Asian Journal of Chemistry; Vol. 33, No. 6 (2021), 1336-1340

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2021.23192

Metabolite Profiling of Different Tissues of *Barleria dinteri* through the GC-MS Analysis

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Received: 4 March 2021; *Accepted*: 10 April 2021; *Published online*: 5 June 2021; AJC-20365

Barleria dinteri is a medicinal plant with distribution in the Limpopo, Gauteng and Mpumalanga provinces of South Africa as well as in Botswana, Swaziland and Namibia with exclusive specific habitation on limestone-rich soil. The plant species is used by traditional healers for wound healing and treatment of some intestinal tumours, as well as to relieve joint pains and toothache. The present study was aimed at the metabolite profiling of the different tissues (branches, flowers, leaves, roots) of *Barleria dinteri* using GC-MS analysis. Different extracts of the plant parts samples were subjected to GC-MS analysis and detected compounds were compared for presence amongst the different tissues. The results of the study revealed that all different parts (branches, flowers, leaves and roots) of *B. dinteri*, possess compounds that are detectable through GC-MS analysis with most compounds detected in the aerial parts, particularly the flowers. The results of the current study could serve as a basis for the possible plant parts substitution of the roots of *B. dinteri* with the aerial parts and the exploration of the pharmacological properties of the flowers for sustainable uses of the plant species for medicinal purposes.

Keywords: *Barleria dinteri*, GC-MS analysis, Metabolite profiling.

INTRODUCTION

Medicinal plants are regarded as a rich source of secondary metabolites with many biological activities such as antioxidant, anti-inflammatory, anticancer, antiviral, antifungal and antibacterial agents [1]. They play a pivotal role in healthcare and are a major source of raw materials for both traditional and conventional medicinal preparations, since people are increasingly choosing herbal medicines over conventional medicines [2]. It is estimated that about 40-90% of people living in developing countries frequently use traditional medicines [3]. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action in the human body [4].

In order to promote the use of medicinal plants as potential sources of important bioactive compounds, it is important to thoroughly investigate their phytochemical compositions (metabolite profiling) and biological activities and thus validate their use [5]. An essential part in the investigation of medicinal plants usually used by traditional doctors for the treatment of various diseases is to identify the phytochemical components present in their parts extracts [6,7]. Spectroscopic methods such as gas chromatography-mass spectrometry have become firmly established as key technological platforms for secondary metabolite profiling in medicinal plants [8,9]. GC-MS affords direct detection and identification of compounds present in medicinal plant parts extracts [10]. In the GC-MS technique, mass spectra of the separated volatile compounds are compared with those of compounds stored in electronic libraries for accurate identification of the compounds in the extracts of the plants under investigation.

The most frequently used parts of medicinal plants in the traditional medicinal practices of many communities of the world are the roots [11]. This practice involves the uprooting of plants with possible endangering of important plants species especially in the changing climatic conditions that are charact-

erized by low rainfall patterns as seen in water-scarce countries like South Africa. The importance and preservation of medicinal plants for continuous usage require sustainable harvesting approaches [12]. Sustainable harvesting approaches are even more important in rare plants species and those of specific habitat requirements as they are often harvested in bulk when located.

Barleria dinteri is one such rare medicinal plant that grows selectively in specific areas. In South Africa, it occurs in rocky areas of some parts of Limpopo, Mpumalanga and Gauteng provinces [13]. The roots and leaves of this plants are used interchangeably in traditional medicine to promote the healing of wounds, treatment of some intestinal tumours, infectious diseases and to relieve joint pains and toothache [14]. *B. dinteri* is a plant species of specific and rare geospatial habitation as it grows exclusively on limestone-rich soil [15]. Considering the commercial value of limestone, the habitation upon which the plant species is growing likely to be eroded in persuasion of economic interests. Scientifically, plant parts substitution, in particular substituting the roots with the aerial parts, is one of the many interventions that could be encouraged for sustainable usage of the flora species for medicinal purposes [16]. Such an approach would only be beneficial if the phytochemical composition profiles of the aerial parts are mostly similar to those of the roots. The current study was therefore aimed at the metabolite profiling of the different parts of *Barleria dinteri* using GC-MS analysis for comparison of the phytoconstituents between the aerial parts and the roots, upon the basis that possession of more similar compounds will likely inform more similar pharmacological properties. The findings of this study would strengthen the encouragement of the usage of the aerial parts rather than the roots in traditional medicine as a contribution to the sustainable use of the plant species.

EXPERIMENTAL

Sample collection, preparation and storage: The different plant tissues, namely branches, flowers, leaves and roots, of *B. dinteri* were collected from their natural habitat at Zebediela in Limpopo Province, South Africa, while in full flowering, using convenient sampling method. The collected plant material was authenticated by Dr. Bronwyn Egan, a taxonomist at the University of Limpopo Herbarium where the voucher specimen was deposited (UNIN 11118). The different parts of *B. dinteri* were separately dried at room temperature, ground to powder using a coffee grinder (Mellerware, South Africa) and stored in the dark in airtight containers until they were used.

Extraction of the plant material: The finely ground powder (5 g) of each plant tissue were extracted with 50 mL of *n*-hexane, dichloromethane, acetone and methanol, respectively in a serial exhaustive extraction procedure using cold maceration extraction procedure. The mixture was allowed to settle and the extracts were then filtered into different preweighed beakers and allowed to dry at room temperature under a stream of air. The dry extracts were then stored in the dark until further usage.

GC-MS analysis: Separation of hydrocarbons and other volatile compounds present in the *n*-hexane, dichloromethane, acetone and methanol extracts of the different parts of *Barleria dinteri* was done with a Shimadzu gas chromatograph coupled to a QP2010 SE mass detector, GC-MS (Shimadzu, South Africa). A Zebron capillary column (ZB-MultiResidue Tm-1) with a length of 30 mm, internal diameter of 0.25 mm ID and 0.25 µm film thickness was used. An electron ionization system with ionizing energy of 70 eV was used for analysis. The initial oven temperature was programmed to 50 ºC for 1.00 min; the temperature was gradually increased to 180 ºC, 240 ºC and 280 ºC at a rate of 20 ºC until reaching the final temperature at 300 ºC for 10 min. The temperature for the injector and detector was kept at 290 ºC. Helium (He) 5.0 was used as the carrier gas at a linear flow rate of 2.21 mL/min with an injection volume of 5 µL. The operation of the MS detector was done at 230 ºC. The scan range was at a rate of 0.30 scan/s from 50 to 700 *m/z*. The solvent delay time was 6.00 min and the total sample run time was 33.5 min. Software adopted to handle mass spectra and chromatogram was a GC-MS SOLUTIONS version 2.6.

Compound identification: All compounds were identified through a mass spectral compound database search using National Institute of Standards and Technology (NIST08) library. The mass spectra of unknown compounds were compared with those of the known components stored in the NIST08 library. The name, molecular weight, and molecular formula of identified compounds were recorded. The detected compounds were grouped based on differences in functional groups and compared amongst the different tissues in a tabular form. In addition, the percentage similarities of the compounds detected in the roots with their presence in the aerial parts were determined.

RESULTS AND DISCUSSION

The extracts of the different parts of *B. dinteri* were subjected to GC-MS and the results showing the detected and identified compounds are shown in Table-1 for hydrocarbons, Table-2 for halogens, Table-3 for esters, Table-4 for alcohols, Table-5 for fatty acids and Table-6 for amines. The results showed higher number of detected hydrocarbon compounds, halogen compounds and ester compounds to be found in the flowers; higher number of detected alcohol compounds in both the flowers and the branches; higher number of detected fatty acid compounds in both the leaves and the roots and higher number of detected amine compounds in the leaves.

In addition, the % similarities of compounds detected in the roots that were also present in the aerial parts were determined and the results are shown in Table-7. The results showed 100% of amine compounds that were detected in the roots to be present in both the branches, flowers and the leaves. For the fatty acid compounds; 10%, 20% and 10% of compounds detected in the roots were also present in the branches, flowers and the leaves, respectively. With regard to the ester compounds; 78% of the compounds in the roots were present in the branches and 44% present in both the flowers and the leaves. Also, 83% of the hydrocarbon compounds detected in the roots were present in the branches and 100% were present in both the flowers and the roots. On average, about 45% of the compounds detected in the roots of *B. dinteri* were also present in the aerial parts.

TABLE-1
COMPARISON OF HYDROCARBONS AMONGST THE DIFFERENT TISSUES OF *Barleria dinteri* DETECTED THROUGH GC-MS ANALYSIS

Compound name	m.w.	m.f.	Plant parts			
			Branches	Flowers	Leaves	Roots
Dodecane	170	$C_{12}H_{26}$	✓	✓		
Tetradecane	198	$C_{14}H_{30}$	✓	✓	✓	✓
Pentadecane	212	$C_{15}H_{32}$	✓	✓	✓	✓
Hexadecane	226	$C_{16}H_{34}$	✓	✓	✓	✓
Heptadecane	240	$C_{17}H_{36}$	✓	✓	✓	✓
Eicosane	282	$C_{20}H_{42}$		✓	✓	✓
Tetracontane-3,5,24-trimethyl	604	$C_{48}H_{88}$			✓	
Tetratetracontane	618	$C_{44}H_{90}$	✓	✓	✓	✓
2,6,10,14,18,22-Tetracosahexane,2,6,10,15,19,23-hexamethyl	410	$C_{30}H_{50}$			✓	
17-Pentatriacontane	490	$C_{35}H_{70}$		✓	✓	
Pentadecane-8-hexyl	296	$C_{21}H_{44}$		✓		
Heptadecane-2,6,10,15-tetramethyl	296	$C_{21}H_{44}$		✓		
Heneicosane	366	$C_{26}H_{54}$		✓		
4-Methyldocosane	324	$C_{23}H_{48}$		✓		
Tritetracontane	604	$C_{43}H_{88}$	✓	✓		
Octadecane-3-ethyl-5(2-ethylbutyl)	366	$C_{26}H_{54}$		✓		
Nonane-4,5-dimethyl	156	$C_{11}H_{24}$		✓		
Octane-3,4,5,6-tetramethyl	170	$C_{12}H_{26}$	✓			
Heptadecane,2,3-dimethyl	268	$C_{19}H_{40}$		✓		
Hexadecane-4-methyl	240	$C_{17}H_{36}$	✓			
Total detected hydrocarbons			9	16	9	6

✓: Compound presence

TABLE-2
COMPARISON OF THE HALOGEN CONTAINING COMPOUNDS AMONGST THE DIFFERENT TISSUES OF *Barleria dinteri* DETECTED THROUGH GC-MS ANALYSIS

Compound name	m.w.	m.f.	Plant parts			
			Branches	Flowers	Leaves	Roots
Sulphurous acid, Pentadecyl-2-propyl ester	334	$C_{18}H_{38}OS$		✓		
Nonadecylpentafluoropropionate	430	$C_{22}H_{39}F_5O$		✓		
Sulphurous acid, butylheptadecyl ester	376	$C_{21}H_{44}OS$	✓	✓		
Sulphurous acid, hexylpentadecyl ester	376	$C_{21}H_{44}O_3S$		✓		
Heptacosane,1-chloro	414	$C_{27}H_{55}Cl$	✓	✓		
1-Octadecanesulphonyl chloride	352	$C_{18}H_{37}ClO_2S$		✓		
Triacontylpentafluoropropionate	584	$C_{33}H_{61}F_5O_2$		✓		
cis-1-Chloro-9-octadecene	286	$C_{18}H_{35}Cl$	✓			
Triacontane-1-bromo	500	$C_{30}H_{61}Br$		✓		
Total halogens detected			3	8	0	0

✓: Compound presence

TABLE-3
COMPARISON OF ESTER COMPOUNDS AMONGST THE DIFFERENT TISSUES OF *Barleria dinteri* DETECTED THROUGH GC-MS ANALYSIS

Compound name	m.w.	m.f.	Plant parts			
			Branches	Flowers	Leaves	Roots
Decanal	156	$C_{10}H_{20}O$	✓	✓	✓	✓
Oxirane, (hexacycloxy) methyl	286	$C_{16}H_{34}O$	✓		✓	✓
i-Propyl, 12-methyl-tridecanoate	270	$C_{17}H_{34}O_2$	✓			✓
1,2-Benzenedicarboxylic acid, butyl-2-ethyl ester	366	$C_{20}H_{30}O_4$	✓	✓		✓
Nonadecyl acetate	326	$C_{21}H_{42}O_2$				✓
1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester	278	$C_{16}H_{22}O_4$	✓	✓	✓	✓
1,2-Benzenedicarboxylic acid, ditridecyl ester	530	$C_{34}H_{58}O_4$	✓	✓	✓	✓
2-Dodecen-1-yl(-) succinic anhydride	266	$C_{16}H_{26}O_3$			✓	
Methyl-14-methyl-eicosanoate	340	$C_{26}H_{52}O_2$		✓	✓	
Methyl-21-methyldocosanoate	368	$C_{24}H_{48}O_2$		✓		
1,2-Benzenedicarboxylic acid, butyl-2-decyl ester	362	$C_{22}H_{34}O_4$			✓	
Eicosyl acetate	340	$C_{22}H_{44}O_2$			✓	
i-Propyl-14-methyl-pentadecanoate	298	$C_{19}H_{38}O_2$	✓		✓	

Nonane,4,5-dimethyl	156	$C_{11}H_{24}$		✓		
Isopropyl myristate	270	$C_{17}H_{34}O_2$		✓		
1,2-Benzenedicarboxylic acid,2-ethoxy-2-exoethyl methyl ester	266	$C_{13}H_{14}O_6$		✓		
I-(+) Ascorbic acid 2,6-dihexadecanoate	652	$C_{38}H_{68}O_2$		✓		
Triacontyl acetate	480	$C_{32}H_{64}O_2$		✓		
Tributyl acetylcitrate	402	$C_{20}H_{34}O_8$				✓
Cyclopenta(c)pyran-4-carboxylic acid, 7-methyl, methyl ester	190	$C_{11}H_{10}O_3$	✓			✓
Methoxyacetic acid, 2-tridecyl ester	272	$C_{16}H_{32}O_3$		✓	✓	
Dihyroartemisinin,10-O-(t-butyloxy)	356	$C_{19}H_{32}O_6$	✓			
Total detected esters			9	12	10	9

✓: Compound presence

TABLE-4
COMPARISON OF ALCOHOL COMPOUNDS AMONGST THE DIFFERENT TISSUES OF *Barleria dinteri* DETECTED THROUGH GC-MS ANALYSIS

Compound name	m.w.	m.f.	Plant parts			
			Branches	Flowers	Leaves	Roots
7,8-Epoxylanostan-11-ol-acetoxy	502	$C_{32}H_{54}O_4$			✓	
Phenol,2,4-*bis*(1,1-dimethylethyl)	206	$C_{14}H_{22}O$			✓	
Stigma-7,22-dien-3-ol-acetate (3-beta,5-alpha 2EE)	454	$C_{31}H_{50}O_2$				✓
1-Hentetracontanol	592	$C_{41}H_{84}O$		✓		
1-Octacosanol-2,4,6,8-tetramethyl (all-R)	466	$C_{32}H_{66}O$		✓		
Ethanol, 2-(didecycloxy)	230	$C_{14}H_{30}O_2$	✓			
Estra,1,3,5(10)-trien-17-beta-ol	256	$C_{18}H_{24}O$	✓	✓		
1-Docosanol, acetate	368	$C_{24}H_{48}O$	✓			
Total alcohols detected			3	3	2	1

✓: Compound presence

TABLE-5
COMPARISON OF FATTY ACID COMPOUNDS AMONGST THE DIFFERENT TISSUES OF *Barleria dinteri* DETECTED THROUGH GC-MS ANALYSIS

Compound name	m.w.	m.f.	Plant parts			
			Branches	Flowers	Leaves	Roots
8-Octadecenoic acid, methyl	296	$C_{19}H_{36}O_2$				✓
(E)-9-Octadecenoic acid, ethyl	310	$C_{20}H_{38}O_2$				✓
Hexanedioic acid, *bis*(2-ethylhexyl)	370	$C_{22}H_{42}O_2$			✓	
Octadecanoic acid, octadecyl	536	$C_{36}H_{72}O_2$			✓	
Pentanoic acid, 2,2,4-trimethyl-1,3-carboxyisopropyl, isobutyl	286	$C_{16}H_{30}O_4$		✓		
Benzenepropanoic acid-3,5-*bis*(1,1-dimethylethyl)-4-hydroxy-methyl	292	$C_{18}H_{28}O_3$	✓	✓	✓	✓
Heptadecanoic acid,10-methyl	298	$C_{19}H_{38}O_2$			✓	
9-Octadecanoic acid, octadecyl	534	$C_{36}H_{70}O_2$				✓
Nonahexacantanoic acid	998	$C_{69}H_{13}O_2$			✓	
7-Hexadecanoic acid, methyl	268	$C_{17}H_{32}O_2$		✓		✓
Total fatty acids detected			1	3	5	5

✓: Compound presence

TABLE-6
COMPARISON OF AMINE COMPOUNDS AMONGST THE DIFFERENT TISSUES OF *Barleria dinteri* DETECTED THROUGH GC-MS ANALYSIS

Compound name	m.w.	m.f.	Plant parts			
			Branches	Flowers	Leaves	Roots
cis-11-Eicosenamide	309	$C_{20}H_{39}NO$	✓	✓	✓	✓
13-Docosenamide	337	$C_{22}H_{43}NO$	✓		✓	
2,2,6,6-Tetramethyl-4-piperidone	155	$C_{20}H_{39}NO$	✓	✓		
18,19-Secoyohimban-19-oic,16,17,20,21-tetrahydro-16-(hydromethyl) methyl	352	$C_{21}H_{24}N_2O$	✓	✓	✓	✓
Total amines detected			4	2	5	2

✓: Compound presence

The GC-MS analysis of the extracts of the different parts of *B. dinteri* enabled the detection and identification of a number of compounds. GC-MS technique is widely used for the detection of bioactive compounds present in plant extracts [8]. The identified compounds included hydrocarbons, halogens, alcohols, esters, fatty acids, and amines. Most of the compounds detected

TABLE-7
% SIMILARITIES OF COMPOUNDS DETECTED IN THE ROOTS OF *Barleria dinteri* WITH THEIR PRESENCE IN THE OTHER TISSUES MAKING UP THE AERIAL PORTION OF THE PLANT SPECIES

Compound group	% Similarities with compounds present in the roots		
	Branches	Flowers	Leaves
Hydrocarbons	100	100	100
Halogens	10	20	10
Esters	0	0	0
Alcohols	78	44	44
Fatty acids	0	0	0
Amines	83	100	100
Average % similarity	45	44	42

in different parts of *B. dinteri* are already reported to possess several pharmacological activities that include antimicrobial, antioxidant, anti-inflammatory and cancer preventive. For example, 7,8-epoxylanostan-11-ol-3-acetoxy is reported to act as an anti-inflammatory agent [17,18]. Also, 2,6,10,14,18,22-tetracosahexaene,2,6,10,15,19,23-hexamethyl is known to possess anti-oxidant and antimicrobial activities whereas, phenol, 2,4-*bis*(1,1-dimethylethyl) possesses antimicrobial activity. Nonadecyl, pentafluoropropionate is used as a surfactant to functionalize carbon nanotubes [19]. Fatty acids and esters have many different applications including acting as antimicrobial agents [20]. Furthermore, 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl)ester has been reported to possess antimicrobial activity [21,22]. Hydrocarbons and alcohols have been found to contain biocidal activity against molds, yeast and bacteria [23], 2-dodecen-1-yl-(succinic anhydride) is reported to act as an antineoplasic agent, antioxidant and possess antimicrobial activity, 9-octadecenoic acid (*Z*) methyl ester is anti-inflammatory, anti-androgenic and cancer preventive [24]. Therefore, the results of the current study demonstrate that the different parts of *B. dinteri*, namely branches, flowers, leaves and roots, possess bioactive compounds that make them suitable for usage as potential herbal remedies.

Conclusion

The rationale of the current study was the determination of possible substitution of the underground part, the roots with the aerial parts, the branches, flowers and leaves of *B. dinteri* for usage in traditional medicine. The results showed most of the detected compounds to be present mostly in the aerial parts. In addition, most of the compounds detected in the roots were also present in one or more of the aerial parts. The results, therefore, suggested that most volatile compounds of the plant species, *i.e.* compounds separable through gas chromatography were found in the aerial parts. The possession of higher numbers of detected compounds by the aerial parts of *B. dinteri*, in particular the flowers, could be useful for possible synergistic effect on the health benefits of their extracts. The findings of the current study therefore provide a basis for substitution of the roots of *B. dinteri* with its aerial parts for usage in traditional medicine purposes, as contribution to the sustainable usage of the plant species. The usage of the flowers of *B. dinteri* for the medicinal purposes has not been reported thus far. Therefore, the findings of the present study provide a solid background for such explorative studies.

ACKNOWLEDGEMENTS

The Departments of Chemistry and Biochemistry of the Sefako Makgatho Health Sciences University are acknowledged for the provision of facilities to undertake the present study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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