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Medicinal Potential of the Root of *Arctotis arctotoides*

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Abstract

The antioxidant and antimicrobial activity of the acetone, methanol, and water extracts from the root of *Arctotis arctotoides* (L.f.) O. Hoffm (Asteraceae) were assessed in an effort to validate the medicinal potential of the subterranean part of the herb. The antioxidant activities of acetone and methanol extracts as determined by the ABTS, DPPH, and FRAP methods were higher than that of water extracts. The extracts showed significant activity against both Gram-positive and Gram-negative bacteria. The strongest activity was found in the acetone extract on *Bacillus cereus*, *Staphylococcus aureus*, *Micrococcus kristinae*, and *Streptococcus pyrogens* with an MIC of 0.1 mg/mL. Although not completely fungicidal, these extracts showed significant growth inhibition against all the fungi tested. Antioxidant and antimicrobial activities of the extracts were strongly correlated with total phenols and to a lesser extent with their flavonoids and proanthocyanidins contents. This study has validated the medicinal potential of the underground part of *A. arctotoides*.

Keywords: Antibacterial activity, antifungal activity, *Arctotis arctotoides*, flavonoids, free radical scavenging activity, polyphenols, proanthocyanidins, reducing capacity.

Introduction

Arctotis arctotoides (L.f.) O. Hoffm (Asteraceae) is a decumbent herb commonly found as roadside weeds in most coastal districts of South Africa. The rural dwellers of the Eastern Cape Province use the shoot of this herb for the treatment of epilepsy, indigestion, and catarrh of the stomach. The Xhosa-speaking people in the province apply the juice from the leaf as

a topical paste to treat wounds (Afolayan, 2003). The ability to inhibit the growth of a wide range of micro-organisms has also been well documented (Afolayan, 2003; Sultana & Afolayan, 2003). Bioassay-guided fractionation of its hexane extract has led to the isolation of sesquiterpene lactones and farnesol derivatives (Sultana & Afolayan, 2003). Despite the well-documented reports on the chemical components and the pharmacological property of the shoots of this herb, no such information is available on its roots. Yet, plant roots are known for a number of medicinal components that may be totally different from or in addition to those found in the shoots. For example, natural antioxidants have been reported to occur in all parts of plants such as flowers, leaves, roots, and bark (Pratt & Hudson, 1990). Root extracts of several medicinal plants have been reported to exhibit anti-inflammatory, anticancer, antimicrobial, antidiarrheal, as well as hepatoprotective effects (Lee et al., 2004; Ilavarasan et al., 2005; Srivastava & Shivanandappa, 2006). The occurrence of simple quinoline alkaloids in the aerial and underground parts of more than 14 species in the Asteraceae family have been reported (Chaudhuri, 1992). A comprehensive investigation into the chemical composition of the volatile oil from *A. arctotoides* also showed the occurrence of sesquiterpenes in its root (Oyedepi et al., 2005). Sesquiterpene lactones constitute a large group of more than 5000 compounds, mainly isolated from the members of Asteraceae family. They display a wide spectrum of biological and pharmacological activity (Feltensteina et al., 2004; Koukoulitsa et al., 2006). In this paper, we present the results of the antioxidant and antimicrobial activities of different extracts from the roots of *A. arctotoides*. The findings from this work may add to the overall value of the medicinal potential of the herb.

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Materials and Methods

Collection of plant roots

The root materials of *A. arctotoides* were collected from plants growing in the natural population around the University of Fort Hare campus. The plant has been previously authenticated by Dr. D.S. Grierson of the Department of Botany, University of Fort Hare, when a voucher specimen was prepared and deposited at the herbarium of the Department of Botany.

Preparation of extracts

The root samples were carefully washed under running tap water, air-dried at room temperature, and pulverized before extraction. Portions (100 g each) of the pulverized samples were extracted separately for 24 h in acetone, methanol, and water. The extracts were filtered through Whatman no. 1 filter paper and evaporated to dryness under reduced pressure at a maximum of 40°C using a rotary evaporator. The extracts were redissolved in their respective solvents to the required concentrations for the bioassay analysis.

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4',4''-disulfonic acid, potassium ferricyanide, catechin, butylated hydroxytoluene (BHT), ascorbic acid, catechin, tannic acid, quercetin, and FeCl₃ were purchased from Sigma Chemical Co. (St. Louis, MO, USA); vanillin was from BDH (Poole, England); and Folin-Ciocalteu phenol reagent and sodium carbonate were from Merck Chemical Supplies (Darmstadt, Germany). All the other chemicals used, including the solvents, were of analytical grade.

Determination of total phenolics

Total phenol contents in the extracts were determined by the modified Folin-Ciocalteu method (Wolfe et al., 2003). An aliquot of the extract was mixed with 5 mL Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 mL (75 g/L) of sodium carbonate. The tubes were vortexed for 15 s and allowed to stand for 30 min at 40°C for color development. Absorbance was then measured at 765 nm using the Hewlett Packard UV-VIS spectrophotometer (Palo Alto, CA). Samples of extract were evaluated at a final concentration of 0.1 mg/mL. Total phenolic contents were expressed as mg/g g tannic acid equivalent using the following equation based on the calibration curve: $y = 0.1216x$, $R^2 = 0.9365$, where x is the absorbance and y is the tannic acid equivalent (mg/g).

Determination of total flavonoids

Total flavonoids were estimated using the method of Ordon ez et al. (2006). To 0.5 mL of sample, 0.5 mL of 2% AlCl₃ ethanol solution was added. After 1 h at room temperature, the absorbance was measured at 420 nm. A yellow color indicated the presence of flavonoids. Extract samples were evaluated at a final concentration of 0.1 mg/mL. Total flavonoid contents were calculated as quercetin (mg/g) using the following equation based on the calibration curve: $y = 0.0255x$, $R^2 = 0.9812$, where x is the absorbance and y is the quercetin equivalent (mg/g).

Determination of total proanthocyanidins

Determination of proanthocyanidin was based on the procedure reported by Sun et al. (1998). A volume of 0.5 mL of 0.1 mg/mL extract solution was mixed with 3 mL of 4% vanillin-methanol solution and 1.5 mL hydrochloric acid; the mixture was allowed to stand for 15 min. The absorbance was measured at 500 nm. Extract samples were evaluated at a final concentration of 0.1 mg/mL. Total proanthocyanidin contents were expressed as catechin equivalents (mg/g) using the following equation based on the calibration curve: $y = 0.5825x$, $R^2 = 0.9277$, where x is the absorbance and y is the catechin equivalent (mg/g).

Determination of antioxidant activity

ABTS radical scavenging assay

For the ABTS assay, the method of Re et al. (1999) was adopted. The stock solutions included 7 mM ABTS^{•+} solution and 2.4 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 mL ABTS^{•+} solution with 60 mL methanol to obtain an absorbance of 0.706 ± 0.001 units at 734 nm using the spectrophotometer. Fresh ABTS^{•+} solution, was prepared for each assay. Plant extracts (1 mL) were allowed to react with 1 mL of the ABTS^{•+} solution, and the absorbance was taken at 734 nm after 7 min using the spectrophotometer. The ABTS^{•+} scavenging capacity of the extract was compared with that of BHT and percentage inhibition calculated as $\text{ABTS}^{\bullet+} \text{ scavenging activity (\%)} = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{(\text{Abs}_{\text{control}})} \times 100$, where $\text{Abs}_{\text{control}}$ is the absorbance of ABTS^{•+} methanol; and $\text{Abs}_{\text{sample}}$ is the absorbance of ABTS^{•+} sample extract/standard.

DPPH radical scavenging assay

The effect of extracts on DPPH radical was estimated using the method of Liyana-Pathirana and Shahidi

(2005). A solution of 0.135 mM DPPH in methanol was prepared and 1.0 mL of this solution was mixed with 1.0 mL of extract in methanol containing 0.02–0.1 mg of the extract. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid and BHT were used as references. The ability to scavenge DPPH radical was calculated by the following equation: DPPH radical scavenging activity (%) = $[(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})] / (\text{Abs}_{\text{control}}] \times 100$, where $\text{Abs}_{\text{control}}$ is the absorbance of DPPH radical + methanol; and $\text{Abs}_{\text{sample}}$ is the absorbance of DPPH radical + sample extract/standard.

Total antioxidant activity (FRAP assay)

A modified method of Benzie and Strain (1996) was adopted for the ferric reducing antioxidant power (FRAP) assay. The stock solutions included 300 mM acetate buffer (3.1 g $\text{C}_2\text{H}_3\text{NaO}_2 \cdot 3\text{H}_2\text{O}$ and 16 mL $\text{C}_2\text{H}_4\text{O}_2$), pH 3.6, 10 mM TPTZ (2,4,6-tripyridyl-*S*-triazine) solution in 40 mM HCl, and 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ, and 2.5 mL $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$. The temperature of the solution was raised to 37°C before use. Plant extracts (150 µL) were allowed to react with 2850 µL of the FRAP solution for 30 min in the dark condition. Readings of the colored product (ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve was linear between 200 and 1000 µM FeSO_4 . Results are expressed in µM Fe (II)/g dry mass and compared with that of BHT, ascorbic acid, and catechin.

Antibacterial assay

Preparation of agar-extract plates

Nutrient agar (Biolab, Johannesburg, South Africa) was prepared in the usual fashion by autoclaving and allowed to cool to about 60°C before the addition of the extracts. The agar medium containing the extracts at final concentrations of 0.1, 0.5, 1.0, 2.5, and 5.0 mg/ml were poured into Petri dishes, swirled carefully until the agar began to set. Plates were left overnight for the solvents to evaporate (Afolayan & Meyer, 1997). Plates containing 5 ml of acetone or methanol were used as controls (Dulger & Ugurlu, 2005).

Bacterial strains and bioassay

Bacterial species used in the study were obtained from the Department of Biochemistry and Microbiology, Rhodes University, South Africa. Five Gram-positive (*Bacillus cereus*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Micrococcus kristinae*, *Streptococcus pyogenes*) and five Gram-negative (*Escherichia coli*, *Salmonella pooni*, *Serratia marcescens*, *Pseudomonas*

aeruginosa, and *Klebsiella pneumoniae*) species were used in this study. Organisms were maintained on nutrient agar plates and were revived for bioassay by subculturing in fresh nutrient broth (Biolab) for 24 h before use.

Organisms were streaked in radial patterns, and the plates were incubated at 37°C for 24 to 48 h. The minimum inhibitory concentration (MIC) values were recorded, where no bacterial growth was observed. Experiments were replicated three-times and results compared with those of known antibiotics (streptomycin and chloramphenicol).

Antifungal assay

Five species of fungi, *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporium*, *Mucor heamalis*, and *Penicillium notatum*, were used for the antimycotic investigation. The fungal cultures were maintained on potato dextrose agar (PDA) (Biolab) and were recovered for testing by subculturing in fresh PDA for 3 days prior to bioassay. The plates were prepared by autoclaving before the addition of the extracts. Each extract was mixed with the molten agar (at 45°C) to final extract concentrations of 0.1, 0.5, 1.0, and 5.0 mg/mL of molten agar and poured into Petri dishes. Blank plates containing only PDA or PDA with the respective solvents served as controls. The prepared plates containing the extracts were inoculated with plugs obtained from actively growing portions of the fungal plates and incubated at 25°C for 5 days. Diameter of the fungal growth was measured and expressed as means of percentage growth inhibition of three replicates (Quiroga et al., 2001; Lewu et al., 2006). Significant differences within the means of treatments and controls were calculated using the LSD statistical test (Steel & Torrie, 1960). LC_{50} (the concentration at which 50% of the growth was obtained) was calculated by extrapolation.

Statistical analysis

The experimental results were expressed as mean \pm standard deviation (SD) of three replicates. Where applicable, the data were subjected to one-way analysis of variance (ANOVA), and differences between samples were determined by Duncan's multiple range test using the Statistical Analysis System (SAS) program. *p* values < 0.05 were regarded as significant and *p* values < 0.01 as very significant. Correlation between polyphenol contents and antioxidant activity was established by regression analysis.

Results and Discussion

Total phenolic, flavonoids, and proanthocyanidin contents

Polyphenols are the major plant compounds with antioxidant activity. This activity is believed to be mainly

Table 1. Polyphenol contents and antioxidant activity of the root extracts from *Arctotis arctotoides*.

Root extracts	Total polyphenol ^a	Flavonoids ^b	Proanthocyanidins ^c	FRAP ^d
Acetone	18.71 ± 0.4 ^a	1.84 ± 0.04 ^a	6.64 ± 0.00 ^a	80.43 ± 1.76 ^a
Methanol	13.73 ± 0.5 ^b	0.40 ± 0.03 ^b	0.98 ± 0.00 ^b	81.86 ± 3.40 ^a
Water	5.33 ± 0.7 ^c	0.07 ± 0.07 ^c	1.72 ± 0.15 ^c	15.28 ± 0.00 ^b
Ascorbic acid	—	—	—	1626.54 ± 17.76 ^c
BHT	—	—	—	62.35 ± 3.01 ^d
Catechin	—	—	—	971.55 ± 1.07 ^c

Analyses were mean of three replicates ± standard deviations. Means along the same column with different superscripts are significantly different, $p > 0.05$.

^aExpressed as mg tannic acid/g of dry plant material.

^bExpressed as mg tannic acid/g of dry plant material.

^cExpressed as mg tannic acid/g of dry plant material.

^dExpressed in units of $\mu\text{mol Fe (II)/g}$.

due to their redox properties (Zheng & Wang, 2001), which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Results obtained in the current study revealed that the level of these phenolic compounds were in the following order: acetone > methanol > water extracts (Table 1).

A good correlation was observed between the total phenol ($R^2 = 0.7753$) and flavonoid content, and a moderate correlation existed between the phenols and proanthocyanidins ($R^2 = 0.4947$). The results strongly suggest that phenolics are important components of *A. arctotoides*, and some of its pharmacological effects could be attributed to the presence of these valuable constituents.

Total antioxidant power

The antioxidant potentials of *A. arctotoides* extracts were estimated from their ability to reduce TPRZ-Fe (III) complex to TPTZ-Fe (II). The reducing ability of the extracts was in the range 15.28–82.87 $\mu\text{mol Fe (II)/g}$ (Table 1). The FRAP values for the acetone and methanol extracts were significantly lower than those of ascorbic acid and catechin but higher than that of BHT and the water extract. Antioxidant activity increased proportionally to the polyphenol content: the linear relationship between FRAP values and total polyphenol, flavonoid, and proanthocyanidin contents were $R^2 = 0.8732$, 0.4277, and 0.1634, respectively (Figs. 1–3). According to recent reports, a highly positive relationship between total phenols and antioxidant activity appears to be the trend in many plant species (Oktay et al., 2003).

ABTS radical scavenging activity

Proton radical scavenging is an important attribute of antioxidants. ABTS, a protonated radical, has characteristic absorbance maxima at 734 nm that decreases with the scavenging of the proton radicals (Matthew &

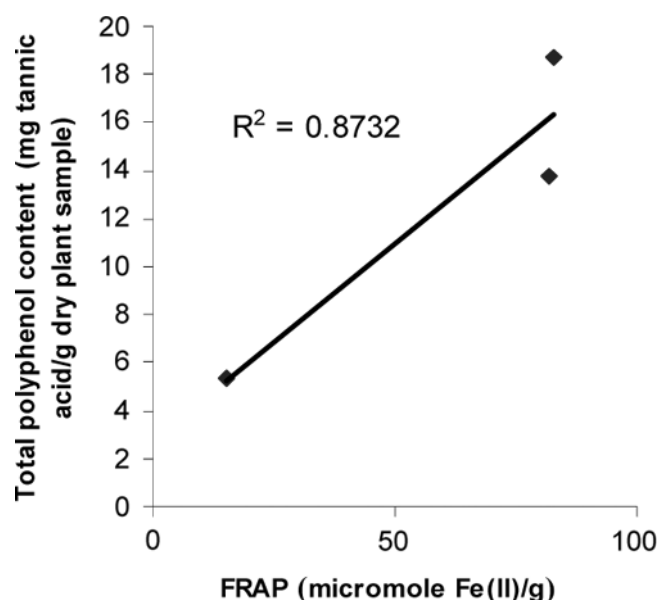


Figure 1. Correlation of FRAP and total polyphenol content of different extracts of *A. arctotoides*.

Abraham, 2006). *A. arctotoides* extracts were fast and effective scavengers of the ABTS radical (Fig. 4) and this activity was comparable with that of BHT. The percentage inhibition was 96.7%, 95.5%, 91.1%, and 96.3% for acetone, methanol, water extracts, and BHT, respectively. Higher concentrations of the extracts were more effective in quenching free radicals in the system. The high correlation between ABTS radical scavenging activity of the extracts and total phenolic content ($R^2 = 0.9641$) might imply that the phenolic compounds may contribute directly to the antioxidative action.

DPPH radical scavenging activity

The effect of antioxidants on DPPH[•] is thought to be due to their hydrogen-donating ability (Baumann et al., 1979). Figure 5 shows the dose-response curve of DPPH

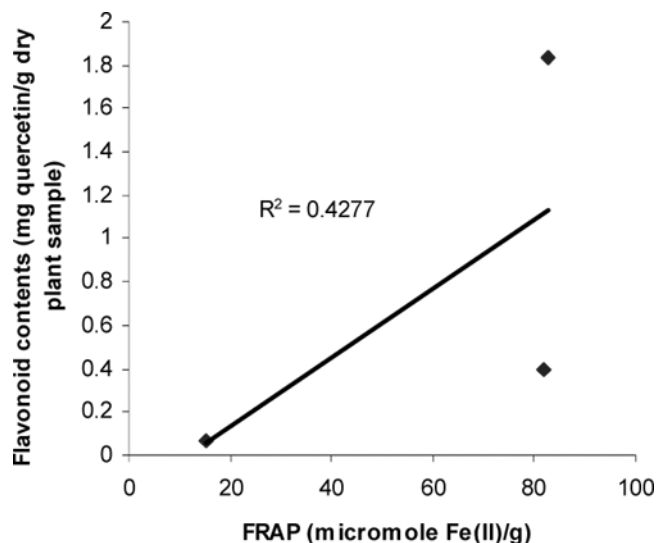


Figure 2. Correlation of FRAP and flavonoid content of different extracts of *A. arctotoides*.

radical scavenging activity of the different extracts of the root of *A. arctotoides* compared with BHT and ascorbic acid. It was observed that methanol extract had the highest activity among the extracts, followed by acetone extract, whereas water extract had the least. At a concentration of 0.1 mg/mL, the scavenging activity of methanol extract reached 68.2%, but at the same concentration, that of acetone and water extracts were only 61.8% and 7.5%, respectively. Although the DPPH rad-

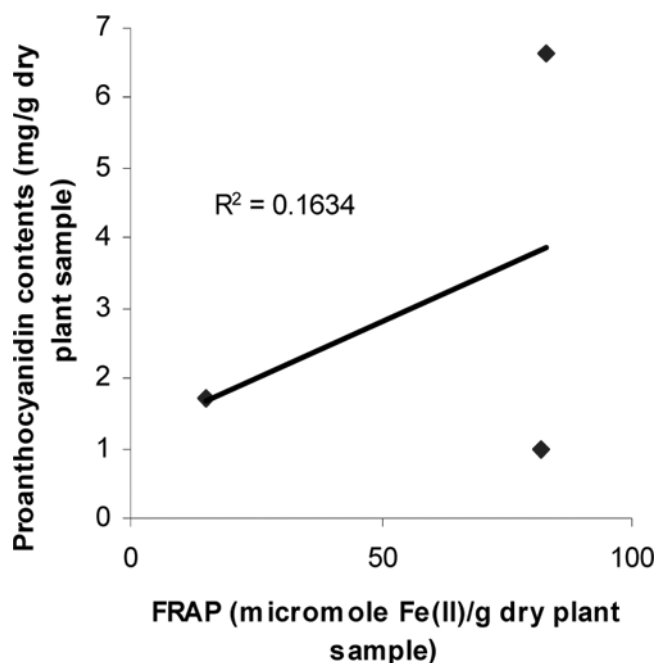


Figure 3. Correlation of FRAP and proanthocyanidin content of different extracts of *A. arctotoides*.

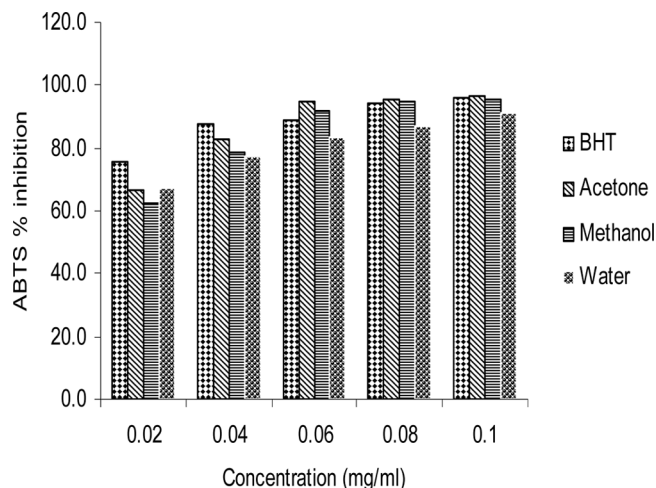


Figure 4. ABTS radical scavenging activities of extracts from the roots of *A. arctotoides*.

ical scavenging abilities of the extracts were significantly lower than those of ascorbic acid and BHT, it was evident that the extracts did show proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. This value correlates highly ($R^2 = 0.7923$) with the total phenolic content of the extracts. The low phenolic content of the water extract might therefore explain the lowest DPPH radical scavenging activity of this extract.

The scavenging of $ABTS^{\cdot+}$ by the extracts was found to be higher than that of DPPH $^{\cdot}$. Factors like stereoselectivity of the radicals or the solubility of the extract in different testing systems have been reported to affect the capacity of extracts to react and quench different radicals (Yu et al., 2002). Wang et al. (1998) found that some compounds that have $ABTS^{\cdot+}$ scavenging activity did not show DPPH scavenging activity. A correlation

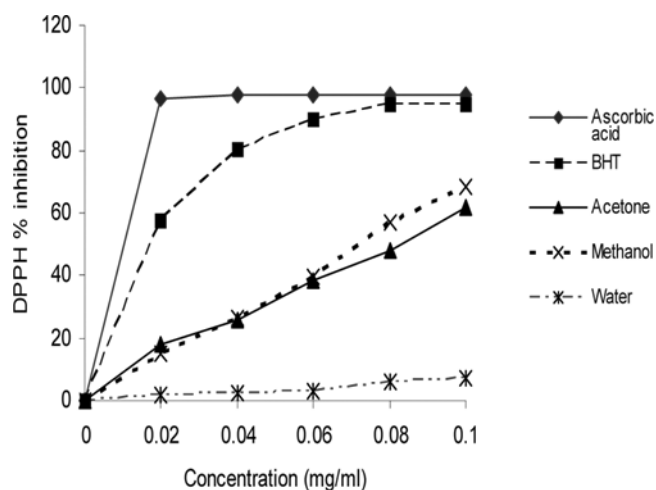


Figure 5. DPPH radical scavenging activities of extracts from the roots of *A. arctotoides*.

Table 2. Antibacterial activity of the root extracts of *Arctotis arctotoides*.

Bacterial species	Minimum inhibitory concentration					
	Gram +/-	Acetone (mg/mL)	Methanol (mg/mL)	Water (mg/mL)	Chloramphenicol ^a	Streptomycin ^a
<i>Bacillus cereus</i>	+	0.1	0.5	10.0	<2	<2
<i>Staphylococcus epidermidis</i>	+	NA	NA	NA	<2	<2
<i>Staphylococcus aureus</i>	+	0.1	5.0	NA	<2	<2
<i>Micrococcus kristinae</i>	+	0.1	5.0	NA	<2	<2
<i>Streptococcus pyrogens</i>	+	0.1	5.0	NA	<2	<2
<i>Escherichia coli</i>	-	5.0	10.0	NA	<2	<2
<i>Salmonella pooni</i>	-	1.0	5.0	NA	<2	<2
<i>Serratia marcescens</i>	-	NA	NA	NA	<2	<2
<i>Pseudomonas aeruginosa</i>	-	NA	NA	NA	<20	<5
<i>Klebsiella pneumoniae</i>	-	NA	NA	NA	<2	<2

NA, no activity.

^aChloramphenicol and streptomycin in µg/mL.Table 3. Antifungal activity of the root extracts of *Arctotis arctotoides*.

Concentrations (mg/mL)	Growth inhibition (%)				
	<i>A. flavus</i>	<i>A. niger</i>	<i>F. oxysporum</i>	<i>P. notatum</i>	<i>M. heamalis</i>
Acetone extracts					
5.0	56.23 ^d	53.05 ^d	60.11 ^e	100.00 ^d	100.00 ^e
1.0	38.56 ^c	41.43 ^c	32.90 ^d	64.90 ^c	90.07 ^d
0.5	33.13 ^c	16.53 ^b	24.38 ^c	46.61 ^c	77.78 ^c
0.1	17.01 ^b	6.00 ^a	12.01 ^b	31.31 ^b	37.62 ^b
Control	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
LC ₅₀ mg/mL	3.59	3.95	3.51	0.59	0.22
Methanol extracts					
5.0	61.35 ^d	52.20 ^c	63.23 ^c	58.09 ^b	72.52 ^d
1.0	44.54 ^c	21.32 ^b	26.01 ^b	13.85 ^a	55.80 ^c
0.5	15.07 ^b	16.37 ^b	5.14 ^a	9.13 ^a	27.18 ^b
0.1	4.77 ^a	6.53 ^a	2.43 ^a	6.51 ^a	11.41 ^a
Control	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
LC ₅₀ mg/mL	2.30	4.72	3.58	4.27	0.87
Water extracts					
5.0	34.89 ^b	16.50 ^b	11.08 ^a	21.97 ^b	64.84 ^b
1.0	29.27 ^b	13.96 ^b	6.97 ^a	7.79 ^a	57.26 ^b
0.5	8.77 ^a	10.97 ^b	2.15 ^a	3.77 ^a	27.07 ^a
0.1	3.87 ^a	5.10 ^a	0.77 ^a	3.16 ^a	13.89 ^a
Control	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
LC ₅₀ mg/mL	>5	>5	>5	>5	0.88

Values are means of percentage growth inhibition of three replicates: values within a column followed by the same superscript are not significantly different at $p < 0.05$.

between these two models was obvious in our study ($R^2 = 0.9223$). This further showed the capability of the extracts to scavenge different free radicals in different systems, indicating that they may be useful therapeutic agents for treating radical-related pathologic damage.

Antibacterial activity

The antibacterial activity of the various extracts is presented in Table 2. With the exception of *Staphylococcus*

epidermidis, acetone and methanol extracts demonstrated activity against all the Gram-positive bacteria tested in this study. The MIC ranged from 1 to 5 mg/mL except for the water extract which was only active against *Bacillus cereus* at a concentration of 10 mg/mL. Generally, the extracts were more active against than the Gram-positive bacteria than against the Gram-negative strains. This observation is not unexpected as, in general, these bacteria are more resistant than the Gram-positive ones (Grierson & Afolayan, 1999; Afolayan, 2003).

Antifungal activity

Though not completely fungicidal, extracts from the roots of *A. arctotoides* have shown appreciable inhibition of growth against species tested in the study (Table 3). The acetone and methanol extracts demonstrated significant activity against the five fungal species used with growth inhibition ranging from 56.23% on *Aspergillus flavus* to 100% on *P. notatum* and *M. heamalis* at 5.0 mg/mL, which was the highest concentration tested. The water extract showed the least inhibitory activity in the study (Table 3). Growth inhibition was, however, generally less on *Aspergillus niger*. When compared with the results obtained using the shoot of this plant (Afolayan, 2003), higher percentage inhibition was obtained in the root with the acetone and methanol extracts against *A. flavus*.

In the current study, there was a positive correlation ($R^2 = 0.8879$; 0.7234) between the phenolic content, antibacterial and antifungal activity of the acetone extract of the root of *A. arctotoides*. The antimicrobial properties of phenolic compounds are well documented (Rauha et al., 2000) and may, in part, account for the observed activity. The presence of antibacterial and antioxidant activities in the extracts from the root of *A. arctotoides* has revealed the potential of the herb as a good medicinal agent. These findings have further validated the use of the herb for the treatment of both infectious diseases and physiologic stress. Information on the antimicrobial and antioxidant properties of medicinal plants, such as this, throws more light onto the understanding of the medicinal potential of such herbal species.

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