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Extracts from the Shoots of *Arctotis arctotoides* Inhibit the Growth of Bacteria and Fungi

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Abstract

Acetone, methanol and water extracts obtained from the shoots of *Arctotis arctotoides* through shaking and homogenization, were investigated for their antimicrobial activities. Growth inhibition using agar dilution assays was determined against ten selected bacterial and six fungal species. Although not fungicidal, extracts from the herb showed significant growth inhibition against all the fungi tested. The homogenized water extract was particularly inhibitory to the growth of the fungi with inhibitory activity ranging from 50.7 to 95.2% on *Aspergillus tamari* and *Penicillum digitatum*, respectively. Acetone and methanol extracts were very active against the Gram positive bacteria. The Gram negative bacteria were, however, more resistant to the extracts than the Gram positive ones. None of the extracts inhibited *Klebsiella pneumoniae* and *Pseudomonous aeruginosa*, both Gram negative bacteria.

Keywords: Antibacterial, antifungal, antimicrobial, *Arctotis arctotoides*, Gram positive bacteria, Gram negative bacteria, homogenized extracts, medicinal, plant extracts, shaken extracts.

Introduction

The increasing resistance of human pathogens to current antimicrobial agents is a serious medical problem. The number of resistant strains of microbial pathogens is growing since penicillin resistant and multiresistant pneumoccoci caused a major problem in South Africa in 1977 (Meurer-Grimes et al., 1996; Eloff, 1998). Hitherto, natural products from microorganisms have been the primary source for antibiotics, but with the increasing acceptance of herbal medicine as an alternative form of healthcare, the screening of medicinal plants for active compounds has become very important and may, as well, serve as promising sources of novel antibiotic prototypes (Meurer-Grimes et al., 1996; Rabe & Van Staden, 1997).

Arctotis arctotoides (L. f.) O. Hoffm., Asteraceae, is a decumbent herb commonly found as roadside weed in most costal districts of South Africa. Ethnomedical information from the indigenous people of the eastern Cape province has revealed that extracts of the herb are widely used among Xhosa speaking people for the treatment of various ailments, ranging from epilepsy to indigestion and catarrh of the stomach (Watt & Breyer-Brandwijk, 1932). No information is available on the antimicrobial potential of extracts from this herb. *In vitro* antimicrobial screening methods have provided the needed preliminary observations necessary to select among crude plant extracts, those with potentially useful properties for further chemical and pharmacological investigations (Mathekga & Meyer, 1998).

The purpose of this study was to investigate *A. arctotoides* for potential antibiotic activity by preliminary bioassay screening. Extracts obtained through shaking and homogenization were tested against representative Gram positive and Gram negative bacteria as well as fungi.

Materials and methods

Plant material

The shoots of *A. arctotoides* were collected from the natural population around the University of Fort Hare (UFH) campus, and identified using the University herbarium. A voucher specimen of the plant (Afol. 99/03) was prepared and deposited at the Giffen Herbarium, UFH.

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Extract preparation

Portions of air-dried plant materials (50 g) were separately shaken for 5 min each in acetone, methanol and water, and filtered through Whatman No.1 filter paper under suction. These filtrates are referred to in this paper as "shaken extracts". The residues were then homogenized separately in the respective solvents and filtered and are referred to as "homogenized extracts". Each extract was concentrated to dryness under reduced pressure at 40 °C and stored at 4 °C until further use.

Addition of the extracts to the nutrient medium

Adopting the previous method of Afolayan and Meyer (1997), nutrient agar (NA) for bacteria and potato dextrose agar (PDA) for the fungi (both Biolab) were prepared in the usual fashion by autoclaving before the addition of the extracts. Each was filtered through sterile 0.22 µm syringe fitted filters. To test an extract at 0.5 mg/ml, 5 mg of the extract was dissolved in 0.1 ml of its solvent of extraction and added to 9.9 ml of molten nutrient medium. This was poured into a Petri dish, swirled carefully until the agar began to set and left overnight for the solvent to evaporate.

Antibacterial test

Ten bacterial species were obtained as previously described (Grierson & Afolayan, 1999). Each organism was maintained on NA slants (Biolab) and was recovered for testing by growth in nutrient broth No. 2 (Biolab) for 24 h at 37 °C. Before streaking, each culture was diluted 1 : 100 with fresh sterile nutrient broth (Afolayan & Meyer, 1997; Grierson & Afolayan, 1999).

The bacteria were streaked in radial patterns on the agar plates (Mitsher et al., 1972; Meyer & Afolayan, 1995). Plates were incubated at 37 °C and examined after 24 and 48 h. Complete suppression of growth by a specific concentration of an extract was required for it to be declared active. Each extract was tested at 5.0, 1.0, 0.5, and 0.1 mg/ml (three replicates). Blank plates containing only NA and another ones containing NA and 2% acetone or methanol served as negative controls (Meyer & Afolayan, 1995; Mathekga & Meyer, 1998).

Antifungal test

Six fungal species were obtained from the Department of Microbiology, UFH. Each culture was maintained on PDA and was recovered for testing by sub-culturing on fresh PDA for 3 days at 25 °C. The prepared plates containing an extract at concentrations of 5.0, 1.0, 0.5 and 0.1 mg/ml, respectively, were inoculated with plugs obtained from the actively growing margin of the fungi plates and incubated at 25 °C for 3 days (Afolayan & Meyer, 1997). The diameter of the fungal growth was measured and expressed as means of percentage growth inhibition of three replicates. Significant differences within the means of the treatments and the control were calculated using the LSD statistical test (Steel & Torrie, 1960).

Results and discussion

Antibacterial property

Acetone, methanol and water extracts from the shoots of *A. arctotoides*, prepared by shaking and homogenization, have demonstrated significant antibacterial activities (Table 1). However, there was a lower degree of activity from the water extract. Generally, the extracts showed a broad spectra of activity, although there was more activity against the Gram positive bacteria than against the Gram negative ones. *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, both Gram negative, were not inhibited by any of the extracts at the tested concentrations.

The observed lower degree of activity of the extracts against the Gram negative bacteria was not surprising as, in general, these bacteria are more resistant than the Gram positive ones (Rabe & Van Staden, 1997; Grierson & Afolayan, 1999). Both methods of extraction, shaking and homogenization, appeared to have a similar activity effect on the bacteria. A careful look at the results shows a generally lower activity in the water extracts. This is noteworthy, because considering the fact that plant extracts are traditionally prepared with water, it is unlikely that the traditional healer adequately extracts those compounds that are responsible for activity in the acetone and methanol extracts.

Antifungal activity

Though not fungicidal, extracts from the shoots of *A. arctotoides* have shown appreciable inhibition of growth against fungi (Table 2). The homogenized water extract was particularly active against the six fungal species with growth inhibitions ranging from 50.7% on *Aspergillus tamarii* to 83.6% on *Penicillium digitatum* at 0.1 mg/ml. *P. digitatum* was especially susceptible to the homogenized water extract with 95.2% growth inhibition at 5.0 mg/ml which was the highest concentration tested. Despite the high antifungal activity shown by the homogenized water extract, the shaken extract showed the least inhibitory activity against the fungi (Table 2).

Both homogenized and shaken extracts, using acetone, demonstrated significant antifungal activity against the six fungal species used. However, their growth inhibition was generally less on *Aspergillus flavus* and *Penicillium digitatum*. The methanol extract showed, more or less, the opposite result obtained from the water extract in that, while the shaken extract of methanol exhibited a strong inhibitory effect on the growth of the fungal species, the homogenized extract was generally less inhibitory to the organisms.

Table 1. Antibacterial activity of *A. arctotoides*.

		Minimum inhibitory concentration (mg/ml)					
		Shaken extracts			Homogenized extracts		
Bacteria	Gram +/–	acetone	methanol	water	acetone	methanol	water
Bacillus cereus	+	0.1*	0.1	na	0.1	0.5	5.0
B. pumilus	+	0.1	0.1	5.0**	0.1	0.5	5.0
B. subtilis	+	0.5	0.1	5.0	0.5	0.5	na
Micrococcus kristinae	+	0.5	0.5	5.0	na	1.0	5.0
Staphylococcus aureus	+	1.0	0.5	5.0	0.5	5.0	5.0
Enterobacter cloacae	–	5.0	0.5	na	5.0	na	na
Escherichia coli	–	1.0	0.5	na	na	na	na
Klebsiella pneumoniae	–	na	na	na	na	na	na
Pseudomomas aeruginosa	–	na	na	na	na	na	na
Serratia marcescens	–	5.0	5.0	na	5.0	1.0	na

na (not active).
* Minimum and ** maximum concentrations of extracts tested respectively.

Table 2. Antifungal property of *A. arctotoides*.

	Growth inhibition (%)											
	Shaken						Homogenized					
Treatment (mg/ml)	*A. fla*	*A. tam*	*C. her*	*C. sph*	*P. dig*	*P. ita*	*A. fla*	*A. tam*	*C. her*	*C. sph*	*P. dig*	*P. ita*
Acetone extracts	9.8^{c}	15.6^{d}	22.2^{b}	30.0^{b}	3.3^{c}	30.4^{a}	10.4^{b}	15.3^{d}	23.1^{b}	31.3^{b}	6.6^{a}	35.1^{b}
0.1	13.3^{b}	24.2^{c}	8.1^{d}	29.9^{b}	7.0^{b}	21.6^{b}	10.0^{b}	21.4^{c}	13.8^{c}	29.0^{b}	3.3^{b}	36.3^{b}
0.5	15.9^{b}	33.3^{b}	16.5^{c}	29.8^{b}	9.3^{b}	18.7^{b}	9.8^{b}	30.8^{b}	16.2^{bc}	27.6^{b}	7.0^{a}	28.6^{c}
1.0	32.7^{a}	46.5^{a}	35.2^{a}	39.1^{a}	15.0^{a}	30.3^{a}	29.4^{a}	41.5^{a}	33.3^{a}	41.9^{a}	8.9^{a}	42.7^{a}
5.0	0.0^{d}	3.3^{e}	$–4.3^{e}$	3.2^{c}	$–2.3^{c}$	4.2^{c}	$–4.2^{c}$	3.1^{e}	$–2.2^{d}$	3.2^{c}	2.4^{b}	0.0^{d}
Control	0.0^{d}	0.0^{e}	0.0^{e}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{e}	0.0^{d}	0.0^{c}	0.0^{c}	0.0^{d}
MeoH extracts												
0.1	14.7^{bc}	18.6^{c}	20.1^{b}	30.0^{b}	6.4^{c}	29.2^{a}	8.0^{b}	10.3^{bc}	6.4^{b}	20.4^{b}	3.3^{b}	17.4^{b}
0.5	18.4^{b}	28.1^{bc}	17.2^{bc}	28.0^{b}	10.3^{b}	23.7^{b}	8.1^{b}	15.4^{b}	7.8^{b}	17.3^{bc}	3.3^{b}	18.8^{b}
1.0	19.9^{b}	37.2^{b}	10.5^{c}	29.8^{b}	12.4^{b}	19.7^{c}	7.3^{b}	15.7^{b}	7.8^{b}	11.0^{d}	6.0^{a}	13.6^{c}
5.0	37.7^{a}	52.5^{a}	33.3^{a}	40.3^{a}	18.0^{a}	28.1^{a}	10.7^{a}	28.1^{a}	14.3^{a}	29.8^{a}	8.8^{a}	21.6^{a}
MeoH	3.3^{d}	3.3^{d}	$–3.3^{d}$	4.1^{c}	0.0^{d}	0.0^{d}	$–2.3^{c}$	3.2^{e}	3.3^{c}	0.4^{e}	2.0^{e}	0.3^{d}
Control	0.0^{d}	0.0^{d}	0.0^{d}	0.0^{d}	0.0^{d}	0.0^{d}	0.0^{c}	0.0^{e}	0.0^{c}	0.0^{e}	0.0^{c}	0.0^{d}
Water extract												
0.1	0.0^{c}	3.3^{c}	0.0^{b}	0.0^{b}	0.0^{d}	10.0^{c}	64.8^{c}	50.7^{d}	65.8^{c}	60.3^{b}	83.6^{c}	70.8^{bc}
0.5	5.0^{b}	3.0^{c}	2.0^{a}	0.0^{b}	17.3^{a}	14.3^{b}	82.1^{b}	64.8^{c}	86.5^{ab}	70.2^{a}	89.2^{b}	80.8^{a}
1.0	7.3^{ab}	8.6^{b}	0.0^{b}	9.0^{a}	3.3^{c}	14.1^{b}	80.0^{b}	73.9^{b}	92.4^{a}	71.1^{a}	91.0^{b}	77.4^{b}
5.0	8.0^{a}	14.1^{a}	0.0^{b}	9.3^{a}	6.7^{b}	20.3^{a}	88.5^{a}	82.9^{a}	90.8^{b}	70.3^{a}	95.2^{a}	77.7^{b}
Control	0.0^{c}	0.0^{d}	0.0^{b}	0.0^{b}	0.0^{d}	0.0^{d}	0.0^{e}	0.0^{d}	0.0^{c}	0.0^{d}	0.0^{d}	0.0^{d}

Values are means of percentage growth inhibition of three replicates; values within a column followed by the same superscript are not significantly different at $P < 0.05$ according to the LSD test (Steel & Torrie, 1960).
A. fla (Aspergillus flavus), A. tam (Aspergillus tamarii), C. her (Cladosporium herbarum), C. sph (Cladosporium sphaerospermum), P. dig (Penicillium digitatum), P. ita (Penicillium italicum).

This observation has justified the homogenization process (grinding) in water employed by the traditional healers during the preparation of *A. arctotoides* for the treatment of microbial infections, such as catarrh of the stomach. The ability of the extracts of this herb to inhibit the growth of all the Gram positive and a number of the Gram negative bacteria used, as well as on the fungal species, is an indication of the potential of the herb as a source of broad spectrum antimicrobial agents. This probably explains the use of the herb by the traditional herbalists of the eastern Cape, South Africa, against a number of infections. A similar study was carried out on *A. auriculata*, also in South African (Salie et al., 1996), in which the herb was found to be active against bacteria and fungi. This type of information is necessary in the current search for novel antimicrobial drugs. Work is in progress on the isolation, purification and structural identification of the bioactive compounds in this herb.

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