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Isolation of an Iridoid glycoside compound from the leaves of *Barleria dinteri* collected from Zebediela sub-region in Limpopo province, South Africa

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Abstract

Barleria dinteri (*Acanthaceae)* is a perennial shrub that is widely distributed in Southern Africa. The leaves of the plant species are used in traditional medicine by South African healers to promote the healing of wounds, treatment of some intestinal tumours, infectious diseases and to relieve joint pains and toothache. The aim of the study was to isolate and identify the active ingredient(s) from the leaves of the South African *B. dinteri* species that contribute to the medicinal importance of the plant species. The ground leaves of *B. dinteri* were extracted with hexane, dichloromethane, acetone and methanol through a serial exhaustive extraction procedure. The obtained extracts were screened for free radical scavenging activity using diphenyl-1-picrylhydrazil (DPPH) bioassay. Furthermore, the methanol extract was fractionated using silica gel 60 column chromatography and the purified fraction was characterized using NMR. All the leaf extracts of *B. dinteri* indicated potential for free radical scavenging. A fraction suggesting to be representing a compound in pure form upon TLC analysis was obtained by the fractionation of the methanol extract using gel 60 column chromatography. Following NMR characterization of the purified fraction, the obtained compound was identified as an iridoid glycoside, Barledinoside. The isolation and identification of barlerin from the leaves of *Barleria dinteri* justifies the usage of the plant species in traditional medicine by local communities of Zebediela sub-region in Limpopo province, South Africa.

Keywords: *Barleria dinteri*, free radical scavenging activity, fractionation, isolation, NMR, iridoid glycoside

1. INTRODUCTION

People of different cultures have for a long time used medicinal plants in traditional medicine across the world. The widespread use of plants parts extracts as healthcare agents is based on the premise that some plants possess compounds with biological activities of medicinal importance [1]. Plants parts possess metabolites classified as primary and secondary metabolites. Primary metabolites comprise of common sugars, amino acids, proteins and chlorophyll that are involved in normal plant metabolism and are present in all plants. Secondary metabolites, in contrast, are present in selected plants and are responsible for defence against pathogens, herbivores, ultra-violet (UV) radiation and are also involved in colour or pigmentation of flowers and in pollination (2, 3). These secondary metabolites consist of phenolic compounds, terpenes, alkaloids, to mention but a few. In most cases, they are unique to individual plant species and usually occur in low concentrations [4].

Phytochemicals are soluble along a wide solvent polarity spectrum ranging from nonpolar to polar solvents, which makes their isolation a mammoth task. Bioassay-guided fractionation is a foremost pre-requisite towards the isolation of compounds with biological activities using targeted bioassay tests [5]. *Barleria dinteri* (*Acanthaceae*) is a perennial shrub that grows up to a height of 1.35 meters that is widely distributed in Southern Africa [6]. The leaves are used in traditional medicine to promote the healing of wounds, treatment of some intestinal tumours, infectious diseases and to relieve joint pains and toothache [7]. Studies on the phytochemical composition of related *Barleria* species, suggest the presence of glycosides, saponins, flavonoids, steroids and tannins [7, 8, 9, 10]. In our recent previous study, we have reported on the presence of alkaloids, flavonoids, saponins and tannins in the leaves of *B. dinteri* collected around the Zebediela sub-region of Limpopo province (South Africa), as well as possession of both the antioxidant and antibacterial properties by the leaves of the plant species [11]. However, the identity of the compound(s) that contribute to the reported biological

activities from the leaves of *B. dinteri* is not known. The current study deals with the isolation and identification of an iridoid glycoside compound from the methanol extract of the leaves of *B. dinteri* from Zebediela sub-region in South Africa, which, justifies the usage of the plant species in traditional medicine.

2. Material And Method

2.1. Plant material

The leaves of *B. dinteri* were collected from Bolahlakgomo village (-24.33, 29.31) in the Zebediela sub-region of the Limpopo province, South Africa. The plant material was collected in the presence of a local traditional healer and its identity authenticated by the University of Limpopo Herbarium were voucher specimen number UNIN 11118 was deposited. The leaves were dried at room temperature (25 $^{\circ}$C), ground to powder using a Retsch1MM 400 ball-milling machine (Monitoring and Control Pty Ltd., Hans, Germany) and stored in a closed container away from light until used.

2.2. Extraction of the plant material

The powdered leaves of *B. dinteri* (500 g) was extracted using hexane, dichloromethane, acetone and methanol respectively through serial exhaustive extraction procedure (SEE). The extracts were filtered through filter paper (Whatman 1, Sigma-Aldrich, South Africa) and concentrated using a Büchi rotary evaporator (Büchi Labortechnik R200, Flawil, Switzerland).

2.3. Isolation of the bioactive compound(s)

The leaf extracts of *B. dinteri* were screened for antioxidant properties using the free radical scavenging DPPH assay as described by Masoko and Eloff (12). The methanol extract was re-dissolved in methanol and subjected to liquid-liquid extraction with n-hexane. The methanol fraction was then further fractionated using gel 60 column chromatography with chloroform: methanol (8:2, v/v) as a mobile phase and obtained fractions were assessed for purity and free radical scavenging activity by TLC analysis.

2.4. Characterization of the fraction isolated in pure form

The fraction (CPD8BM) that was deemed to be in pure form upon TLC analysis and also exhibited free radical scavenging activity against DPPH was subjected to NMR characterization using CD_3OD as solvent. ^{1}H and ^{13}C NMR spectral profiles of the fraction were obtained on a Varian MRS 400 Hz NMR instrument (SMM Instruments). The 2D NMR spectra (COSY and gHMBC) were used for assignment of proton and carbon peaks. The UV absorption profile was determined using a Varian Cary 50 UV-Vis Spectrophotometer (Agilent Technologies, USA). The obtained NMR profiles were compared with those reported in the literature for the identification of the isolated fraction.

3. Results And Discussion

The schematic representation for the bioassay-guided fractionation procedure for isolation of a compound from the leaves of *B. dinteri* is shown in Figure 1. Extraction of the powdered leaves of *B. dinteri* through a serial exhaustive extraction procedure afforded 0.12 g, 0.92 g, 1.21 g and 2.81 g of dry hexane, dichloromethane, acetone and methanol extract, respectively. As expected, the highest yield was obtained with methanol as an extracting solvent. All four extracts exhibited some free radical scavenging activity as evidenced by the discoloration of the purple DPPH stain to yellow by bands of compounds resolved on TLC plates. Compound bands within the methanol extracts appeared to possess stronger free radical scavenging activity than those of other extracts as suggested by the higher intensity of the displayed discoloration of the DPPH stain. The suggested stronger free radical scavenging activity displayed by the methanol extract served as the basis for its further fractionation. After liquid-liquid extraction of the methanol extract with n-hexane, fractionation of the methanol fraction using gel 60 silica column chromatography afforded the isolation of a fraction (CPD8BM, 0.15 g) with free radical scavenging activity in its pure form. The purity of the fraction (CPD8BM) was confirmed by a single band with *Rf* value of 0.38 (CHCl3: MeOH 4:1, v/v) that was obtained on TLC plate upon visualization using vanillin in sulphuric and UV light.

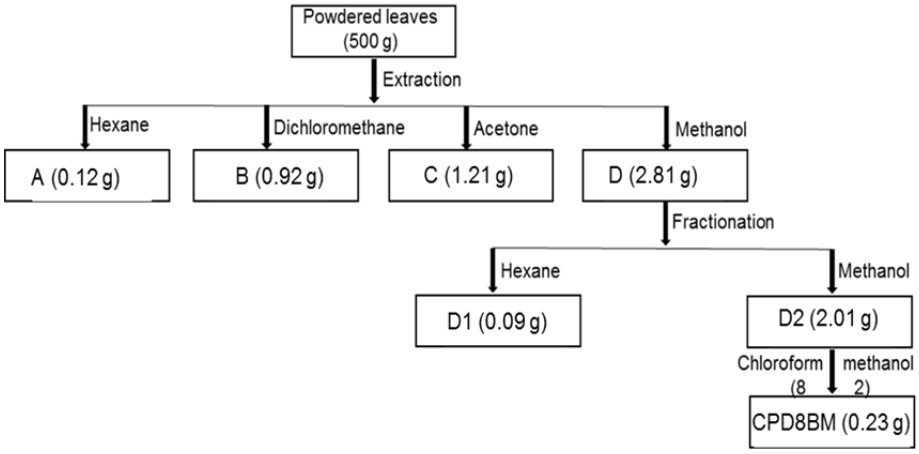

Figure 1: Schematic representation of the bioassay-guided fractionation procedure for the isolation of an iridoid glycoside compound from the leaves of *B. dinteri*.

The structural features of the isolated fraction were determined through UV-vis and NMR characterization. The UV absorption spectrum of CPD8BM showed λ_{max} at 236, suggestive of the presence of α, β-unsaturated carbonyl moiety within the structure of the isolated compound.

^{1}H-NMR (CD_3OD): 7.43 (H-3), 5.91 (H-1), 4.89 (H-1'), 3.87 (H-6), 3.66 ($COOOCH_3$), 3.25 – 4.32 (H-2' – H-6'), 3.05 (H-5), 2.16 (H-9), 2.03 ($OCOOCH_3$), 2.00 (H-7), 1.50 (H-10).

^{13}C-NMR (CD_3OD): 94.26 (C-1), 152.31 (C-3), 108.34 (C-4), 40.87 (C-5), 74.49 (C-6), 46.21 (C-7), 88.34 (C-8), 48.51 (C-9), 29.0 (C-10), 20.79 (CH_3), 50.41 (CH_3O), 167.57 (C=O), 171.79 (C=O), 98.92 (C-1'), 73.23 (C-2'), 76.56 (C-3'), 76.89 (C-5'), 70.17 (C-4'), 61.51 (C-6')

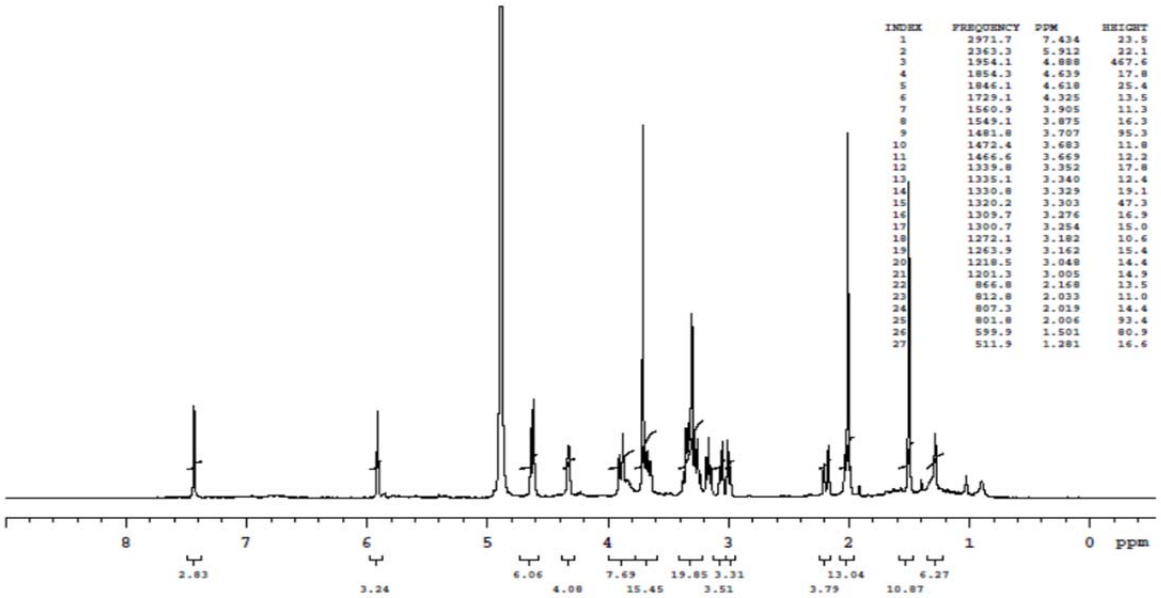

Figure 2: ^{1}H NMR spectrum (CD_3OD) of the fraction (CPD8BM) isolated from the methanol leaf extract of *B. dinteri.*

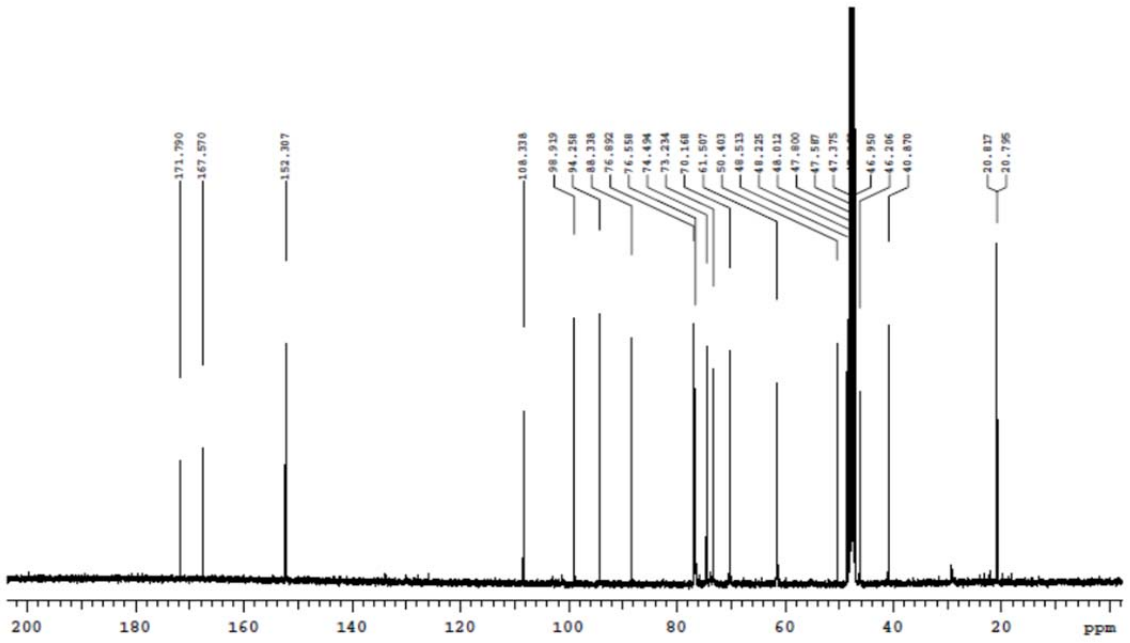

Figure 3: ^{13}C NMR spectrum (CD_3OD) of fraction (CPD8BM) isolated from the methanol leaf extract of *B. dinteri.*

Proton NMR spectrum (Figure 2) of CPD8BM showed a proton signal δ_H 2.03 ppm that is characteristic of an acetyl group and a signal at δ_H 1.50 indicating a tertiary methyl group. The peak signal at δ_H 4.89 suggest the presence of an anomeric proton for a β-glucose moiety. In addition to the anomeric proton, other proton signals δ_H 3.25 – 4.32 confirmed the sugar unit. Proton signals at δ_H 7.43 (H-3) and 3.66 ($COOCH_3$) indicate the presence of an enol-ether backbone with a carbomethoxy group [13]. In addition to the signals attributable to an acetyl group, an enol-ether backbone with a carbomethoxy group, a methyl group and a glucose moiety, proton signals of two oxymethines (δ_H 5.91 and 3.87), two methines (δ_H 3.05 and 2.16) and a methylene group (δ_H 1.50). The methoxyl group is also confirmed by the peak signal at δ_C 50.41 on the ^{13}C NMR spectrum (Figure 3).

Carbon peak signals at δ_C 171.79 and 167.57 indicate the presence of carbonyl carbons confirming the presence of acetyl groups earlier suggested with ^1H NMR peaks [14]. The peak signal at δ_C 152.31 indicates the presence of an olefinic bond within the structure. Carbon peak signals at δ_C 61.51, 70.17, 73.23, 76.56, 76.89 and 98.92 confirms the β-glucose moiety [15]. Other carbon signals were those of two methyl group, C-10 and C-12 at □ 20.79, one methylene C-7 at δ_C 46.21 and five methyl carbons (C-1, C-3, C-5, C-6, C-9) at□δ_C 94.26, 152.31, 40.87, 74.49 and 48.51 respectively thus bringing the total number of carbons in CPD8BM to 19.

Assignment of proton and carbon peaks was based on 2D NMR spectrum (COSY and gHMBC) The HMBC experiment revealed that C-13 correlated to a methoxy proton at 50.41 ppm. The presence of methoxy carbonyl carbon in CPD8BM and knowledge of compounds that have been previously reported from the genus, *Barleria*, arouse the possibility that CPD8BM could contain an iridoid skeleton *O*-glycosylating to a sugar unit. Hence, a possible HMBC linkage between the methoxy group, the carbonyl carbons (C-13 and C-14),) and the presumed iridoid skeleton was investigated.

To confirm our suspicion, long *J*-signals for the methoxy proton at □ 3.66 connected to C-13 carbonyl carbon at □ 167.57 ppm was observed. The HMBC correlation of H-3 at □ 7.43 to the carbon at 108.34 and the anomeric carbon at 98.92 ppm was in favour of an iridoid glycoside [16]. Comparison of the NMR spectra profile of CPD8BM with those reported in the literature (Table 1) suggested the identity of the isolated compound to be barlerin (8-O-Acetylshanzhiside methyl ester).

Table 1: ^1H and ^{13}C NMR (CD_3OD) chemical shift values (ppm) of compound CPD8BM compared with the ones from the literature (17).

Atom (C/H) Position	^{13}C NMR Experimental (CD_3OD)	^{13}C NMR Literature (CD_3OD) (Kaur et al., 2014)	^1H NMR Experimental (CD_3OD)	^1H NMR Literature (CD_3OD) (Kaur et al., 2014)
1	94.26	94.29	5.91	5.82
3	152.31	152.40	7.43	7.35
4	108.34	108.37		
5	40.87	40.85	3.05	3.08
6	74.49	74.53	3.87	3.80
7	46.21	46.29	1.50	1.93
8	88.34	88.42		
9	48.51	48.49	2.16	2.09
10	□ 29.0	29.41	1.28	1.41
CH3	20.79	20.80	1.50	1.51
$COO\underline{C}H_3$	50.4	50.47	3.66	3.62
$O\underline{C}OCH_3$	167.75	167.75		
$OCO\underline{C}H_3$	171.8	172.02	2.03	2.08
O-Glucose				
1’	98.92	98.88	4.89	4.80
2’	73.23	73.24		
3’	76.56	76.54		
4’	70.17	70.18		
5’	76.89	76.88		
6’	61.51	61.47		

(Assignments made using COSY and gHMBC correlations, □: peak present in small intensity).

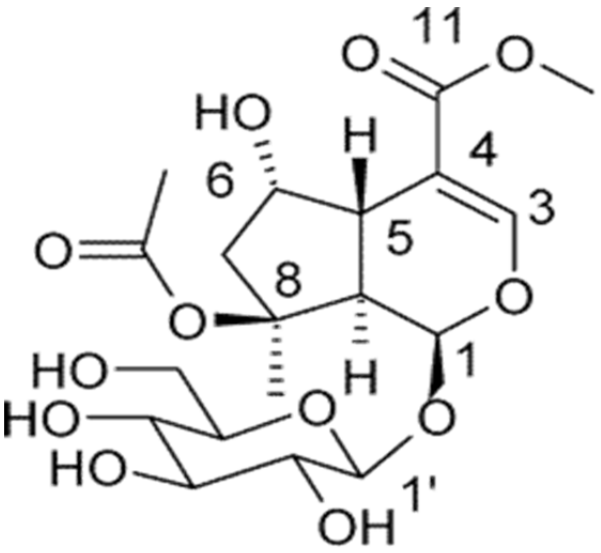

Figure 3: Structure of barlerin (8-*O*-acetylshanziside methyl ester), whose NMR profile was found to be similar to that of CPD8BM.

4. CONCLUSION

Fractionation of the methanol extract of the leaves of *B. dinteri* using silica gel 60 column chromatography, afforded the isolation of an iridoid glycoside compound, barlerin. Iridoid glycosides have been reported to possess biological activities that include antibacterial, antidiabetic and anti-inflammatory properties [14]. This compound, barlerin, was initially isolated from *Barleria prionitis* [18] and its isolation from *Barleria dinteri* was not reported before this study.

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