg/ml) of the extracts were spotted onto the MHA plates. The plates were incubated at 37 °C for 18 hours, and after incubation, the plates were read to determine antibacterial activity, which was denoted by clear zones in the area where the extracts were spotted.

Results

Qualitative phytochemical screening demonstrated the presence of several phytochemical classes in the leaf extracts (Table 1). The methanolic extract tested positive for carbohydrates, phenolics, tannins, alkaloids, amino acids, cholesterol, anthocyanins/flavonoids, while the pattern of

detection differed across the hexane and chloroform extracts.

GC-MS analysis of the methanolic leaf extract identified 50 compounds (Table 2; Figure 1). The identified compounds accounted for 93.38% of the chromatogram. The major constituents by relative abundance included 13-docosenamide (9.81%), hexadecanoic acid 2-hydroxy-1-(hydroxymethyl) ethyl ester (9.44%), n-tetracosanol-1 (9.30%), 1,10-cycloicosanedione (8.41%), and 5,7-dihydroxy-4-methylcoumarin (6.94%).

The methanolic leaf extract demonstrated antibacterial activity against all six bacterial strains tested (Table 3). The reported inhibition zones were 20 ± 0.62 mm for *Escherichia coli*, 18 ± 0.51 mm for *Klebsiella pneumoniae*, 17 ± 0.62 mm for *Salmonella typhimurium*, 15 ± 0.62 mm for *Pseudomonas aeruginosa*, 14 ± 0.47 mm for *Staphylococcus aureus*, and 11 ± 0.35 mm for methicillin-resistant *S. aureus* (MRSA). Methanol used as the solvent control showed no antibacterial activity.

Table 1: Phytochemical tests showing the presence and absence of major compounds within leaves of *Plumbago auriculata*.

Test	Reagents/ Substrate	Hexane	Methanol	Chloroform
Carbohydrates	Mollsch	+	+	+
	Fehling's	-	-	-
	Benedict's	-	+	-
Phenolics	Ferric Trichloride	+	+	+
	Potassium dichromate	-	+	+
Tannins	Lead Acetate	+	+	+
Alkaloids	Mayer's	-	+	-
	Hager's	-	-	+
Amino acids	Ninhydrin	-	+	+
Mucilage	Ruthenium Red	-	-	+
Cholesterol	Salkowski's	-	+	-
Anthocyanins,	Concentrate	-	+	-
Flavanones,	d sulphuric	-	+	+
Flavones,	acid	-	+	+
Flavanoids	Sodium hydroxide solution	-	-	-
Fixed oils and fats	Filter paper	+	-	+

¹ + indicates presence/ – indicates absence

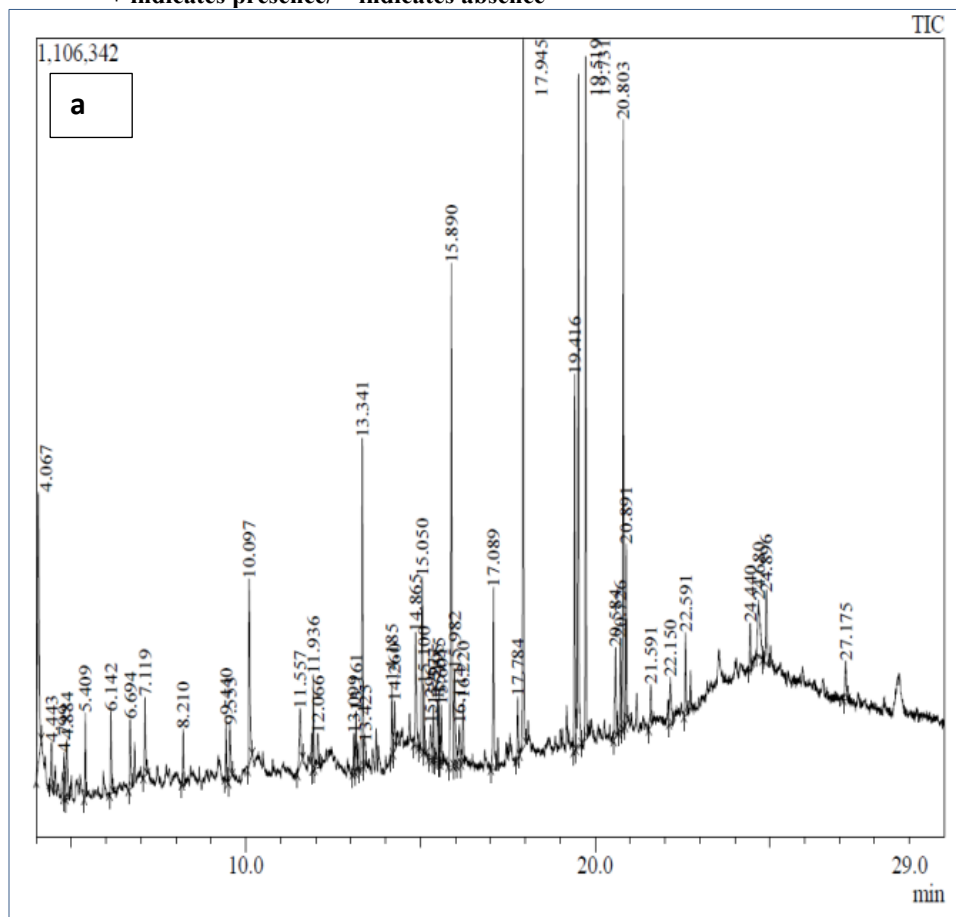


Figure 1: GC-MS chromatogram of methanolic extract of *Plumbago auriculata* leaves

Table 2: Compounds identified in the methanolic leaf extract of *Plumbago auriculata* in GC-MS.

Sl.NO	Name of the compounds	% of compound	Retention Time	Retention Index	Molecular Weight
1	1-ethyl-3-methylbenzene,	3.75	4.0	1006	120
2	1,2,3-trimethylbenzene	0.63	4.4	1020	120
3	1-methyl-3-propyl- Benzene	0.63	4.7	1106	134
4	1,4-Diethylbenzene	0.83	4.8	1106	134
5	Tridecane	1.17	5.4	1115	184
6	2-Propyl-tetrahydropyran-3-ol	1.14	6.1	1156	144
7	Tetrahydropyran-2-carbinol	0.96	6.6	1012	116
8	4-Ethoxystyrene	1.09	7.1	1172	148
9	Dodecane	0.78	8.2	1214	170
10	1-Tridecene	0.90	9.4	1304	182
11	2-Decenoic acid	0.75	9.5	1380	170
12	2-Hydroxy-4-methylbenzaldehyde	2.58	10.0	1316	136
13	3-Hydroxy-4-methoxybenzoic acid	0.79	11.5	1560	168
14	1-Octadecene	1.31	11.9	1801	252
15	2-(Carboxymethyl)-6-methylbenzoic acid	0.79	12.0	1832	194
16	Z, E-2,13-Octadecadien-1-ol	1.39	13.0	2069	266
17	(3Z)-3-Octadecenyl acetate	0.47	13.1	2185	310
18	Octadecanal	0.54	13.3	1999	268
19	2-Ethyl-5-propylphenol	0.83	13.4	1426	164
20	Nonadecanol	4.55	14.1	2153	284
21	2-methyltetracosane	0.32	14.2	2442	352
22	Pentadecanoic acid	1.03	14.8	1869	242
23	3,7-Dimethyl-1-[2-(vinylloxy)ethyl]-	0.68	15.0	2159	250

24	3,7-dihydro-1H-purine-2,6-dione	1.57	15.1	2423	366
25	Heptadecyl dichloroacetate	2.41	15.2	2409	292
26	n-Octyl-.beta.-D-glucopyranoside	0.94	15.3	2081	276
27	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	0.48	15.4	2061	268
28	Z-2-Octadecen-1-ol	0.75	15.5	1878	270
29	Hexadecanoic acid, methyl ester	0.85	15.6	2134	292
30	Methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate	0.94	15.8	2366	312
31	Eicosanoic acid	0.81	15.9	1987	226
32	Cyclopentadecanol	6.94	16.1	1905	192
33	5,7-Dihydroxy-4-methylcoumarin	1.33	16.2	1773	388
34	Hexadecyl pentafluoropropionate	0.55	17.0	2351	256
35	1-Heneicosanol	0.89	17.7	2694	372
36	Octadecanoic acid, 2-(2-hydroxyethoxy)ethyl ester	2.52	17.9	2021	255
37	Hexadecanamide	0.85	19.4	2525	312
38	Phthalic acid, 3-methylbenzyl butyl ester	9.81	19.5	2625	337
39	13-Docosenamide	5.20	19.7	2220	283
40	Octadecanamide	9.30	20.5	2650	354
41	n-Tetracosanol-1	9.44	20.7	2498	568
42	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	1.24	20.8	2561	330
43	1,2-Benzenedicarboxylic acid, dicyclohexylester	1.27	21.0	2704	390
	Bis (2-ethylhexyl) phthalate				

44	1,10-Cycloicosanedione	8.41	21.5	2741	308
45	9-Hexacosene	2.48	22.1	2614	364
46	cis-9,10-Epoxyoctadecanamide	0.58	22.5	2272	297
47	Stigmasta-4,7,22-trien-3.alpha.-ol	0.64	24.4	2750	410
48	19-Hydroxy-19-nortestosterone	1.15	24.6	2216	290
49	4,6-Cholestadien-3.beta.-ol	0.66	24.8	2579	384
50	Ergost-5-en-3-yl acetate	0.76	24.7	2771	442

Table 3:	Antibacterial activity of <i>Plumbago auriculata</i> methanolic extracts	
Microorganism	Leaf extract	Methanol
<i>Escherichia coli</i> -	20 ± 0.62	-
<i>Staphylococcus aureus</i> +	14± 0.47	-
<i>Klebsiella pneumonia</i> -	18 ± 0.51	-
<i>Salmonella typhimurium</i> -	17 ± 0.62	-
<i>methicillin-resistant S. aureus</i> , MRSA +	11 ± 0.35	.-
<i>Pseudomonas aeruginosa</i> -	15 ± 0.62	-

Discussion

The phytochemical profile observed in this study supports previous reports that *P. auriculata* contains diverse secondary metabolites with potential biological activity (Lakshmanan et al., 2016; Singh et al., 2018; Singh et al., 2019). The detection of phenolics, alkaloids, flavonoid-related compounds, tannins, and other constituents provides a plausible phytochemical basis for the antibacterial activity observed in the crude leaf extract.

The GC-MS profile further demonstrated substantial chemical diversity in the methanolic leaf extract. Several of the major compounds identified have previously been associated with biological activities. For example, 13-docosenamide has been reported in biological systems in studies of fatty-acid amides (Ben-Shabat et al., 1998), while 2-palmitoylglycerol-related compounds may interact with endocannabinoid signalling (Gallily et al., 2000; Walker et al., 2002). Long-chain alcohols such as n-tetracosanol-1 and nonadecanol have also been reported in plant extracts exhibiting antimicrobial or cytotoxic activity (Kuppuswamy et al., 2013). These associations should, however, be interpreted cautiously because the present study evaluated

crude extracts and did not isolate individual compounds or establish their specific contribution to antibacterial activity. The antibacterial findings indicate activity against both Gram-positive and Gram-negative organisms, with the largest reported inhibition zone observed against *E. coli* and the smallest against *MRSA*. This broad activity is consistent with earlier reports of antimicrobial properties associated with *P. auriculata* and plumbagin-containing plant material (Padhye et al., 2010; Singh et al., 2018). Differences in susceptibility among the tested bacteria may reflect differences in cell-envelope structure, intrinsic resistance mechanisms, and the concentrations of active constituents in the crude extract.

Although these findings support the medicinal potential of *P. auriculata*, they should not be interpreted as evidence of clinical efficacy. Further fractionation and compound-level testing are required to determine which constituents are responsible for the observed antibacterial effects and to establish potency, selectivity, toxicity, and mechanisms of action.

Conclusion

The findings demonstrate that *P. auriculata* leaves contain a diverse range of phytochemical constituents and that the methanolic leaf extract exhibits antibacterial activity against the tested Gram-positive and Gram-negative bacteria. The results support further investigation of *P. auriculata* as a potential source of antibacterial lead compounds, while recognising that crude-extract activity alone is insufficient to establish therapeutic efficacy.

Study limitations

The study was limited to leaf material collected from a single geographic location and a relatively small panel of bacterial strains. The use of crude extracts means that the antibacterial effects cannot be attributed to individual compounds. In addition, cytotoxicity testing, bioassay-guided isolation, mechanistic assays, and *in vivo* efficacy and safety studies were not performed. These limitations restrict the generalisability of the findings and the extent to which therapeutic conclusions can be drawn.

Recommendations

Future research should evaluate *P. auriculata* material from additional populations, isolate and characterise the active constituents through bioassay-guided fractionation, determine antibacterial potency using standardised quantitative assays, and assess cytotoxicity, mechanisms of action, and *in vivo* safety and efficacy. These steps are recommended before the plant, or its constituents, are considered for therapeutic development.

List of abbreviations

ATCC – American Type Culture Collection; CB1 – cannabinoid receptor type 1; CB2 – cannabinoid receptor type 2; GC–MS – gas chromatography–mass spectrometry; MHA – Mueller–Hinton agar; MIC – minimum inhibitory concentration; MRSA – methicillin-resistant *Staphylococcus aureus*; TNF- α – tumour necrosis factor alpha.

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Conflict of interest

The author declares that there are no financial, personal, or other conflicts of interest that could have inappropriately influenced the conduct or reporting of this study.

Credit authorship contribution

Karishma Singh: Conceptualisation; Investigation; and writing of the manuscript.

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Data availability

The author confirms that the data supporting this study and its findings are available within the article and its listed references.

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