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Antibacterial and antifungal activities of *andrachne cordifolia* muell

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Abstract

The crude methanolic extract of *Andrachne cordifolia* Muell. (Euphorbiaceae) and its various fractions in different solvent systems (chloroform, ethyl acetate and *n*- butanol) were screened for antibacterial and antifungal activities. Crude extract and subsequent fractions demonstrated moderate to excellent antibacterial activities against the tested pathogens. Highest antibacterial activity was displayed by both chloroform and ethyl acetate fractions (100%) followed by the crude extract (68%) against *Salmonella typhi*. Similarly, crude extract and its subsequent fractions showed mild to excellent activities in antifungal bioassay with maximum (76%) antifungal activity against *Microsporum canis* by the chloroform fraction followed by the crude extract (65%).

Keywords: Andrachne cordifolia *Muell, antibacterial, antifungal*

Introduction

Plants, as extracts and in various other forms, have been used for centuries in different traditional systems of medicine for the treatment of human ailments, particularly those caused by pathogenic bacteria and fungi. The antiseptic qualities of aromatic and medicinal plants and their extracts have been recognized since antiquity, while attempts to characterize these properties in the laboratory date back to the early 1900s [1–3]. The growing antimicrobial resistance of certain microorganisms is now a worldwide concern. Acquisition and further spread of antibiotic resistance determinants among virulent bacterial populations is the most relevant problem for the treatment of infectious diseases [4,5]. It is because of these reasons that the search for plant products having antimicrobial properties has intensified in recent years [6,7].

In herbal medicine, the genus, *Andrachne* is used for the treatment of eye sores and to improve eyesight [8] and also has a stimulating action on respiration and the blood pressure of the dog and cat, spasmolytic activity on the tracheal muscle of the cat and anti histaminic activity a guineapig illium [9]. Interest in the phytochemical exploration of *Andrachne cardifolia* Muell began from 1983 when M.Ikram et al., isolated two bisbenzylisoquinoline alkaloids, cocsulin and penduline, from the roots of *Andrachne cardifolia* Muell [10]. This achievement was followed by the isolation of a new triterpene alcohol glut-5 (10)-en-1β -ol [11] and a pentacyclic triterpenic ketone, Glut-5 (10)-en-one [12], from the whole plant of *Andrachne cardifolia* Muell. Since then, this plant has not been extensively subjected to isolation of its active constituents and characterization, though it deserves to be thoroughly investigated for its beneficial uses in the light of present scientific advancement in the field of natural products. The methanolic extract and subsequent fractions of *Andrachne cardifolia* Muell have been screened for their enzyme inhibitory activites in our laboratory and showed outstanding activity against lipoxygenase [13]. Here, these extracts are examined for their antibacterial and antifungal activities.

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Table I. Antibacterial Activities Of Crude Extracts and Various fractions of *Andrachne Cardifolia* Muell.

Name of Bacteria	Zone of Inhibition of standard (Imipinem) 10 μg/mL	Crude Extract		$CHCl_3$ Fraction		CH_3COOEt Fraction		BuOH Fraction	
		Zone of Inhibition (mm)	Inhibition (%)	Zone of Inhibition (mm)	Inhibition (%)	Zone of Inhibition (mm)	Inhibition (%)	Zone of Inhibition (mm)	Inhibition (%)
E.coli,	30	13.00	43%	Nil	Nil	Nil	Nil	17	53%
B. subitilis	33	15.00	46%	Nil	Nil	Nil	Nil	Nil	Nil
Sp aurous	33	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Shigella flexenari	24	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
P. aerugenosa	24	11	46%	15	63%	15	63%	Nil	Nil
S. typhi	25	17	68%	25	100%	25	100%	7	28%

The plates were inculated at a concentration of 3 mg/mL of DMSO

Methods and materials

Plant material

Andrachne cordifolia Muell, locally known as Krachay (Pushto) belong to the Euphorbiaceae family. The whole plant was collected from Dir district, N.W.F.P (Pakistan) in 2004, and identified by Professor Dr. Jahander Shah, Plant Taxonomist, Vice Chancellor University of Malakand, Chakdara Dir. A voucher specimen (CA-012) has been deposited in the herbarium of the University of Malakand.

Extraction

The shade dried plant material was chopped into small pieces and pulverized into a fine powder. The plant material (15 kg) was soaked in methanol with occasional shaking, at room temperature. After 15 days, the methanol soluble materials were filtered off. The filtrate was concentrated under vacuum at low temperature (40°C) using a rotary evaporator. A blackish crude extract (295 g) was obtained.

Fractionation

The crude methanolic extract (295 g) was suspended in distilled water (500 mL) and sequentially partitioned with *n*-hexane (3 × 500 mL), chloroform (3 × 500 mL), ethyl acetate (3 × 500 mL) and *n*-butanol (3 × 500 mL) to yield the *n*-hexane (33 g), chloroform (69 g), ethyl acetate (36 g), *n*-butanol (41 g) and aqueous (75 g) fractions, respectively.

Antibacterial activity

The crude extract along with its fractions was screened against various human pathogens including *Escherichia coli, Bacillus subtilis, Klebsiella pneumonae, Shigella flexenari, Staphylococcus aurous, and Salmonella typhi* by the agar well diffusion method [14]. In this method, 10 mL aliquots of nutrient broth (Sigma-Aldrich, Germany) was inoculated with the test organism and incubated at 37°C for 24 h. Using a sterile pipette, 0.6 mL of the broth culture of the test organism was added to 60 mL of molten agar, which had been cooled to 45°C, mixed well and poured into a sterile Petri dish (for the 9 cm Petri dish, 0.2 mL of the culture was added to 20 mL of agar). Duplicate plates of each organism were prepared. The agar was allowed to set and harden and the required number of wells were dug in the medium with the help of a sterile metallic cork borer ensuring proper distribution of the wells in the periphery and one in the center. Agar plugs were removed. Stock solutions of the test samples at a concentration of 1 mg/mL were prepared in the sterile dimethyl sulfoxide (DMSO) and 100 μL and 200 μL of each dilution was added to the respective wells. The control well received only 100 μL and 200 μL of DMSO. Imipinem was used as a standard drug. The plates were left at room temperature for 2 h to allow diffusion of the samples and then incubated face upwards at 37°C for 24 h. The diameter of the zones of inhibition was measured to the nearest mm (the well size also being noted).

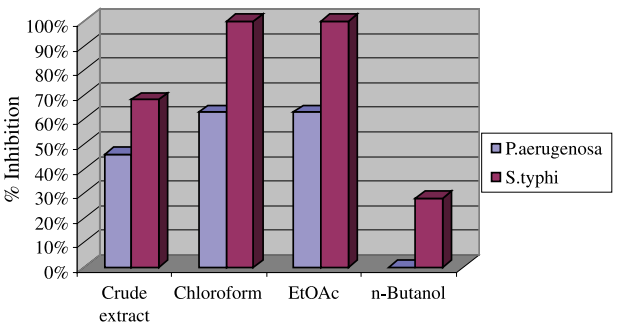

Figure 1. Antibacterial activity towards *P. aerugenosa* and *S. typhi* of crude extract and subsequent fractions of *Andrachne Cardifolia* Muell. The extracts were used at a concentration of 3 mg/mL of DMSO.

Table II. Antifungal activities of crude extracts and various fractions of *Andrachne cardifolia* Muell.

Name of fungi	Control Linear Growth (mm)	Crude Extract		$CHCl_3$ Fraction		CH_3COOEt Fraction		BuOH Fraction		Standard Drugs	
		Linear Growth (mm)	Inhibition (%)	Linear Growth (mm)	Inhibition (%)	Linear Growth (mm)	Inhibition (%)	Linear Growth (mm)	Inhibition (%)	Name	MIC µl/mL
T. longi fusus	100	44	56%	30	50%	25	50%	70	30%	Miconazole	65
C. albicans	100	100	0%	100	0%	65	0%	80	20%	Miconazole	110.8
A. flavus	100	100	0%	35	0%	30	0%	100	0%	Amphotericin - B	24
M. canis	100	35	65%	30	76%	25	40%	75	25%	Miconazole	97
F. solani	100	50	50%	100	0%	100	0%	100	0%	Miconazole	85
C. glaberata	100	100	0%	100	0%	100	0%	100	0%	Miconazole	100.8

The concentration of relavant extract used was 24 mg/mL of DMSO.

Antifungal activity

The antifungal activity of the extracts was evaluated by the agar tube dilution method [14]. The samples, 24 mg/ mL, were dissolved in sterile (autoclaved) dimethyl sulfoxide (DMSO, Merck), which served as a stock solution. Sabouraud dextrose agar (SDA, Sigma-Aldrich, Germany) was prepared by mixing 32.5 g sabouraud, 4% glucose agar and 4.0 g of agar-agar in 500 mL distilled water thoroughly with a magnetic stirrer. Then a 4 mL aliquot was dispensed into screw cap tubes, which were autoclaved at 120°C for 15 min and then cooled to 15°C. The non-solidified SDA media was mixed with stock solution (66.6 µL) giving a final concentration of 400 µg of the extract per mL of SDA. The tubes were then allowed to solidify in the slanted position at room temperature and then inoculated with a piece (4 mm diameter) of an inoculum removed from a seven days old culture of fungi to determine non-mycelial growth; an agar surface streak was employed. Other media supplemented with dimethyl sulfoxide (DMSO) and reference antifungal drugs served as negative and positive control respectively. Inhibition of fungal growth was observed visually after 7 days of incubation at 28 ± 1°C. Humidity (40–50%) was controlled by placing an open pan of water in the incubator.

Results and discusion

The crude methanolic extract and fractions of *Andrachne cordifolia* Muell were screened against various human pathogens and the results of their antibacterial activities are shown in Table I. The results show that the crude extract displayed low activity against *E. coli* (43%), *P. aeruginosa* (46%), *B. subtilis* (46%) and *S. typhi* (68%). No activity against *S. aurous* and *S. flexenari* was seen.

The chloroform fraction presented outstanding activity against *S. typhi* (100%) *and P. aeruginosa* (63%) (Figure 1). However it exhibited no activity against *S. aurous, B. subtilis, S. flexenari and E. coli*. The ethyl acetate fraction had similar activity to that of the chloroform fraction i.e. against *S. typhi* (100%) *and P. aeruginosa* (63%). The *n*-butanol fraction showed moderate activity against *E. coli* (53%) *and S. typhi* (28%), while against *S. aurous, B. subtilis, S. flexenari and P. aeruginosa* it exhibited no activity.

Antifungal activities of the crude extract and various fractions of *Andrachne cardifoliaI* Muell were evaluated against *Trichophyton longi fusus, Candida albicans, Aspergilus flavus, Icrosporum canis, Fusarium solani, and Candida glaberata,* in comparison with miconazole and amphotericin-B and the results are shown Table II.

The crude extract displayed good antifungal activity against *T. longi fusus* (56%), *M. canis* (65%) and *F. solani* (50%) (Figure 2) while inactive *C. albicans, A. flavus and C. glaberata*. The $CHCl_3$ fraction showed

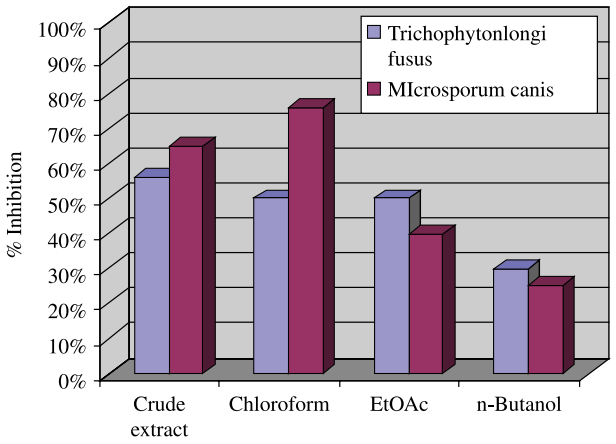

Figure 2. Antifungal activity towards *T. longifusus* and *M. cansis* of crude extract and subseqent fractions of *Andrachne cardifolia* Muell. Final concentration of relavant extract was 24 mg/mL of DMSO.

good to excellent result against *T. longi fusus* (50%) and *M. canis* (76%) but showed no activity against *C. albicans, F. solani, A. flavus* and *C. glaberata*. The activities of the EtOAc Fraction were similar to those of the chloroform fraction except against the *M. canis* (40%). The *n*-butanol fraction also showed antifungal activity against *T. longi fusus* (30%), *C. albicans (20%) and M. canis* (25%) but no activity against *A. flavus, C. glaberat* and *F. solani* was observed.

The results presented in Figure 1 show that the chloroform and ethyl acetate fractions of *Andrachne cordifolia* Muell had an outstanding activity (100%) against *S. typhi* which is an intracellular pathogen that causes diseases ranging from self-limiting enteritis to typhoid fever, the latter being a global health problem although its real impact is difficult to estimate because the clinical picture is confused with those of many other febrile infections. Worldwide, enteric infections rank third among all causes of the disease burden, being responsible for some 1.7–2.5 million deaths per year, mostly in young children and infants in developing countries [15–18].

On the other hand, multidrug-resistant (MDR) *Salmonella* Typhi (resistant to chloramphenicol, ampicillin, and trimethoprim–sulphamethoxazole) and isolates with reduced susceptibility to fluoroquinolones (indicated by resistance to nalidixic acid) can be treated with alternative synthetic drugs which are reasonably effective but quite expensive and have many side effects [19,20]. Development of new effective and safe products for the treatment of typhoid fever is urgently required. Therefore, this plant species could be an excellent natural source for the treatment of typhoid fever and a potential target for the activity-guided isolation of its active constituents in order to explore the mechanism of this activity and potentially relevant uses.

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