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IDENTIFICATION OF PHYTOCHEMICALS FROM *BORRERIA ALATA* (AUBL.) DC. USING LC-MS-QTOF

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Abstract

The present investigation determined the phytochemical components of *Borreria alata* through the utilisation of LC-MS. A total of 88 phytochemicals were identified with a retention time ranging from 1 to 21 minutes. Ten phenolic compounds, two flavonoids, six amines and six amino acids were identified among them. The results of this study showed the medicinal and allelopathic potential of *B. alata*.

Introduction

Plants synthesise a wide range of phytochemical substances, including flavonoids, phenolics, alkaloids, terpenoids, benzoxazinoids, etc (Motmainna *et al.* 2021a). These molecules serve as a defence mechanism against illnesses, herbivory, unfavourable environmental circumstances, and competition. Phytochemicals are found in various parts of plants, and can be used as bioherbicides and phytomedicines (Hasan *et al.* 2021, Motmainna *et al.* 2021b). Phytochemicals have the ability to function as insecticides, herbicides, and antimicrobial agents that regulate the development and growth of crops. Certain phytochemicals have a mode of action comparable to herbicides, and most of these compounds are soluble in water (Hasan *et al.* 2024).

Plant extraction is an essential step in the process of finding potential bioactive chemicals in plants. Phytochemical screening is essential for the identification of novel herbicides or medicines derived from natural products. Liquid chromatography-electrospray ionisation tandem mass spectrometry (LC-ESI-MS) is a key analytical tool used to detect and characterise known and undiscovered substances in natural products (Motmainna *et al.* 2024). A mass spectrometry experiment can examine the structure of the discovered precursor ion (Kind *et al.* 2018). Time-of-flight (TOF) mass spectrometry (MS) enables the determination of the composition of components and precise measurement of the mass of molecules and fragment ions. Overall, LC -QTOF-MS/MS is a reliable and effective technique for analysing complicated samples.

Borreira is a genus of Rubiaceae that is widely distributed in tropical and subtropical regions of America, Africa, Asia, and Europe. *Borreria* species have diverse medicinal properties (Wong *et al.* 2015). Several research studies have been reviewed to confirm their biological activities. Based on current understanding, previous research on the phytochemical profile of these weeds has been somewhat limited. Thus, this study aims to identify the active phytochemical components in *B. alata* extract using LC-ESI-QTOF-MS/MS.

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Materials and Methods

A slightly modified version of the procedure outlined by Aslani *et al.* (2014) was used to prepare the *B. alata* methanol extract. The entire plant was crushed, chopped, and passed through a 40-mesh sieve. One hundred grams of powder were immersed in 1L of a mixture of methanol and distilled water [80:20 (v/v)] in a conical flask wrapped with parafilm. The flask was repeatedly shaken using an orbital shaker and four layers of cheesecloth were used to filter the solution, then 0.2- µm, 15- mm syringe filters were used to re-filter the solution. The methanol was evaporated from the collected supernatant using a rotary evaporator. Prior to usage, all extracts were stored in the refrigerator at 4°C.

The crude sample (20 mg) was dissolved in 100% HPLC grade methanol (20 mL) and filtered with 0.2- µm, 15- mm syringe filters for LC-QTOF-MS/MS analysis. The methanol extract of *B. alata* were analyzed using the approach described by Ismail *et al.* (2019), with minor adjustments. The analysis of chemical components was performed using an Agilent 1290 Infinity LC system connected to an Agilent 6520 Accurate-Mass Q-TOF mass spectrometer equipped with a dual ESI source. Chromatographic separation was carried out using an ACQUITY UPLC BEH C18 column (150 mm × 2.1 mm × 3.5 µm), maintained at 50°C, with a steady flow rate of 0.4 mL/min to ensure rapid and efficient separation at reduced backpressure. The total LC run time was 26 minutes. A gradient elution technique was employed, utilizing LC-MS grade water with 0.1% formic acid as solvent A, and LC-MS grade acetonitrile with 0.1% formic acid as solvent B. For the MS/MS experiments, the nebulizer pressure was set to 40 psi, with a drying gas flow rate of 10 L/min and a temperature of 325°C. Both positive and negative ionization modes were explored at varying collision energies (CE) to enhance ionization efficiency and maximize structural information within the m/z range of 100 to 3200 m/z. The MassHunter Qualitative Analysis software was utilized for data processing, and peak identification was conducted by comparing the results with literature values and an online database (Abu Bakar *et al.* 2020).

Results and Discussion

In *Borreria alata*, 88 compounds were identified using LC-MS analysis. Among the compounds, most of the known compounds were identified in positive ionization mode and the retention time was 1 to 21 minutes (Table 1). The resulting chromatograms are shown in Figs 1 and 2. In this study, 10 phenolic compounds (Fumaric acid, Osthole, Kaempferol, Kaempferol-3-o-rutinoside, Rhabduscin, Ferujol, Shogaol, Phthalic anhydride, Octadecenedioic acid and 3-[[(E)-4-(dimethylamino)but-2-enoyl]amino]-N-[4-[[4-(2-phenylpyrazolo[1,5-a] pyridin-3-yl) pyrimidin-2yl]amino] phenyl]benzamide) were identified.

Two flavonoids, namely Kaempferol ($C_{15}H_{10}O_6$) at 286.0482 m/z and Kaempferol-3-o-rutinoside ($C_{27}H_{30}O_{15}$) at 594.159 m/z were identified in positive and negative ionization mode, respectively. Indole type alkaloids e.g., Indoline (RT 2.61 min) and Indole-3-acrylic acid (RT 3.641 min) exhibited a $[M+H]^+$ ion at 119.0744 and 187.0634 m/z, respectively. A total of 6 amines (Tetradecylamine, Dioctylnitrosamine, Laurixamine, Myristamidopropyl dimethylamine, *Piptamine* and Diundecylamine) were detected and identified in positive ESI mode. Fragment ion 243.256 m/z was determined for Laurixamine at 2.539 min. Four fatty acids (Angoletin, Dodecanamide, Hexadecanamide and 2-Arachidonoylglycerol) were also detected in positive ionization mode. Angoletin and Hexadecanamide were determined at 14.242 and 19.916 min with 300.1348 and 255.2561 m/z. A total of six amino acids (D-Norvaline, Hexadecasphinganine, Phytosphingosine, 15-Methylhexadecasphinganine, Sphinganine and Eicosasphinganine) were identified, which usually provide $[M+H]^+$ ion. Among the identified amino acids, D-Norvaline ($C_5H_{11}NO_2$) at the retention time of 1.44 min (117.0788 m/z) produced the fragment ions of $[M+H]^+$.

Table 1. LC-MS profile of methanol extract of *Borreria alata.*

RT (min)	Determined compounds	Molecular formula	Mass fragment (m/z)	Polarity	Error (ppm)
1.24	4-Chlorothiazole-5-sulfonamide	$C_3H_3ClN_2O_2S_2$	197.9330	Positive	-3
1.235	4-Chloro-2-methylthiopyrimidine	$C_5H_5ClN_2S$	159.9857	Negative	3.27
1.414	2-Nitro-5-[2-(triazol-1-yl)ethylamino]benzonitrile	$C_{11}H_{10}N_6O_2$	258.0878	Positive	-5.01
1.416	2-Amino-5-hydroxy-3,4,6-trimethoxyhexanal;hydrochloride	$C_9H_{20}ClNO_5$	257.1029	Positive	0.33
1.419	Fumaric acid	$C_4H_5NO_3$	115.0268	Positive	1.55
1.439	2-Pyridin-4-ylacetohydrazide;2,4,6-trinitrophenol	$C_{13}H_{12}N_6O_8$	380.0725	Positive	-2.2
1.44	D-Norvaline	$C_5H_{11}NO_2$	117.0788	Positive	1.39
1.449	Pentyl 2-(4-hydroxyphenyl)sulfonyloxybenzoate	$C_{18}H_{20}O_6S$	364.0982	Positive	-0.51
1.514	1,3,5-Triazine-2,4,6-triamine oxalate	$C_5H_8N_6O_4$	216.0613	Positive	-2.94
1.754	Benzofuran	C_8H_6O	118.0404	Positive	12.75
2.61	Indoline	C_8H_9N	119.0744	Positive	-7.32
3.641	Indole-3-acrylic acid	$C_{11}H_9NO_2$	187.0634	Positive	-0.13
4.077	Asperulosidic acid	$C_{18}H_{24}O_{12}$	432.127	Negative	-0.53
6.88	Loliolide	$C_{11}H_{16}O_3$	196.1099	Positive	0.17
8.046	Carbocyclic-3'-amino-ara-adenosine	$C_{11}H_{16}N_6O_2$	264.1362	Positive	-10.2
8.126	Osthole	$C_{15}H_{16}O_3$	244.1103	Positive	-1.55
8.409	Kaempferol	$C_{15}H_{10}O_6$	286.0482	Positive	-1.5
8.414	N-{1-[(Allylamino)carbonyl]-2-[(4-chlorophenyl)sulfanyl]-2-phenylvinyl}benzamide	$C_{25}H_{21}ClN_2O_2S$	448.1011	Positive	0.18
8.493	Kaempferol-3-o-rutinoside	$C_{27}H_{30}O_{15}$	594.159	Negative	-0.93
11.499	Ethyl 5-[4-[[[4-azido-1-(1-tert-butyltriazol-4-yl)butyl]amino]methyl]triazol-1-yl]pentanoate	$C_{20}H_{34}N_{10}O_2$	446.2856	Positive	2.21
11.64	Dimethylmatairesinol	$C_{22}H_{26}O_6$	386.1726	Positive	0.79
11.838	Tri-Boc-hydrazinoacetic acid	$C_{17}H_{30}N_2O_8$	390.2003	Positive	-0.27
11.983	Hexadecasphinganine	$C_{16}H_{35}NO_2$	273.2669	Positive	-0.6
12.021	Phytosphingosine	$C_{18}H_{39}NO_3$	317.2936	Positive	-2
12.162	Dihydroxyethyl lauramine oxide	$C_{16}H_{35}NO_3$	289.2616	Positive	0.36
12.165	3-ethoxy-2-phenylhydrazono-butyraldehyde phenylhydrazone	$C_{18}H_{22}N_4O$	310.1795	Positive	-0.4
12.18	Dodecyldimethylamine oxide	$C_{14}H_{31}NO$	229.2406	Positive	-0.26
12.216	Tetradecylamine	$C_{14}H_{31}N$	213.2454	Positive	1.1
12.303	Dioctylnitrosamine	$C_{16}H_{34}N_2O$	270.2664	Positive	2.52
12.305	15-Methylhexadecasphinganine	$C_{17}H_{37}NO_2$	287.2825	Positive	-0.08
12.308	Rhabduscin	$C_{17}H_{20}N_2O_5$	332.1367	Positive	1.64
12.331	N-(Decanoyl)piperidine	$C_{15}H_{29}NO$	239.2244	Positive	2.06
12.334	Tetrabutylurea	$C_{17}H_{36}N_2O$	284.2828	Positive	-0.19
12.335	Kobusone	$C_{14}H_{22}O_2$	222.16 1	Negative	4.27
12.339	Lauryl aminopropylglycine	$C_{17}H_{36}N_2O_2$	300.2774	Positive	0.93
12.445	Myristyl sulfate	$C_{14}H_{30}O_4S$	294.1866	Negative	-0.5
12.539	Laurixamine	$C_{15}H_{33}NO$	243.256	Positive	1.07
12.607	6-(6-Piperidin-1-ylpurin-9-yl)hexyl nitrate	$C_{16}H_{24}N_6O_3$	348.1909	Positive	0.15
12.688	Aminopregnane	$C_{21}H_{37}N$	303.2931	Positive	-1.75
12.969	2-Dodecylbenzenesulfonic acid	$C_{18}H_{30}O_3S$	326.1922	Negative	-1.88
13.037	N,N'-Dicarbamimidoyltetradecanediamide	$C_{16}H_{32}N_6O_2$	340.2582	Positive	1.47

RT (min)	Determined compounds	Molecular formula	Mass fragment (m/z)	Polarity	Error (ppm)
13.048	1,3-Bis{[(4-methyl-1H-imidazol-5-yl)methyl]amino}-2-propanol	$C_{13}H_{22}N_6O$	278.1864	Positive	-3.27
13.195	Olomoucine	$C_{15}H_{18}N_6O$	298.1554	Positive	-3.88
13.2	Panaxytriol	$C_{17}H_{26}O_3$	278.1886	Positive	-1.36
13.218	6-(Cyclopentylamino)-2-[(3-hydroxypropyl)amino]-9-isopropylpurine	$C_{16}H_{26}N_6O$	318.217	Positive	-0.55
13.252	Sphinganine	$C_{18}H_{39}NO_2$	301.2976	Positive	1.75
13.288	Ferujol	$C_{19}H_{24}O_4$	316.1664	Positive	3.46
13.29	(4-Oxo-1,2,3-benzotriazin-3-yl)methyl 1-phenyl-3-pyridin-3-ylpyrazole-4-carboxylate	$C_{23}H_{16}N_6O_3$	424.1294	Positive	-2.5
13.291	4-Azido-N-(2,2,2-tripyridin-4-ylethylideneamino)pyrimidin-2 amine	$C_{21}H_{16}N_{10}$	408.1558	Positive	0.43
13.316	Estriol	$C_{18}H_{24}O_3$	288.1734	Positive	-3.09
13.337	1-(2,5-Diazidophenyl)-1,4,8,11-tetrazacyclotetradecane	$C_{16}H_{26}N_{10}$	358.2333	Positive	2.48
13.675	Shogaol	$C_{17}H_{24}O_3$	276.1727	Positive	-0.46
13.685	Stearic acid hydrazide	$C_{18}H_{38}N_2O$	298.2985	Positive	-0.15
13.718	N-Decylcyclohexanecarboxamide	$C_{17}H_{33}NO$	267.2562	Positive	0.22
13.729	Myristamidopropylamine oxide	$C_{19}H_{40}N_2O_2$	328.3099	Positive	-2.92
13.988	Myristamidopropyl dimethylamine	$C_{19}H_{40}N_2O$	312.3133	Positive	2.47
14.15	Piptamine	$C_{23}H_{41}N$	331.324	Positive	5.3
14.242	Angoletin	$C_{18}H_{20}O_4$	300.1348	Positive	4.63
14.25	N-[4-Acetamido-5-(4,5-diacetamido-1,2,4-triazol-3-yl)-1,2,4-triazol-3-yl]acetamide	$C_{12}H_{16}N_{10}O_4$	364.1346	Positive	2.7
14.626	1,1'-[(4-Methoxyphenyl)methylene]dipiperazine	$C_{16}H_{26}N_4O$	290.2121	Positive	-4.86
14.687	Phthalic anhydride	$C_8H_4O_3$	148.0168	Positive	-5.21
15.007	3-Piperazin-1-yl-2-[(2-piperazin-1-ylacetyl)amino]propanamide	$C_{13}H_{26}N_6O_2$	298.2134	Positive	-5.47
15.101	Diundecylamine	$C_{22}H_{47}N$	325.3708	Positive	0.03
15.15	Dodecanamide	$C_{12}H_{25}NO$	199.1934	Positive	0.86
15.257	3-Azido-1-methyl-1-[6-[4-(4-methylpiperazin-1-yl)anilino]pyrimidin-4 yl]urea	$C_{17}H_{22}N_{10}O$	382.1977	Positive	0.34
15.354	Eicosasphinganine	$C_{20}H_{43}NO_2$	329.33	Positive	-2.0
15.588	Purin-6(3H)-one, 8-diethylaminomethyl-3,7-dimethyl-2-dimethylamino-	$C_{14}H_{24}N_6O$	292.2011	Positive	0.09
15.62	9,10-Bis[(2,4-dimethylphenyl)methyl]anthracene	$C_{32}H_{30}$	414.2332	Positive	3.73
16.478	octadecenedioic acid	$C_{18}H_{32}O_4$	312.2285	Positive	4.88
17.036	Dodecyl sulfate	$C_{12}H_{26}O_4S$	266.1551	Negative	0.47
17.068	Lagochilin	$C_{20}H_{36}O_5$	356.2551	Positive	3.32
17.177	[7-Amino-5-[[(2S,4S)-1-[3-cyclohexyl-2-(pyrimidine-2-carbonylamino)propanoyl]-4-[5-(2-hydroxypropan-2-yl)triazol-1-yl]pyrrolidine-2-carbonyl]amino]-6,7-dioxoheptyl]carbamic acid	$C_{32}H_{46}N_{10}O_8$	698.3497	Positive	0.43
17.364	3-[[(E)-4-(dimethylamino)but-2-enoyl]amino]-N-[4-[[4-(2-phenylpyrazolo[1,5-a]pyridin-3-yl)pyrimidin-2-yl]amino]phenyl]benzamide	$C_{36}H_{32}N_8O_2$	608.2646	Positive	0.33
17.384	4-[5-[[3-[1-(3-Aminopropyl)pyrazol-4-yl]triazolo[4,5-d]pyrimidin-5-yl]amino]-2,3-dihydropyridin-3-yl]butanoic acid	$C_{19}H_{24}N_{10}O_2$	424.2081	Positive	0.6

RT (min)	Determined compounds	Molecular formula	Mass fragment (m/z)	Polarity	Error (ppm)
19.008	6-Amino-2-[[2-[[2-[(2,4-diamino-4-oxobutanoyl)amino]-3-(4-hydroxyphenyl)propanoyl]amino]-3-methylpentanoyl]amino]hexanoic acid	$C_{25}H_{40}N_6O_7$	536.2964	Positive	-0.96
19.119	1,4-Dimethylcyclohexane;ethane;3,4,5,6,7-pentamethylnonane	$C_{26}H_{58}\ N_{10}$	510.4833	Positive	2.61
19.124	3-[5-(3-Dimethylamino-1,2,4-thiadiazol)-yl]quinuclidine	$C_{11}H_{18}N_4S$	238.1249	Positive	1.18
19.125	1,2-Dinaphthalen-1-ylhydrazine	$C_{20}H_{16}N_2$	284.1301	Positive	4.29
19.526	2-[[4,6-Bis(dimethylamino)-1,3,5-triazin-2-yl]-butylamino]ethanol	$C_{13}H_{26}N_6O$	282.2175	Positive	-2.37
19.762	2,2-Bis(azidomethyl)-3-decoxypropan-1-ol	$C_{15}H_{30}N_6O_2$	326.2436	Positive	-1.62
19.825	Stearyldiethanolamine	$C_{22}H_{47}NO_2$	357.3609	Positive	-0.68
19.916	Hexadecanamide	$C_{16}H_{33}NO$	255.2561	Positive	0.49
20.226	(2S)-2-[[4-[(2,4-Diaminopteridin-6-yl)methyl-methylamino]benzoyl]amino]-N,N'-dipropylpentanediamide	$C_{26}H_{36}N_{10}O_3$	536.2967	Positive	0.91
20.387	4-Undecylbenzenesulfonic Acid	$C_{17}H_{28}O_3S$	312.1758	Negative	0.3
20.869	Acetylresibufogenin	$C_{26}H_{34}O_5$	426.2409	Positive	-0.63
21.133	N-(3-Aminopropyl)-N-[2-[3-aminopropyl-(2-azidoacetyl)amino]ethyl]-2-azidoacetamide	$C_{12}H_{24}N_{10}O_2$	340.2073	Negative	3.28
21.263	2-Arachidonoylglycerol	$C_{23}H_{38}O_4$	378.2756	Positive	3.61
22.168	Methyl dodecylbenzenesulphonate	$C_{19}H_{32}O_3S$	340.2071	Negative	0.28

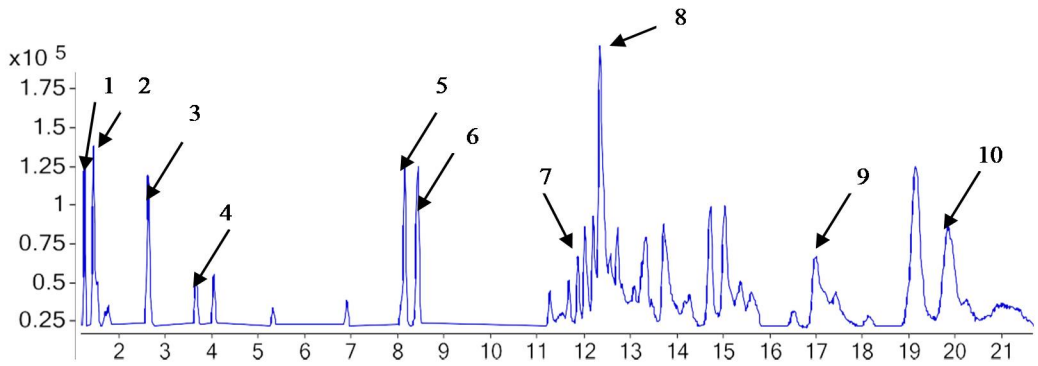

Fig. 1. LC-MS chromatogram chemical compounds of *Borreria alata* in positive ion mode. 1. fumaric acid, 2. D-norvaline, 3. indoline, 4. indole-3-acrylic acid, 5. osthole, 6. kaempferol, 7. dimethyl matairesinol, 8. laurixamine, 9. lagochilin, and 10. hxadecanamide.

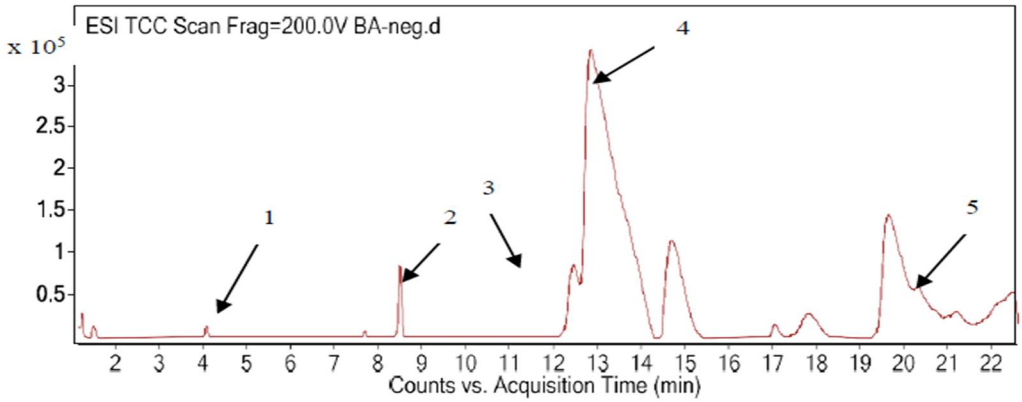

Fig. 2. LC-MS chromatogram chemical compounds of *Borreria alata* in negative ion mode.

Among the detected compounds, two flavonoids, namely Kaempferol and Kaempferol-3-o-rutinoside were identified in *B. alata*. The medicinal and allelopathic properties of identified phytochemicals have been reported by many scientists so that they can be used as a good source of valuable drugs and pesticides. Further study is essential for isolating and purifying those phytochemicals and analysing their individual and combined effects.

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