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Antifungal, acetylcholinesterase inhibition, antioxidant and phytochemical properties of three *Barleria* species

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Abstract

This study was aimed at evaluating the antifungal, acetylcholinesterase inhibition and antioxidant activities of petroleum ether, dichloromethane, ethanol and methanol extracts from different parts of *Barleria prionitis*, *Barleria greenii* and *Barleria albostellata*. Their phytochemical properties and the possibility of plant-part substitution as a conservation strategy against destructive harvesting (use of aerial parts and roots) of these species for medicinal purposes were also investigated. Microtitre plate assays were used in determining their antifungal (against *Candida albicans*) and acetylcholinesterase inhibition activities. All the extracts demonstrated both fungistatic and fungicidal activities with the minimum inhibitory concentration ranging from 0.78 to 9.38 mg/ml and minimum fungicidal concentration ranging from 1.17 to 12.50 mg/ml. The higher inhibitory activity of *B. greenii* leaf extracts in most cases compared to similar extracts of the stems and roots suggest the potential of *B. greenii* leaves in plant-part substitution. At the lowest extract concentration (0.156 mg/ml), the leaf extract of *B. greenii* demonstrated a significantly higher acetylcholinesterase (AChE) inhibition than its stem and root extracts. In *B. albostellata*, the AChE inhibitory activity demonstrated by the stem was significantly greater than that recorded in its leaf extract. These findings suggest that the idea of plant part substitution may be species and/or biological activity dependent. In the DPPH radical scavenging assay, different parts of *Barleria* species showed free radical scavenging activity with EC_{50} values ranging from 6.65 to 12.56 μg/ml. The ability of the extracts from different plant parts to reduce ferric ion/ferricyanide complex to the ferrous form and decrease carotenoid bleaching rate suggests the presence of antioxidant compounds capable of donating electrons and hydrogen atoms in their reaction mechanisms. Flavonoids, iridoids and tannins were detected in the different parts of these *Barleria* species. These phytochemicals might be responsible for the observed biological activities. The isolation of specific bioactive compounds through bioassay-guided fractionation and their characterization as well as studies evaluating their safety may be necessary in the exploration of these species for potential new therapeutic drugs or drug leads.
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Keywords: AChE; Alzheimer's disease; *Candida albicans*; Candidiasis; DPPH radical scavenging; Secondary metabolites

1. Introduction

The use of natural products in disease prevention and control as well as in drug development has received increased attention in recent times. According to Rates (2001), about 25% of globally prescribed drugs are obtained from plants. The author observed that 11% of the 252 drugs considered as basic and essential by the World Health Organisation are solely of plant origin. In many parts of the world, especially in Africa, the increasing use of plants in traditional medicines by a growing population, among other reasons, has resulted in the over-exploitation of many of our plant resources.

Plants, in addition to their therapeutic use in herbal preparations, can serve as important sources of new drugs, new drug leads and new chemical entities (Balunas and Kinghorn, 2005). However, a large percentage of the estimated 350,000 plant species on earth is yet to be investigated for their pharmacological and phytochemical potential (Hostettman and Marston, 2002). Shai et al. (2008) noted that some South

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African indigenous plant species are at risk of becoming extinct before the investigation and application of their potential as sources of therapeutic drugs can be carried out. Some of these plant resources (including those rapidly disappearing) could contain novel, active chemotypes that can serve as leads for effective drug development (Cragg et al., 1997).

The dual chemical–biological screening approach has been recommended as the fastest method of discovering important plant-derived bioactive compounds (Hostettman and Marston, 2002). The localisation of such active compounds, however, requires the use of simple but sensitive target-specific bioassays, since plant extract often contains low concentrations of active compounds (Hostettman and Marston, 2002; Rates, 2001). Considering the fact that plant extracts often contain different chemicals with different pharmacological activities, the use of a series of pharmacological tests has been recommended in order to get a complete bioactivity spectrum of a plant extract (Houghton et al., 2007). The use of a series of pharmacological tests might also be helpful in understanding the mechanism of action involved in the therapeutic effect of a particular plant extract since the aetiology of many disease states is often due to more than one factor (Houghton et al., 2007).

Opportunistic fungal infections such as candidiasis caused by *C. albicans* have been reported to be common especially among immuno-compromised persons with AIDS (Motsei et al., 2003; Shai et al., 2008). Alzheimer's disease (AD) is the most common type of neurodegenerative disease, characterized primarily by impaired memory and cognitive dysfunction, and at advanced stages, language deficit, depression, agitation, mood disturbances and psychosis (Houghton et al., 2007). The inhibition of the acetylcholinesterase enzyme (AChE), leading to the maintenance of acetylcholine levels and enhanced cholinergic function has become the standard approach in the symptomatic treatment of AD (Howes and Houghton, 2003; Vinutha et al., 2007). The undesirable side effects such as hepatotoxicity in the therapeutic use of some AChE inhibitors (like tacrine) coupled with their limitation to only alleviate symptoms without any long-lasting improvement has necessitated further search for more potent drugs (Ferreira et al., 2006; Howes and Houghton, 2003; López et al., 2002).

Free radical reactions have been implicated in the pathology of a large number of disease states such as cancer, AD, diabetes, inflammation and several cardiovascular diseases (Houghton et al., 2007). According to Howes and Houghton (2003), the use of antioxidants has been shown to slow AD progression and neuronal degeneration. The realization of antioxidant roles in the management of some disease states has led to the inclusion of antioxidant tests in many pharmacological screenings of plant extracts and isolated compounds.

The leaves, tender twigs and roots of *Barleria prionitis* L. (Family: Acanthaceae) are used in folk medicine to treat infection-related ailments, fever, toothache, body ache, inflammation, dropsy, liver congestions and in irritation control (Kosmulalage et al., 2007; Singh et al., 2003; Upadhyay et al., 2010; Verma et al., 2005). In our previous studies, extracts of *Barleria greenii* M.-J. Balkwill & K. Balkwill, *B. prionitis* and *Barleria albostellata* C.B. Clarke showed good anti-inflammatory and broad-spectrum antibacterial activities (Amoo et al., 2009a). *B. greenii* is critically endangered and endemic to a small area in the KwaZulu-Natal province, South Africa (Raimondo et al., 2009). The current study was aimed at further exploring the medicinal potential of South African *Barleria* species. The specific objectives include evaluating the antifungal, acetylcholinesterase inhibition and antioxidant activities of extracts from different parts of these species; investigating the possibility of plant-part substitution as a conservation strategy against destructive harvesting of these species for medicinal purpose; and exploring the phytochemical properties of different parts of these species.

2. Materials and methods

2.1. Plant material

B. albostellata, *B. greenii* and *B. prionitis* were collected from the University of KwaZulu-Natal Botanical Garden, Indigenous Nursery at the Natal Botanical Garden, and Val Lea Vista Nursery respectively, all in Pietermaritzburg, South Africa. Voucher specimens (S. Amoo 03 NU, 02 NU and 04 NU respectively) were deposited in the University of KwaZulu-Natal Herbarium, Pietermaritzburg. After separating into leaves, stems and roots, plant materials were oven-dried at 50 °C, ground and stored in airtight containers at room temperature in the dark.

2.2. Preparation of extracts

For the antifungal assay, sequential extraction of the dried, ground plant materials was done with 20 ml/g of petroleum ether (PE), dichloromethane (DCM) and 80% ethanol (EtOH) in a sonication bath containing ice water for 1 h each. Methanolic extracts (MeOH) (used in acetylcholinesterase and antioxidant assays) were obtained by extracting plant materials with 20 ml/g of 50% methanol using a sonication bath containing ice water for 20 min. In each case, the extracts were filtered through Whatman No. 1 filter paper, concentrated *in vacuo* at 40 °C using a rotary evaporator and air-dried at room temperature.

2.3. Antifungal activity

Antifungal activity against *C. albicans* (ATCC 10231) was performed using the micro-dilution assay (Eloff, 1998) as modified for fungi (Masoko et al., 2007) and described by Amoo et al. (2009b). Amphotericin B was used as a positive control while the solvent 80% ethanol was included as a negative control. After 48 h of incubation, minimum inhibitory concentration (MIC) values were recorded as the lowest concentrations that inhibited fungal growth. Minimum fungicidal concentration (MFC) values were recorded after adding Yeast Malt broth (50 µl) to the clear wells and incubating for another 24 h. The assay was repeated twice with two replicates each. The minimum inhibitory dilution (MID) (ml/g) as well as minimum fungicidal dilution (MFD) (ml/g), indicating the

volume to which the extract derived from 1 g can be diluted and still inhibit the growth of or kill the fungal cells (Eloff, 2004) respectively, were also determined.

2.4. Acetylcholinesterase (AChE) inhibition

A microtitre plate assay based on the colorimetric method described by Ellman et al. (1961) and outlined by Eldeen et al. (2005) was used to determine the AChE inhibition activity by the plant extracts. The assay was done in duplicate, and the rate of reaction was calculated for each of the plant extracts, the blank (water) and positive control (galanthamine). The percentage inhibition by the plant extracts was calculated using the formula:

$$\text{Inhibition}\ (\%) = \left[1 - \frac{\text{Sample reaction rate}}{\text{Blank reaction rate}}\right] \times 100$$

2.5. Antioxidant activities

2.5.1. DPPH (1,1-Diphenyl-2-picrylhydrazyl) radical-scavenging activity

The method described by Karioti et al. (2004) was followed with slight modifications as outlined by Fawole et al. (2010). The negative control had methanol in place of the extract while ascorbic acid and butylated hydroxytoluene (BHT) were used as positive controls. Background solutions with methanol in place of DPPH were included for each extract, in order to correct any absorbance due to extract colour. The tests were carried out in triplicate. The radical scavenging activity (RSA) was calculated using the equation:

$$RSA(\%) = \left[1 - \left(\frac{A_{\text{extract}} - A_{\text{background}}}{A_{\text{control}}}\right)\right] \times 100$$

where A_{extract}, $A_{\text{background}}$ and A_{control} are the absorbances of the extract, background solution and negative control, respectively. The EC_{50}, which is the concentration of the extract required to scavenge 50% of DPPH radical, was determined for each extract.

2.5.2. Ferric-reducing power assay

The reducing power of the extracts was determined according to the method described by Kuda et al. (2005) with slight modifications as outlined by Fawole et al. (2010). The negative control had methanol in place of the extract while ascorbic acid and BHT were used as positive controls. The experiment was done in triplicate.

2.5.3. β-Carotene-linoleic acid model system

The method described by Amarowicz et al. (2004) was followed with slight modifications as described by Moyo et al. (2010). The negative control had 50% methanol in place of the extract while BHT was used as a positive control. Each sample was prepared in triplicate.

Antioxidant activities were expressed as ANT (%), ORR and *AA* (%) at $t=60$ or 120 min (Parejo et al. 2002; Amarowicz et al. 2004) using the following equations:

$$\text{Rate of}\ \beta\text{−carotene bleaching} = \ln\left(\frac{A_{t=0}}{A_{t=t}}\right) \times \frac{1}{t}$$

$$\text{ANT}(\%) = \left(\frac{R_{\text{control}} - R_{\text{sample}}}{R_{\text{control}}}\right) \times 100$$

$$\text{ORR} = \frac{R_{\text{sample}}}{R_{\text{control}}}$$

$$\text{AA}(\%) = \left[1 - \left(\frac{A_E^{t=0} - A_E^{t=t}}{A_W^{t=0} - A_W^{t=t}}\right)\right] \times 100$$

where $At=0$ is the initial absorbance at $t=0$ min, $At=t$ is the absorbance at time $t=30$, 60 or 90 min, ANT (%) is the percentage inhibition of the rate of β-carotene bleaching relative to negative control, R_{sample} and R_{control} are the average bleaching rates (at 30, 60 and 90 min) of β-carotene in the emulsion with plant extract and 50% methanol respectively, ORR is the oxidation rate ratio, AA is the antioxidant activity, $A_E^{t=0}$ and $A_W^{t=0}$ are the absorbances of extract and negative control respectively at 0 min, $A_E^{t=t}$ and $A_W^{t=t}$ are the absorbances of extract and negative control respectively at $t=60$ or 120 min.

2.6. Phytochemical properties

Phenolic compounds were extracted using the method described by Makkar (2000) with slight modifications. Two grams of ground, dried plant samples were extracted with 10 ml of 50% aqueous methanol in a sonication bath containing ice-cold water for 20 min. The extracts were then filtered through Whatman No. 1 filter paper. The filtrates were kept on ice for subsequent analysis.

Total iridoid content was determined following the method described by Levieille and Wilson (2002), which was adapted from Haag-Berrurier et al. (1978). The method was based on the characteristics of glucoiridoids to form a fulvoiridoid complex when reacted with aldehydes (such as vanillin) in an acidic medium (Levieille and Wilson, 2002). HPLC-grade harpagoside (Extrasynthèse, France) was used as a standard for the calibration curve. The total iridoid content, expressed in μg harpagoside equivalents (HE) per gram dry weight (DW) was determined for each extract using their differential absorbance values correlated with the calibration curve.

Total phenolic content was determined using a colorimetric method described by Makkar (2000) with modifications as outlined by Fawole et al. (2009). Standards for the calibration curve were prepared using gallic acid. Each sample was tested in triplicate and the results were expressed in mg gallic acid equivalents (GAE) per gram DW.

Proanthocyanidins (condensed tannins) were quantified using the butanol-HCl method (Makkar, 2000) with modifications as outlined by Fawole et al. (2009). Each sample was tested in triplicate. Proanthocyanidins (% per dry matter) as leucocyanidin equivalents were calculated using the formula described by Porter et al. (1985):

$$\text{Proanthocyanidins (\% dry matter)} = \left(\frac{A_{550nm} \times 78.26 \times \text{Dilution factor}}{\text{\% dry matter}}\right) \times 100$$

where $A550$ nm is the absorbance of the sample at 550 nm.

The determination of gallotannin concentration was done using the rhodanine assay as outlined by Makkar (2000). Each sample was tested in triplicate. Gallic acid was used as a standard for the calibration curve. Gallotannin concentration was expressed in μg gallic acid equivalents (GAE) per gram DW.

Vanillin assay as described by Ndhlala et al. (2007) was used to determine flavonoid content of the samples. Each extract was tested in triplicate. A standard for the calibration curve was prepared using catechin and the flavonoids were expressed in mg catechin equivalents (CE) per gram DW.

2.7. Statistical analysis

The percentage values were log-transformed before they were subjected to statistical analysis. Data were subjected to one-way analysis of variance (ANOVA). Where there were significant differences ($P=0.05$), the mean values were further separated using Duncan's Multiple Range Test (DMRT). The analysis was done using SPSS (version 15.0) software. Regression analysis and the determination of EC_{50} values were done using GraphPad Prism (version 4.03) software.

3. Results and discussion

3.1. Antifungal activity

Table 1 presents the antifungal activity of extracts from different parts of *Barleria* species against *C. albicans*. In general, all the extracts demonstrated both fungistatic and fungicidal activities against *C. albicans*. The MIC of the extracts ranged from 0.78 to 9.375 mg/ml while the MFC ranged from 1.17 to 12.5 mg/ml. The highest inhibitory and lethal activities, as defined by the MIC and MFC values, were recorded in PE and DCM extracts of *B. albostellata* stems. The EtOH extracts had the highest MID and MFD in all cases. The PE, DCM and EtOH extracts of *B. greenii* leaves had higher inhibitory activity compared respectively to similar extracts of the roots (except PE extract) and stems. In the same vein, the leaf EtOH extract of *B. greenii* had the highest MID compared to all other extracts and demonstrated a fungicidal activity similar to the EtOH extracts of its roots and stems. The fungistatic activity of *B. greenii* leaf EtOH extract was higher than the EtOH extract of its stems or roots. These results suggest the potential of *B. greenii* leaves in plant-part substitution. The leaves can be sustainably harvested for medicinal purposes without the destructive harvesting of the roots which could threaten the survival of this critically endangered plant species (Matu and Van Staden, 2003; Zschocke et al., 2000). The higher inhibitory and fungicidal activities demonstrated by the stem extracts of *B. albostellata* indicate their potency when compared to their leaf extracts. However, the leaf extracts may be more

Table 1
Antifungal activity of crude extracts from different parts of three *Barleria* species against *Candida albicans*.

Plant species	Plant part	Extract	Minimum inhibitory concentration (MIC) (mg/ml)	Minimum fungicidal concentration (MFC) (mg/ml)	Minimum inhibitory dilution (MID) (ml/g)	Minimum fungicidal dilution (MFD) (ml/g)
B. prionitis	Stems	PE	3.125	4.688	1.158	0.772
		DCM	3.125	4.688	5.050	3.366
		EtOH	6.250	6.250	8.947	8.947
	Roots	PE	4.688	4.688	0.478	0.478
		DCM	2.343	4.688	1.417	0.708
		EtOH	6.250	6.250	6.848	6.848
B. greenii	Leaves	PE	7.813	12.500	2.768	1.730
		DCM	**0.975***	9.375	7.928	0.825
		EtOH	3.515	9.375	58.506	21.936
	Stems	PE	9.375	12.500	1.873	1.405
		DCM	6.250	12.500	1.213	0.606
		EtOH	6.250	9.375	13.606	9.071
	Roots	PE	3.125	4.688	0.998	0.666
		DCM	3.125	3.125	1.574	1.574
		EtOH	6.250	9.375	21.269	14.179
B. albostellata	Leaves	PE	4.688	6.250	11.817	8.864
		DCM	1.170	4.688	15.949	3.980
		EtOH	4.688	6.250	49.714	37.29
	Stems	PE	**0.780**	1.560	5.103	2.551
		DCM	**0.780**	1.170	4.487	2.991
		EtOH	3.125	3.125	16.979	16.979
Amphotericin B (μg/ml)			0.048	0.193		

*Values boldly-written are considered very active (<1 mg/ml).

efficient from a conservation point of view since they contained more extractable antifungal agents per gram compared to the stem as indicated by their higher MID and MFD.

3.2. Acetylcholinesterase inhibition

The AChE inhibition activity observed in the MeOH extracts of *Barleria* species are presented in Fig. 1. All the extracts evaluated showed a dose-dependent inhibition. In general, at the highest extract concentration (0.625 mg/ml), the leaf and stem extracts of both *B. greenii* and *B. prionitis* exhibited higher inhibitory activities than their root extracts. The leaf extracts of *B. greenii* and *B. albostellata* showed the highest (68%) and lowest (22%) AChE inhibition respectively, at the highest extract concentration evaluated. In the same vein, at the lowest extract concentration (0.156 mg/ml), the leaf extracts of *B. greenii* and *B. albostellata* showed the highest (38%) and lowest inhibition (3%) respectively, compared to other extracts. The inhibitory activity shown by the leaf extract of *B. greenii* at the lowest extract concentration was in fact significantly higher ($P=0.05$) than the activities recorded in the stem and root extracts of the same species at the lowest concentration. The findings indicate that *B. greenii* leaves can potentially be substituted for its stems or roots in the inhibition of the AChE enzyme. In *B. albostellata*, the inhibitory activity demonstrated by the stem at the lowest extract concentration was significantly greater than the activity recorded in its leaf extract. This observation suggests that the idea of plant-part substitution may be species and/or biological activity dependent.

3.3. Antioxidant activities

3.3.1. DPPH radical scavenging activity

Fig. 2 shows the dose–response radical scavenging activities observed in the MeOH extracts of different parts of *Barleria* species. In all the extracts, there was an increase in the DPPH radical scavenging activity with increasing extract concentration. From their dose–response activities, their EC_{50} values were obtained and presented in Table 2. The EC_{50} values for the different extracts of *Barleria* species ranged from 6.65 to 12.56 μg/ml. The EC_{50} values of *B. greenii* leaf and all *B. prionitis* extracts were in fact not significantly different from the EC_{50} of ascorbic acid, a standard antioxidant agent used as a positive control in this study. In the case of *B. greenii*, the leaf extract had a lower EC_{50} value compared to the stem and significantly, the root. This observation correlates well with AChE inhibitory activities observed in the leaves, stems and roots of *B. greenii* (Fig. 1). There was no significant difference in the EC_{50} values of the different parts of *B. prionitis*, an observation that also correlates well with their AChE inhibitory activities (Fig. 1). According to Houghton et al. (2007), free radical reactions are involved in the pathology of many diseases like Alzheimer's disease, cancer and inflammation.

3.3.2. Ferric-reducing power assay

The ferric ion reducing power assay is an assay based on an electron transfer reaction (Huang et al., 2005). In the assay, the presence of reductants (antioxidants) in the tested extracts results in the reduction of the ferric ion/ferricyanide complex to the ferrous form, with a characteristic formation of Perl's Prussian blue, which is measured spectrophotometrically (Chung et al., 2002). The degree of colour change is directly proportional to the antioxidant concentrations in the extracts (Huang et al., 2005).

Fig. 3 presents the reducing power of extracts from different parts of three *Barleria* species. All the extracts evaluated showed an increase in reducing power activity with an increase in extract concentration. However, the reducing activities of the extracts were significantly lower than the ascorbic acid and BHT standard controls. The ferric reducing power activity of the leaf and stem extracts of both *B. prionitis* and *B. greenii* were higher than that of their root extracts, indicating a potential

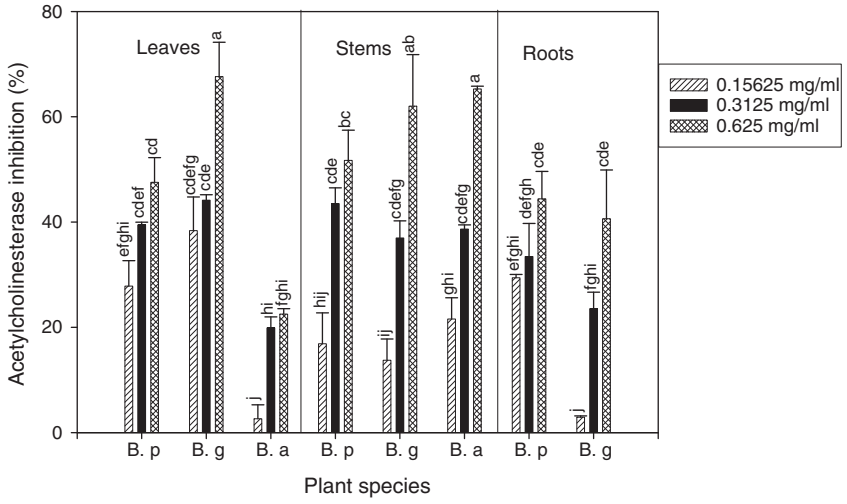

Fig. 1. Dose-dependent acetylcholinesterase inhibition by different parts of three *Barleria* species. B.p=*Barleria prionitis*, B.g=*Barleria greenii*, B.a=*Barleria albostellata*. Bars bearing different letters are significantly different ($P=0.05$) according to Duncan's Multiple Range Test (DMRT). The AChE inhibition activities by galanthamine at 0.5, 1.0 and 2 μM were 49.24, 59.81 and 77.03%, respectively.

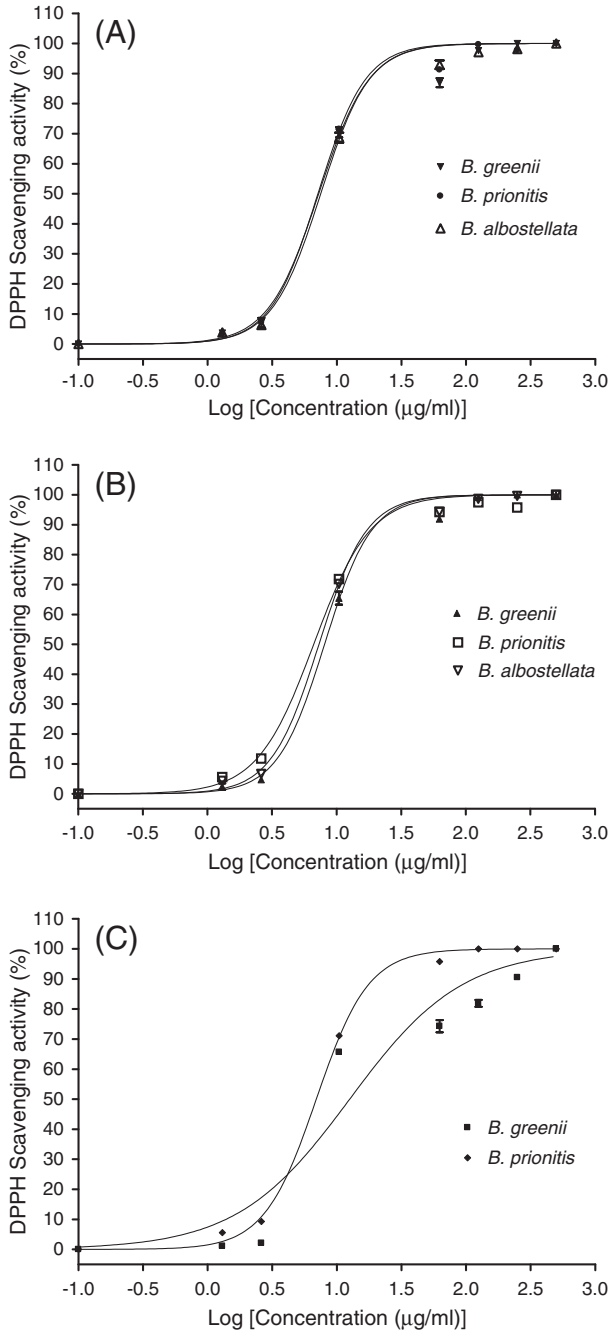

Fig. 2. Dose-dependent nonlinear curve of DPPH radical-scavenging activity of different parts of three *Barleria* species. (A) Leaves. (B) Stems. (C) Roots.

plant-part substitution of the leaves or stems for the roots. The results suggest the presence of antioxidant compounds with electron-donating ability in the different plant parts evaluated, which the assay is known to measure semi-quantitatively (Amarowicz et al., 2004; Rumbaoa et al., 2009). The presence of these compounds perhaps in an impure form or in small amounts in the extracts may be responsible for the generally low activity demonstrated by the extracts.

3.3.3. β-Carotene-linoleic acid model system

This assay involves the heat-induced bleaching of carotenoids and is based on hydrogen atom transfer reactions (Huang et al., 2005). According to Amarowicz et al. (2004), the abstraction of a hydrogen atom from linoleic acid during oxidation results in the formation of a pentadienyl free radical. This free radical subsequently attacks the highly unsaturated β-carotene molecules, leading to the loss of conjugation of the β-carotene molecules and the characteristic orange colour of the carotenoids (Amarowicz et al., 2004). The presence of antioxidants capable of donating hydrogen atoms can however, prevent or reduce carotenoid bleaching by quenching free radicals formed within the system (Amarowicz et al., 2004; Burda and Oleszek, 2001).

Table 2
DPPH free radical-scavenging activity of methanolic extracts obtained from different parts of three *Barleria* species.

Plant species	Plant part	DPPH radical scavenging	
		EC_{50} (µg/ml)	R^2
B. prionitis	Leaves	7.14 ± 0.056^{bcd}	0.9949
	Stems	6.65 ± 0.037^{bc}	0.9965
	Roots	6.94 ± 0.033^{bc}	0.9984
B. greenii	Leaves	7.24 ± 0.326^{bcd}	0.9881
	Stems	8.09 ± 0.266^{d}	0.9954
	Roots	12.56 ± 0.401^{e}	0.9307
B. albostellata	Leaves	7.52 ± 0.169^{cd}	0.9952
	Stems	7.31 ± 0.175^{cd}	0.9973
Ascorbic acid		6.17 ± 0.434^{b}	0.9550
Butylated hydroxy toluene		3.75 ± 0.764^{a}	0.9027

Mean values followed by different letters are significantly different ($P=0.05$) according to Duncan's Multiple Range Test. R^2 is the coefficient of determination which quantifies the goodness of fit of nonlinear regression curve.

The antioxidant activities of different parts of three *Barleria* species in the β-carotene-linoleic acid model system are shown in Fig. 4. The antioxidant activity of the different parts based on the average β-carotene bleaching rate ranged from 52 to 77%. The antioxidant activity of *B. prionitis* roots was significantly higher than that of its leaves and stems (Fig. 4A). There was no significant difference observed in the antioxidant activity (based on average β-carotene bleaching rate) of the different parts of either *B. greenii* or *B. albostellata* evaluated. The presence of significantly comparable antioxidant activity between the different plant parts suggests the potential to substitute the leaves of *B. greenii* and *B. albostellata* for their stems or roots. Another indicator of the antioxidant activity of the different plant parts is the oxidation rate ratio (Fig. 4B). In this case, the lower the oxidation rate ratio, the higher the antioxidant activity. The oxidation rate ratio for the different plant parts ranged from 0.23 to 0.48. The lowest oxidation rate ratio which was recorded in *B. prionitis* roots was significantly equal to that of BHT. According to Amarowicz et al. (2004), the antioxidant activity at $t=60$ or 120 min possibly demonstrates the antioxidant activity of an extract more accurately than the oxidation rate ratio or antioxidant activity based on the average β-carotene bleaching rate. With the exception of *B. prionitis* leaf and *B. greenii* stem extracts, the antioxidant activities at $t=60$ or 120 min of the different plant parts was significantly the same as that of BHT (Figs. 4C and D). There was no significant difference between the antioxidant activities of *B. greenii* leaves and roots as well as the BHT standard control at either $t=60$ or

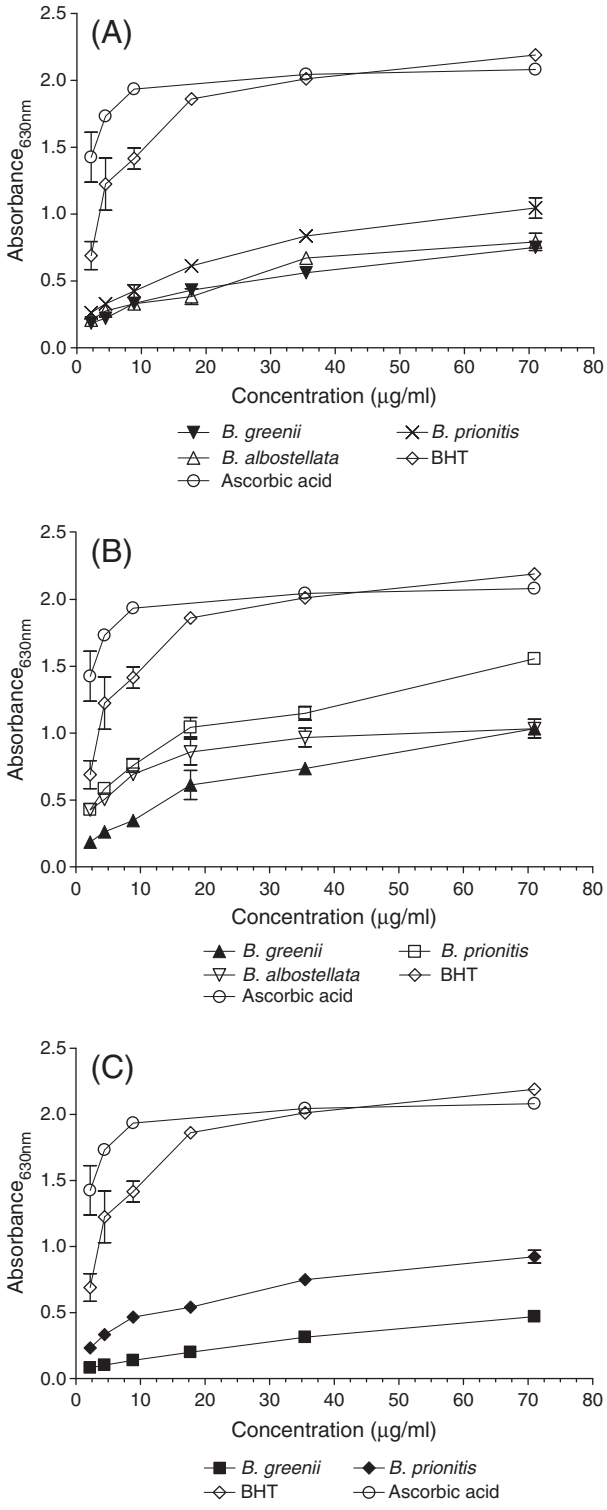

Fig. 3. Ferric ion reducing power activity of different parts of *Barleria* species. (A) Leaves. (B) Stems. (C) Roots. An increase in absorbance value indicates increase in reducing power.

120 min. BHT is one of the synthetic antioxidants widely used as a food preservative (Hassas-Roudsari et al., 2009). The toxicity of these synthetic antioxidants has, however, raised concern about their health safety, resulting in the increased search for naturally occurring antioxidants useful in food and cosmetic industries and as nutraceuticals (Abdel-Hameed, 2009; Orhan et al., 2009). The findings from the current study suggest that *B. albostellata* leaves and stems, *B. greenii* leaves and roots as well as *B. prionitis* stems and roots possibly contain antioxidant agents with activity equivalent to that of BHT, which can potentially be exploited as alternatives in the food and cosmetic industries. The purification of the antioxidant agents present in these plant materials could perhaps improve their antioxidant capacity. The use of *B. albostellata* and *B. greenii* leaves is more sustainable and can potentially substitute for their stems and roots respectively, owing to their equivalent antioxidant activity in this assay.

3.4. Phytochemical properties

The total iridoid, phenolic, flavonoid, gallotannin and condensed tannin contents of different parts of the three *Barleria* species are presented in Table 3. The highest total iridoid content was recorded in *B. albostellata* leaves with 801.4 µg HE/g DW while the lowest was found in *B. prionitis* roots with 30 µg HE/g DW. In each of the *Barleria* species, the total iridoid content of the leaves was significantly higher than that of the other parts. There was no significant difference between the iridoid contents of the stems of the three species. The total iridoid content of *B. greenii* roots was significantly higher than that of *B. prionitis* roots. Ata et al. (2009) reported the isolation of seven iridoids (barlerinoside, shanzhiside methyl ester, 6-*O*-*trans*-*p*-coumaroyl-8-*O*-acetylshanzhiside methyl ester, barlerin, acetylbarlerin, 7-methoxydiderroside and lupulinoside) with different levels of AChE inhibitory and free radical scavenging activities from aerial parts of *B. prionitis*. The AChE inhibitory and radical scavenging activities recorded in the *Barleria* species evaluated in this study could be due to the presence of these or other iridoid compounds. The presence of iridoid compounds even at a low concentration in all parts of the three *Barleria* species could perhaps play a role in the biological activities observed in all the extracts evaluated. Since the leaves of each of these *Barleria* species contained more iridoid compounds, two- to twelve-fold higher than their stems or roots, they may possibly be a potential sustainable source for iridoid compounds. They may also be a potential source of important pharmacological alkaloids since iridoids are known to be precursors in the biosynthesis of alkaloids (Didna et al., 2007; Tundis et al., 2008).

In all three species, the highest total phenolic content was observed in the leaves, compared to other plant parts (Table 3). In *B. prionitis* and *B. greenii*, the stem phenolic contents were significantly higher than that of the root. The highest (12.79 mg GAE/g DW) and lowest (1.95 mg GAE/g DW) total phenolic contents were recorded in *B. prionitis* leaves and *B. greenii* roots, respectively. Phenolic compounds include flavonoids, condensed tannins and hydrolysable tannins, many of which are reported to have antimicrobial, anti-inflammatory and antioxidant activities (Marcucci et al., 2001; Polya, 2003). According to Samak et al. (2009), the redox property of phenolic compounds is an important underlying factor for their antioxidant activity, giving them the ability to act as hydrogen

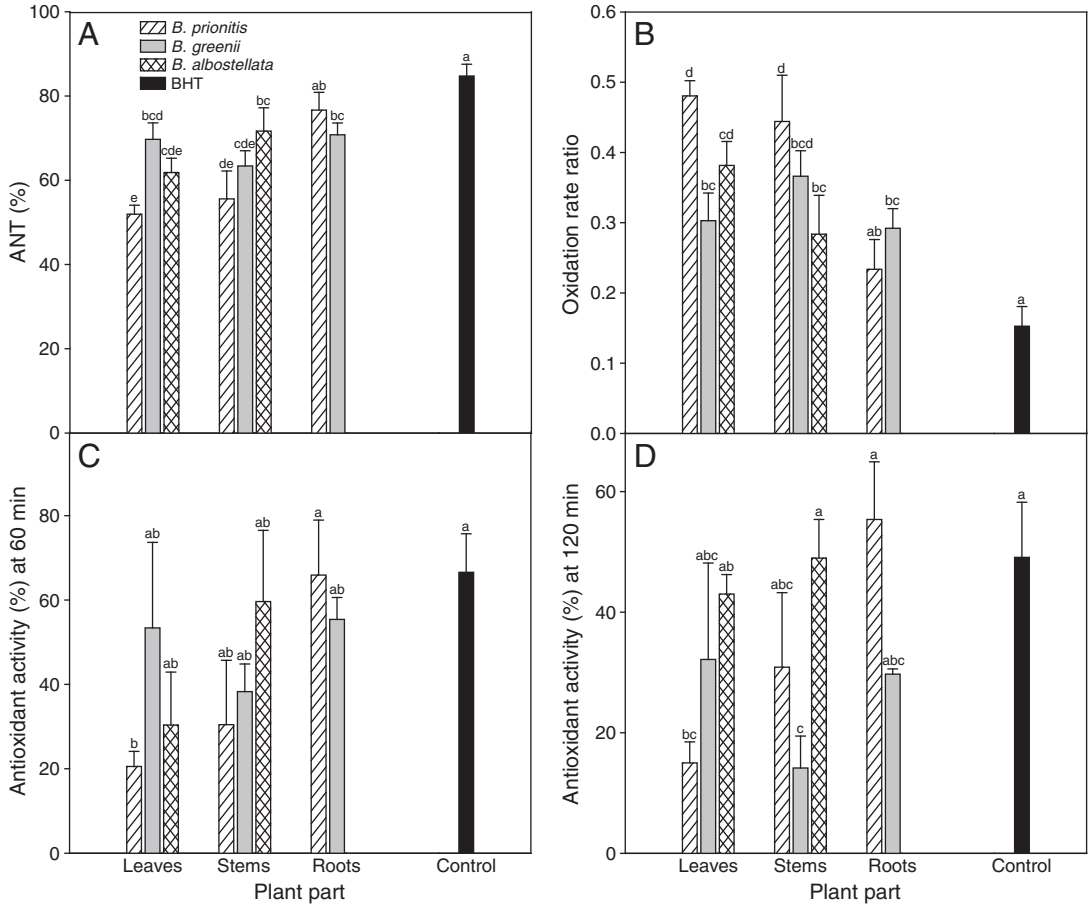

Fig. 4. Antioxidant activities of different parts of three *Barleria* species in β-carotene-linoleic acid model system. Bars bearing different letters in each graph are significantly different ($P=0.05$) according to DMRT. (A) Antioxidant activity (ANT) based on the average β-carotene bleaching rate. (B) Oxidation rate ratio (ORR). (C) Antioxidant activity (AA) at $t=60$ min. (D) Antioxidant activity (AA) at $t=120$ min.

donors, reducing agents and singlet oxygen quenchers. In the light of the total phenolic content recorded in the different parts of the plant species in the current study, the amounts of some particular groups of phenolic compounds were further evaluated in the different parts of the studied species. This could possibly help in underpinning the specific phenolic groups likely to be responsible for the observed pharmacological activities.

The highest (3.92 mg CE/g DW) and lowest (0.35 mg CE/g DW) flavonoid contents were recorded in *B. greenii* leaves and *B. albostellata* stems, respectively (Table 3). In general, higher flavonoid content was observed in the leaves compared to the other plant parts in each of the species. Ren et al. (2010) described a naturally occurring flavonoid, 6-hydroxyflavone, found in the leaves of *B. prionitis* as a promising drug candidate for the treatment of anxiety-like disorders. In an extensive review on the effects of naturally occurring flavonoids on mammalian cells, Middleton et al. (2000) observed that flavonoids demonstrate a noteworthy array of biochemical

Table 3
Total iridoids, phenolics, flavonoids, gallotannins and proanthocyanidins of different parts of three *Barleria* species.

Plant species	Plant part	Total iridoid (μg HE/g DW)	Total phenolics (mg GAE/g DW)	Flavonoids (mg CE/g DW)	Gallotannins (μg GAE/g DW)	Proanthocyanidins (% per g DW)
B. prionitis	Leaves	166.90 ± 4.231^{d}	12.79 ± 0.127^{a}	1.69 ± 0.273^{b}	80.60 ± 2.708^{b}	0.000 ± 0.0000^{e}
	Stems	74.72 ± 4.775^{e}	7.66 ± 0.388^{c}	0.52 ± 0.000^{c}	38.76 ± 8.332^{d}	0.007 ± 0.0036^{e}
	Roots	30.05 ± 8.023^{f}	6.86 ± 0.128^{d}	1.02 ± 0.018^{bc}	57.71 ± 2.420^{c}	0.071 ± 0.0248^{d}
B. greenii	Leaves	521.90 ± 5.229^{b}	11.69 ± 0.262^{b}	3.92 ± 0.615^{a}	87.43 ± 1.589^{b}	1.229 ± 0.0019^{a}
	Stems	82.61 ± 2.457^{e}	3.18 ± 0.073^{f}	0.42 ± 0.020^{c}	41.84 ± 4.507^{d}	0.170 ± 0.0049^{c}
	Roots	201.89 ± 24.174^{c}	1.95 ± 0.039^{g}	1.66 ± 0.044^{b}	145.33 ± 3.291^{a}	0.874 ± 0.0202^{b}
B. albostellata	Leaves	801.43 ± 8.516^{a}	5.27 ± 0.324^{e}	0.78 ± 0.042^{c}	142.83 ± 8.369^{a}	0.023 ± 0.0028^{e}
	Stems	66.39 ± 8.703^{e}	3.76 ± 0.084^{f}	0.35 ± 0.030^{c}	74.92 ± 5.914^{b}	0.000 ± 0.0000^{e}

Mean values within a column followed by different letters are significantly different ($P=0.05$) according to Duncan's Multiple Range Test.

and pharmacological actions, most notable being their antioxidant, anti-inflammatory and antiproliferative effects. Many other researchers have reported the antioxidant, anti-inflammatory and antimicrobial activities of flavonoids or flavonoid-rich extracts (Burda and Oleszek, 2001; Havsteen, 2002; Pattanayak and Sunita, 2008; Tunalier et al., 2007). In the current study, the presence of flavonoids in the different parts of the studied species likely contributed to the observed pharmacological activities in these species. In addition to their quantity, however, the quality or nature of the flavonoid present in the different plant parts could make a difference in their therapeutic potential.

The roots of *B. greenii* had the highest gallotannin content with 145.33 μg GAE/g DW compared to all other *Barleria* species parts (Table 3). In all three species, the gallotannin content of the leaves was significantly higher than that of the stems. The gallotannin contents of the leaves and stems of *B. albostellata* were significantly higher than that of the leaves and stems, respectively of other *Barleria* species. Tian et al. (2009) reported antioxidant and antibacterial activities of gallotannin-rich extracts from *Galla chinensis*. Engels et al. (2009) also reported antimicrobial activities of gallotannin-rich extracts and gallotannins isolated from *Mangifera indica* kernels. The antibacterial activity of hydrolysable tannins (the group to which gallotannins belong) isolated from medicinal plants used in treating gastric disorders against *Helicobacter pylori* has been demonstrated (Funatogawa et al., 2004). Some gallotannins have also been reported to act as inhibitors of particular enzymes such as the COX enzymes involved in the inflammatory pathway (Polya, 2003). The presence of gallotannins in different parts of the species evaluated in this study could, at least, partly contribute to their antioxidant as well as anti-inflammatory and antibacterial activities (Amoo et al., 2009a).

Proanthocyanidins are made up of oligomeric and polymeric flavan-3-ols, and they have been reported to demonstrate strong antioxidant capacity (Shyu et al., 2009). The proanthocyanidin content of different parts of the three *Barleria* species evaluated in this study is presented in Table 3. No proanthocyanidin was detected in *B. prionitis* leaves and *B. albostellata* stems. Where proanthocyanidin was recorded, the content was generally low (ranging from 0.007 to 1.2% per g DW) in all parts of the three *Barleria* species evaluated. All the different parts of *B. greenii* had a significantly higher proanthocyanidin content compared to any part of *B. prionitis* and *B. albostellata*. These findings suggest that the pharmacological activities recorded in *B. prionitis* leaves and *B. albostellata* stems are likely not due to proanthocyanidins. In other parts of *Barleria* species, however, their proanthocyanidin content could possibly contribute, at least to a small extent, to their antioxidant activities.

4. Conclusions

Overall, the results from this study demonstrate the therapeutic potential of the *Barleria* species evaluated. As far as can be ascertained, this is the first report on the antifungal, AChE inhibition and antioxidant activities of *B. greenii* and *B. albostellata*. The observed activities might be due to the presence of flavonoids, iridoids and tannins in the different parts of these species. In some of the pharmacological assays, the leaves demonstrated activities higher than or equal to the other plant parts, suggesting their potential in plant-part substitution. The harvesting of leaves or stems as a conservation strategy is certainly more sustainable than the destructive use of the roots of these plant species. However, the concept of substituting plant parts for sustainable exploitation appeared to be dependent on the species and/or biological activity evaluated. The isolation of specific bioactive compounds through bioassay-guided fractionation and their characterization as well as studies evaluating their safety may be necessary in the exploration of these species for potential new therapeutic drugs or drug leads.

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