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## Comparative Qualitative Phytochemical analysis of the different parts of *Barleria dinteri* (Oberm): A contribution to sustainable use of the plant species

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### ABSTRACT

The objective of the study was to undertake a comparative qualitative phytochemical analysis of the different parts of *Barleria dinteri*, a traditional herb used against several diseases. The different parts of *B. dinteri* were extracted using cold maceration method through a serial exhaustive exclusion procedure. The extracts were subjected to qualitative phytochemical analysis through chemical screening, thin layer chromatography and UV-visible spectrophotometric analysis. Qualitative phytochemical analysis through chemical screening showed mostly similarities in the phytochemical compositions of the different plant parts with only few differences observed. Anthraquinones were not detected in the extracts of all different plant parts. The TLC profiles of samples, depicted through the  $R_f$  values of resolved compound bands, also showed mostly similarities with few differences. The UV-visible spectrum of the samples showed mostly similar maximum absorbance wavelengths amongst similar extracts of the different plant parts. From the results of this study it can be concluded that the qualitative phytochemical profiles of branches, flowers, leaves and roots of *B. dinteri* are more similar. As such, the aerial parts of *B. dinteri* could be recommended to substitute the usage of the roots in traditional medicine as a contribution to sustainable usage of the plant species.

**KEYWORDS:** *Barleria dinteri*; phytochemicals; plant parts; chemical screening tests; thin layer chromatography; UV-vis spectrophotometry.

### INTRODUCTION

Plants possess a unique richness and diversity of metabolites [1]. Natural products isolated from plants have been postulated to remain an essential part of the search for novel medicines against human diseases. Although the plant kingdom has remained poorly explored, today there is growing interest in chemical composition of plant based medicines [2].

Medicinal plants contain naturally-occurring phytochemicals that have defence mechanisms and protection from various diseases [3]. Most phytochemicals have been known to bear valuable therapeutic activities such as insecticidal, antibacterial, antifungal, anti-constipative, spasmolytic, anti-plasmodial and antioxidation [4, 5, 6]. Majority of the population

in developing countries depends on traditional medicine for their primary health care [7]. Population growth, urbanization and unrestricted collection of medicinal plants from the wild are resulting in an over-exploitation of natural resources in Southern Africa [8].

Collection of underground parts, the roots, of medicinal plants leads to mass scale uprooting of plants from their natural habitat [9]. This may lead to depletion of plant resources in the near future due to which, unavailability of plants for use in traditional system of medicine may arise. While some of the species may have certain traits that enable them to recover quickly and are able to adapt to continuous harvesting, others exhibit traits that make them very sensitive to uncontrolled harvesting and these species may not recover for a long time [10]. Conservation of medicinal plants has become a subject of primary importance considering a constant expansion of the rare or endangered plant species list [11]. Substitution of the under surfaced plant parts with aerial parts may contribute to the prevention of the rapid addition of more medicinal plants on the plant red list [12, 13]. However, plant parts substitution may only be pursued provided the substituting part possess similar or more beneficial properties, which may be assessed in terms of the comparative phytochemical composition.

Comparison of the phytochemical composition of different plant parts may lead to the utilization of plants parts, in particular the aerial parts, with minimum adversity to the conservation of the plants. In a study by Srivastava et al [14], the major bioactive constituents of the different parts (roots, stem and flowers) of *Taraxacum officinale* were analysed for comparison. Saponins, flavonoids, alkaloids, phenols were highly concentrated in the stems, roots and flowers, with higher concentration of flavonoids in the flower extract. In another separate study, the phytochemical composition of the root and leaf parts of the medicinal herb, *Hypochaeris radicata* L. were also investigated [15]. The findings of the study showed that alkaloids, cardiac glycosides, phenols, resins, saponins, steroids, tannins, terpenoids and triterpenoids were found in both the leaf and root extracts.

*Barleria dinteri* is a rare medicinal plant with specific habitation that belongs to the family Acanthaceae and used in traditional medicine

for treatment of diseases such as intestinal tumours and bacterial infections. It is found in specific areas in three provinces of South Africa, namely, Limpopo, Mpumalanga and Gauteng; Swaziland, Botswana and Namibia. *B. dinteri* is one medicinal plant whose roots and leaves are used interchangeably in traditional medicine (healing) in Limpopo province, South Africa [16]. In this regard, the primary aim of the study was to undertake the qualitative phytochemical analysis of different parts (namely, flowers, leaves, branches and roots) of *Barleria dinteri* with intention of motivation for usage of plant parts with less adverse implications for the survival of the plant species.

## MATERIALS AND METHODS

### Chemicals

All chemicals, reagent and solvent used during the experimentation were of analytical grade and TLC plates were purchased from Merck (Pty) Ltd, (Halfway House, South Africa).

### Collection and Identification of the plant material

The flowers, leaves, branches and roots of *B. dinteri* (Voucher specimen: UNIN 11118) were collected from Zebediela in Limpopo Province, South Africa during the full bloom season, using convenient sampling method. The different plant parts were dried at room temperature, ground to powder and stored in the dark until used.

### Extraction

The ground branches, flowers, leaves and roots (5 g) of *B. dinteri* collected from Zebediela in Limpopo Province, South Africa were each extracted with 50 ml of *n*-hexane, dichloromethane, acetone and methanol using cold maceration method in a serial exhaustive extraction procedure. The resultant extracts were filtered and the solvents evaporated under a stream of air.

### Experimental procedure

#### *Phytochemical screening using chemical reactions*

Chemical screening tests for the detection of bioactive chemical constituents in extracts of the different parts of *B. dinteri* were carried out using the standard procedures described below [17]:

#### *Test for Alkaloids- Hager's test*

To 1 ml of the sample, few drops of Hager's reagent (Picric acid) was added. Formation of a yellow precipitate indicates the presence of alkaloids.

#### **Test for Anthraquinones**

A small fraction of the sample was mixed with 10 ml chloroform; the mixture was shaken and filtered. Thereafter, 5 ml of 10 % NH<sub>3</sub> solution was added to the filtrate. The appearance of pink/red colour confirms the presence of anthraquinones.

#### **Test for Coumarins**

Few drops of 10 % NaOH was added to the extract, after which chloroform was added. Observation of yellow colour which shows the presence of coumarins.

#### **Tests for Flavonoids - (Shinoda test-Magnesium hydrochloride reduction test)**

To the test solution, few fragments of magnesium ribbon and concentrated hydrochloric acid were added drop wise. Observation of reddish to pink colour indicates the presence of flavonoids.

#### **Test for Tannins**

A 2ml amount of distilled water was added to 1 ml extract solution and then few drops of ferric chloride solution were added. Gallic tannin solution is observed through blue colour and catecholic tannin solution gets indicated through green black colour.

#### **Tests for Steroid and Terpenoids**

Aliquots comprising 4 ml extracts were treated with 0.5 ml acetic anhydride and 0.5 ml chloroform, then concentrated H<sub>2</sub>SO<sub>4</sub> added slowly. Steroid solution is indicated through green blue colour and terpenoid solution is indicated through red violet colour.

#### **Test for Saponins- Foam Test**

1 ml of the extract was added to 2 ml of distilled water and shaken vigorously for a few minutes in a test tube. Formation of a 1 cm of persistent foam for 10 min indicates the presence of saponins.

#### **Thin Layer Chromatography analysis**

Extracts of the different parts of *B. dinteri* were subjected to TLC analysis using silica gel 60 (F<sub>254</sub>) TLC plates as described by Alebiosu and Yusuf [18], with modifications. Aliquots (10 mg/ml) of extracts were loaded on TLC plates and resolved using three mobile phases of different polarities, namely, Hexane: Ethyl

acetate (9:1 v/v) for low polarity; Chloroform: Methanol (9:1 v/v) for intermediate polarity, and Ethyl acetate: Methanol: Water (8:4:1 v/v/v) for high polarity. Compound bands within extracts were located and circled on TLC plates upon eye visualization, under UV light, as well as after spraying with vanillin in sulphuric acid. The retention factors (*R<sub>f</sub>*) of the resolved compound bands were calculated as follows:

$$R_f = \frac{\text{distance travelled by solute or compound}}{\text{distance travelled by solvent}}$$

#### **Ultraviolet- visible spectrophotometric analysis**

The extracts of the different parts of *B. dinteri* were subjected to UV-vis spectrophotometric analysis [18]. Each sample of the plant parts extracts was diluted ten-fold and scanned along the wavelength range of 200-750 nm using a CECIL 1021 spectrophotometer (LASEC, S.A.). Wavelengths of peaks showing maximum absorbance were then noted and recorded.

#### **RESULTS**

Extracts of the different parts, namely: branches, flowers, leaves and roots of *B. dinteri* underwent phytochemical screening using chemical tests and the results are shown in Table 1. Phytochemical screening showed the alkaloids, coumarins, flavonoids, saponins, steroids, tannins and terpenoids in one or more extracts of the different plant parts, although anthraquinones were not observed in any extract of all the plant parts. Alkaloids were across the extracts found to be present in the branches, flowers and roots, while absent in the leaves. Anthraquinones were not observed in any extract of the different parts of the plant species. Coumarins, saponins and terpenoids were on average presently in all plants parts when wholly considering the extracts of the different plant parts. Flavonoids were mainly found in polar extracts, whereas steroids were mainly found in non-polar extracts. Tannins were present in all extracts of the different plants except in their *n*-hexane extracts.

**Table 1: Qualitative phytochemical analysis of the extracts of different parts of *B. dinteri* through chemical screening**

Phytochemicals	n-Hexane				Dichloromethane				Acetone				Methanol			
	B	F	L	R	B	F	L	R	B	F	L	R	B	F	L	R
Alkaloids	-	+	-	-	+	+	-	-	+	-	-	+	+	+	-	+
Anthraquinones	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Coumarins	-	+	+	-	+	-	-	-	+++	+	++	+++	+++	++	+	+++
Flavonoids	-	-	-	-	-	-	-	-	-	+	+	+++	-	+	-	+
Saponins	+	+	+	+	-	+	+	-	+++	-	+++	+	-	+	+	+
Steroids	-	+++	+++	-	++	++	+++	-	-	-	-	-	-	-	-	-
Tannins	-	-	-	-	+	-	++	-	++	+	+++	+	+++	+++	+++	+++
Terpenoids	+	-	-	+	-	-	-	+	+	+	++	+++	+++	+++	+	+++

(-: absence; +: presence in traces; ++: presence in moderates; +++: presence in abundance); (**B**: branches; **F**: flowers; **L**: leaves; **R**: roots)

Extracts obtained from the different parts of *B. dinteri* were subjected to TLC analysis using mobile phases of different polarities and the results in the form of  $R_f$  values of resolved compound bands are shown in Tables 2.1, 2.2 and 2.3. The TLC chromatograms, as depicted through  $R_f$  values showed mostly similarities in the extracts of the different plant parts. From the chromatogram developed using a mobile phase of lower polarity (Table 2.1) only two distinct compound bands with  $R_f$  values of 0.38 and 0.51 were located in the *n*-hexane extract of the roots as compared to the similar extracts of the other plant parts. In addition amongst the hexane extracts, the compound band with  $R_f$  value of 0.21~ 0.22 was located only in the aerial parts; i.e. branches, flowers and leaves; while the other compared band with  $R_f$  values of 0.29 was located only in the leaves. Furthermore, the compound band with  $R_f$  value of 0.83~ 0.85 was located only within the *n*-hexane extracts of the flowers and leaves. The compound band with  $R_f$  value ~ 1.00 was absent in both the *n*-hexane and dichloromethane extracts of the roots,

whereas present in both extracts of the other plant parts.

From the chromatogram developed using a mobile phase of intermediate polarity (Table 2.2), a compound band with  $R_f$  value of 0.81 was located only in the flowers amongst the *n*-hexane extracts of the different parts of the plant species. Amongst the dichloromethane extracts of the plant parts, compound bands with  $R_f$  values of 0.39 and 0.50 were located exclusively in the flowers and leaves, respectively. Still amongst the dichloromethane extracts, a compound band with  $R_f$  value of approximately 0.90 was located only in the flowers and the roots. In addition, a compound band with  $R_f$  value of ~ 0.70 (i.e. 0.73 and 0.69 on the chromatogram) was located only in the dichloromethane extract of the flowers and the acetone extract of the leaves. From the chromatogram developed using a mobile phase of high polarity (Table 2.3), very few differences could be spotted as compound bands with  $R_f$  values of 0.26 and 0.60 were only located in the flowers and the roots, respectively.

**Table 2.1:  $R_f$  values showing compound bands within the different parts extracts of *B. dinteri* resolved using a lower polarity mobile phase.**

Mobile phase: Hexane: Ethyl acetate (9:1): Lower polarity															
n-Hexane extracts				Dichloromethane extracts				Acetone extracts				Methanol extracts			
B	F	L	R	B	F	L	R	B	F	L	R	B	F	L	R
-	0.00	0.00	-	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
-	0.14	-	-	-	0.05	-	0.11	-	-	-	-	-	-	-	-
0.21	0.22	0.22	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	0.29	-	-	-	-	-	-	-	-	-	-	-	-	-
-	0.35	0.35	0.35	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	0.38	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	0.51	-	-	-	-	-	-	-	-	-	-	-	-
-	0.85	0.83	-	-	-	-	-	-	-	-	-	-	-	-	-
1.00	1.00	0.95	-	-	-	-	-	-	-	-	-	-	-	-	-

(-: absent; **B**: branches; **F**: flowers; **L**: leaves; **R**: roots)

**Table 2.2:  $R_f$  values showing compound bands within the different parts extracts of *B. dinteri* resolved using an intermediate polarity mobile phase**

Mobile phase: Chloroform: Methanol (9:1): Intermediate polarity															
n-Hexane extracts				Dichloromethane extracts				Acetone extracts				Methanol extracts			
B	F	L	R	B	F	L	R	B	F	L	R	B	F	L	R
-	-	0.00	-	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
-	-	-	-	0.08	0.06	0.06	0.06	-	-	-	-	-	-	-	-
-	-	-	-	0.23	0.21	0.21	0.23	-	-	-	-	-	-	-	-
<b>0.32</b>	0.29	0.29	0.29	-	-	-	0.32	-	-	-	-	-	-	-	-
-	-	-	-	-	0.39	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	0.50	-	-	-	-	-	-	-	-	-
-	-	-	-	0.64	0.62	0.62	-	-	-	-	-	-	-	-	-
-	-	-	-	-	0.73	-	-	-	-	0.69	-	-	-	-	-
-	0.81	-	-	-	0.85	0.82	-	-	-	-	-	-	-	-	-
-	-	-	-	-	0.94	-	0.91	-	-	-	-	-	-	-	-

(-: absent; **B**: branches; **F**: flowers; **L**: leaves; **R**: roots)

**Table 2.3:  $R_f$  values showing compound bands within the different parts extracts of *B. dinteri* resolved using a higher polarity mobile phase**

Mobile phase: Ethyl acetate: Methanol: Water (8:4:1): Higher polarity															
n-Hexane extracts				Dichloromethane extracts				Acetone extracts				Methanol extracts			
B	F	L	R	B	F	L	R	B	F	L	R	B	F	L	R
0.00	0.00	0.00	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	0.26	-	-
-	-	-	-	-	-	-	-	-	0.40	-	0.40	-	-	-	0.43
-	-	-	-	-	-	-	-	-	-	-	0.51	-	-	-	0.54
-	-	-	-	-	-	-	-	-	-	-	0.60	-	-	-	-
-	-	-	-	-	-	-	-	0.67	-	0.67	-	0.66	0.66	0.66	0.68
-	0.72	-	0.71	-	0.71	0.72	0.72	0.72	0.72	0.72	0.73	-	-	-	-

-	-	-	-	0.75	-	-	-	0.76	-	0.77	0.77	-	-	-	-
-	-	-	-	0.84	0.82	0.82	0.80	0.79	0.79	0.81	0.83	-	-	-	-
-	-	-	-	0.89	0.89	0.89	0.89	-	0.88	-	0.88	-	-	-	-
-	-	-	-	-	-	0.92	0.92	-	-	-	0.94	-	-	-	-

(-: absent; **B**: branches; **F**: flowers; **L**: leaves; **R**: roots)

The extracts of the different parts of *B. dinteri* were subjected to UV-visible spectrophotometric analysis and the results of the maximum absorbance wavelength of the components of the extracts are shown in Table 3. The maximum absorbance wavelengths ( $\lambda_{\max}$ ) of similar extracts were mostly similar for the different parts although, they differed in the obtained

absorbance values. The exception was only seen in the dichloromethane extracts whereby the extracts of the branches and flowers showed two  $\lambda_{\max}$  (i.e., 200 nm and 240 nm), whereas the extracts of the leaves and the roots showed only one  $\lambda_{\max}$  (i.e., 200 nm), as well as in the methanol extracts whereby  $\lambda_{\max}$  of 320 nm was only seen in branches.

**Table 3: Maximum absorbance wavelengths ( $\lambda_{\max}$ ) of the extracts of the different parts of *B. dinteri***

Extracts Solvent	Branches	Flowers	Leaves	Roots
	$\lambda_{\max}$ (Abs. value)	$\lambda_{\max}$ (Abs. value)	$\lambda_{\max}$ (Abs. value)	$\lambda_{\max}$ (Abs. value)
n-Hexane	200 nm (0.699)	200 nm (1.184)	200 nm (1.184)	200 nm (1.456)
Dichloromethane	200 nm (1.972) 240 nm (1.844)	200 nm (1.812) 240 nm (0.781)	200 nm (2.980) -	200 nm (2.990) -
Acetone	200 nm (1.470) 260 nm (2.960)	200 nm (0.839) 260 nm (2.215)	200 nm (1.198) 260 nm (2.365)	200 nm (1.870) 260 nm (2.980)
Methanol	200 nm (2.275) 320 nm (0.866)	200 nm (2.720) -	200 nm (2.940) -	200 nm (2.460) -

(Abs.: absorbance; -: absent)

## DISCUSSION

The results of the phytochemical screening of the different parts of *B. dinteri* showed the presence of all tested phytochemicals except anthraquinones. The phytochemical screening results also showed mostly similarities in the phytochemical compositions of the different plant parts with only few differences observed. The results of the TLC analysis of the extracts of the different parts of the plant species depicted through the  $R_f$  values of resolved compound bands showed mostly similarities with only few differences where some compound bands were present in extracts of certain plant parts while absent in similar extracts of other parts. In TLC analysis, phytochemicals within extracts are separated based on their polarities. As such, the results of the current study suggest that the

polarities of phytochemicals present in the different parts of *B. dinteri* are mostly similar.

The UV-vis analysis of the plant extracts is of importance for the characterization of the components of the extracts, such as the detection of the presence of compounds with unsaturated bonds [18]. The results of the present study indicate mostly similarities in the UV-vis spectral profiles of similar extracts of the different parts of *B. dinteri* as shown by mostly similar  $\lambda_{\max}$  of the plant parts extracts. The results thus suggest similarities in phytochemical compositions amongst the different parts of the plant species. The nature of the components of the plants extracts informs their inherent biological activities [4]. The presence and characteristics, such as chemical reaction, polarity and light absorbance

properties of phytochemicals in plants parts give credence to their medicinal importance due to the many biological activities they effect in physiological systems of animals and humans [19]. Since the observed phytochemical composition of the different parts of *B. dinteri* as determined through phytochemical screening chemical tests, TLC and UV-Vis spectrophotometric analysis appear to be mostly similar, thus their biological activities are also likely to be more similar.

## CONCLUSION

Qualitative phytochemical analysis of plant parts extracts is important as it indicate the nature of phytochemicals that are possessed by such medicinal plants. The results of the current study suggest more similarities in the phytochemical compositions of the different parts of *B. dinteri* which, is likely to contribute to some similarities in their biological activities. Thus, the substitution of the roots of *B. dinteri* with the aerial parts could be encouraged as contribution to the sustainable usage of the plant species in traditional medicine.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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