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Evaluation of Antibacterial and Antioxidant Activities in Ethanol Extract of Wild Bush Tea (*Athrixia phylicoides* (DC.))

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ABSTRACT

South Africa is well known for its indigenous herbal tea production such as honeybush and rooibos tea. Bush tea, like any other herbal tea, has been used for many years as a herbal or medicinal tea by traditional African people. To validate its medicinal potential, an ethanol extract from wild bush tea leaves was used *in vitro* for antibacterial and antioxidant activity determination. The antibacterial assay showed inhibitory activity against five bacterial strains evaluated with minimum inhibitory concentration (MIC) values between 3.13 and 6.12 mg·ml^{-1}. The extract demonstrated the potent antioxidant activity by effectively scavenging DPPH, a free radical, with an EC_{50} value of 28.12 µg·ml^{-1}.

Keywords: Minimum inhibitory concentration (MIC); 2,2-diphenyl-1-picrylhydrazyl (DPPH); Gram-negative; Gram-positive; Phenolic compounds

INTRODUCTION

Bush tea leaves contains 5-hydroxy-6,7,8,3′,4′,5′-hexamethoxy flavon-3-ol considered to be a new flavonoid (Mashimbye *et al*. 2006). Bush tea leaves have no caffeine contents or pyrrolizidine alkaloids (McGaw *et al*. 2007), thus supporting the development of bush tea as a healthy beverage alternative to caffeine-containing tea (Mudau *et al*. 2007a).

Agronomic practices such as mineral nutrition have been reported to increased growth and total polyphenols of bush tea under a controlled environment (Mudau *et al*. 2006, 2007b), although excessive application showed to have an adverse effect on tea quality (Mogotlane *et al*. 2007; Mudau *et al*. 2007b, 2007c). Data that describe the antibacterial and antioxidants activities in wild bush tea leaves are lacking. However, it is known that bush tea harvested from wild having the highest concentrations of condensed tannins were when collected during autumn (4.82%) compared with winter (2.44%), spring (2.66%) and summer (3.04%) (Mudau *et al*. 2007d). The hydrolysable tannins were lowest during summer (0.01%) compared with autumn and winter (0.14%) and spring (0.13%) (Mudau *et al*. 2007d). The authors also reported that polyphenols of wild bush tea leaves were lowest in March and April (autumn) and September (mid-spring) and highest in June and July (mid-winter). Therefore, the objective of this study was to determine the antibacterial and antioxidant activities of wild bush tea leaves harvested from the wild.

MATERIALS AND METHODS

Plant material and extraction

The aerial parts (branches and leaves) of bush tea used traditionally as herbal tea were collected from Venda in the Limpopo Province in South Africa during July (mid-winter). A voucher specimen was prepared and identified for botanical authenticity at the H.G.W.J. Herbarium, University of Pretoria. The dried plant materials (1500 g) were extracted with methanol at room temperature for 3 days. The filtrates were evaporated to dryness under reduced pressure and the residues were dissolved in 1% DMSO to achieve a concentration of 100 mg·ml^{-1}.

Micro-organisms used for antibacterial assay

The micro-organisms tested were *Staphylococcus aureus* (ATCC 12600), *Bacillus cereus* (ATCC 11778), *Enterecoccus faecalis* (ATCC 292192), *Escherichia coli* (ATCC 11775), and *Mycobacterium smegmatis* (ATCC X) and they were obtained from the Department of Microbiology, University of Pretoria. The micro-organisms were evaluated for antibacterial activity.

Antibacterial assay

The minimum inhibition concentrations (MIC) of the crude extract were determined using a micro-dilution method on 96 well microplates, as previously described by Eloff (1998). The trials were performed in triplicate and the MIC was defined as the lowest concentration of the extract that inhibited the growth of the bacteria.

Antioxidant assay (DPPH radical scavenging activity)

The free radical scavenging activity of the crude extract was determined using the DPPH method as previously described by Rangkadilok *et al.* (2007) with slight modifications. A stock solution (29 mg·ml^{-1}) of crude extract was prepared in ethanol solvent. Briefly, 100 µl of distilled water was added to the wells of a microtitre plate as medium. Serial dilutions of plant extract (10 µl) was mixed with 90 µl of 90 µM DPPH methanolic solution to give a final concentration of 7.8, 15.6, 31.3, 62.5, 125, 250, 500 and 1000 µg of extract per ml of DPPH solution. Ascorbic acid (vitamin C) was prepared in the same way as extracts and used as a standard control. The plate was covered with aluminium foil and left to stand for an hour at room temperature and the absorbances were measured with BIO–TEK Power Wave Multiwell plate reader (A.D.P., Weltevreden Park, South Africa) at 550 nm. The experiment was performed in triplicate and the average absorption was recorded for each concentration. The free radical-scavenging acti-

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Short Communication

vity was calculated as a percentage inhibition of the DPPH radical by a sample or ascorbic acid according to the formula:

% inhibition = $(A_C - A_S)/ A_C \times 100$, where A_C is the absorbance of the control solution (containing only DPPH), A_S is the absorbance of the sample in the DPPH solution. The percentage of DPPH radical-scavenging was plotted against the plant extract concentration ($\mu g \cdot ml^{-1}$) to determine the concentration of extract required to scavenge DPPH by 50% (called EC_{50}).

For the qualitative assay, ethanol extract (10 µl) was applied onto a TLC plate (Merck Silica gel 60 F_{254}). The plate was then developed using the solvent system hexane/ethyl acetate (7:3). After drying in a stream of cold air, the plate was sprayed with 0.2% methanolic DPPH and was incubated for 30 minutes at room temperature to detect the number of antioxidant compounds present in the extract. The presence of antioxidants compounds were revealed within 5 minutes as white spots against a purple background on the plate.

Statistical analysis

Data were analysed using SPSS Demo (SPSS Inc., Chicago, IL, USA) and EC_{50} was estimated by sigmoid non-linear regression.

RESULTS AND DISCUSSION

Anti-bacterial trials

The *in vitro* antibacterial screening demonstrated that the crude extract possesses inhibitory activities against all tested micro-organisms (**Table 1**). The Gram-positive bacteria appeared to be more susceptible to the inhibitory effect of the extract than Gram-negative ones. Similar observations were observed by Lall and Meyer (2000), Afolayan and Meyer (1995) and Tshikalange *et al*. (2005), while evaluating the antibacterial activity of *Hyptis veriticillata*, *Helichrysum aureonitens* and selected medicinal plants used in treatment of sexually transmitted diseases, respectively. All these studies demonstrated weak activity of plant extracts against Gram-negative bacterial strains. The weak activity obtained against Gram-negative bacteria was not surprising, as in general, these bacteria are more resistant than Gram-positive as reported by Paz *et al*. (1995) and Rabe and van Staden (1997). The reason for the different sensitivity between Gram-positive and -negative bacteria could be ascribed due to the morphological difference between them (Nikaido and Vaara 1985; Palombo and Semple 2001). The difference in cell wall structure between them was attributed by the fact that Gram-negative is more resistant than Gram-positive and Gram-negative bacteria have a cell wall impermeable to many environmental substances, including antibiotics (Palombo and Semple 2001). The MIC values of the extracts against different micro-organisms are also indicated in **Table 1** ranging from 3.13 to 6.25 $\mu g \cdot ml^{-1}$. The lowest MIC value of 3.13 $\mu g \cdot ml^{-1}$ was obtained with *S. aureus* and *E. faecalis*.

Table 1 Minimum inhibitory concentrations (MIC) of bush tea ethanol extract.

Bacterial species	Gram +/-	MIC ($\mu g \cdot ml^{-1}$)[z]
Staphylococcus aureus	+	3.13
Bacillus cereus	+	6.25
Enterecoccus faecalis	+	3.13
Escherichia coli	-	6.25
Mycobacterium smegmatis	Mycobacterium	6.25

[z]Minimum inhibitory concentration.

Table 2 Antioxidant activity (% inhibition and EC_{50} ± standard deviation) of bush tea ethanol extract.

Concentration ($\mu g \cdot ml^{-1}$)	% inhibition	EC_{50} ($\mu g \cdot ml^{-1}$)[z]
7.8	0.000 ± 0.036	
15.6	18.553 ± 0.020	
31.3	57.233 ± 0.010	28.12 ± 0.400
62.5	71.855 ± 0.007	
125	77.987 ± 0.002	
250	79.874 ± 0.006	
500	81.132 ± 0.001	
1000	81.604 ± 0.000	

[z]EC_{50}: The concentration of extract required to scavenge DPPH by 50%.

Antioxidant activities

Results in **Table 2** and **Fig. 1** demonstrated that at the highest concentration of the extract (1000 $\mu g \cdot ml^{-1}$), the inhibition of DPPH by the extract was 81.6%; when the lowest concentration of the extract (7.8 $\mu g \cdot ml^{-1}$) was used the percentage inhibition was 0.000% (**Table 2**).

Vitamin C showed a higher percentage of inhibition (82.497%) at a high concentration and 81.081% at a low concentration (**Fig. 1**). Tested extract and Vitamin C showed the same percentage of DPPH inhibition at higher concentration, but the extract did not inhibit DPPH at low concentration.

The extract also showed a concentration-dependent radical scavenging activity. The results of investigation demonstrated that at higher concentration of an extract, there is a low amount of DPPH remaining and high free radical scavenging activity. The same results were reported by Paixão *et al*. (2007), while evaluating the relationship between antioxidant capacity and total phenolic content of red, rose and white wines.

The EC_{50} value of extract was 28.12 $\mu g \cdot ml^{-1}$ and it is comparable with the studies done by Santoh *et al*. (2005), which demonstrated the percent DPPH radical scavenging activities of various teas (roasted, green, oolong and black) at EC_{50} values between 25 and 50 $\mu g \cdot ml^{-1}$. The concentration of an antioxidant needed to decrease the initial DDPH concentration by 50% (EC_{50}) is a parameter widely used to measure antioxidant activities (Sanchez *et al*. 1998) thus, the lower the EC_{50} the higher the antioxidant activity (Atoui *et al*. 2005).

The present findings in the TLC-based qualitative antioxidant assay, the extract showed the antioxidant compounds appeared as white spots on the purple background on the plate as a major antioxidant indicator. It has been reported that some medicinal plants are reputed to reduce or cure diseases and these have been attributed to the antioxidant properties of phenolic compounds present in the extract (Inova *et al*. 2005). In conclusion, the present results demonstrated that the *in vitro* antibacterial assay showed inhibitory activity against six bacterial strains evaluated with MIC values between 3.13 and 6.12 $mg \cdot ml^{-1}$. The extract also showed the potent antioxidant activity, effectively scavenging the DPPH free radical with an EC_{50} value of 28.12 $\mu g \cdot ml^{-1}$.

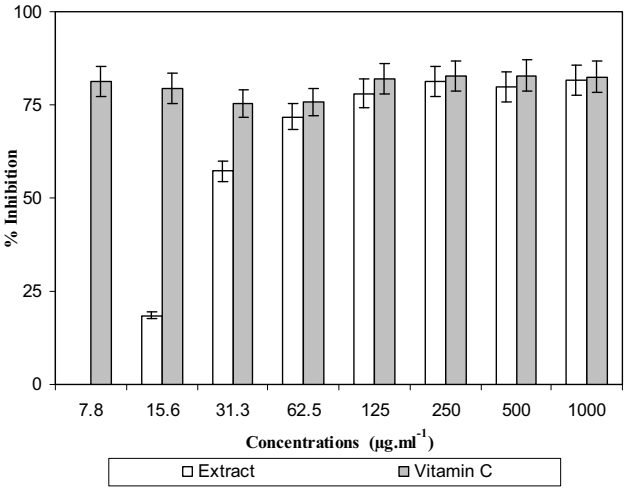

Fig. 1 Antioxidant activity of bush tea ethanol extract versus Vitamin C.

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