REPORT

Antibacterial and antifungal activities of leaf extract of *Achyranthes aspera* (Amaranthaceae) from Pakistan

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Abstract: Alcoholic extract and various fractions of *Achyranthes aspera* leaves, traditionally used in Pakistan for treatment of infectious diseases was screened for in vitro antibacterial and antifungal activity. The chloroform and butanol fractions were found to be the most active among the fractions, showing considerable antibacterial activity against *Shigella flexneri* and *Escherichia coli*. The highest activity was found in the ethylacetate fraction (17 mm zone of inhibition) against gram-negative (*Salmonella typhi*) bacteria, with MIC value as 0.29 mg/mL. In antifungal screening, moderate activity was shown by the chloroform fraction (50 % inhibition) against *Microsporum canis*, with MIC value as 0.25mg/mL. Considerable level of antifungal activity was depicted by crude extract, hexane and butanol fractions against *Aspergillus flavus* and *Microsporum canis*. The ability of various extracts of *Achyranthes aspera* to inhibit different strains of fungi and bacteria indicates its potential use for the treatment of microbial infections.

Keywords: Antibacterial, antifungal activities, *Achyranthes aspera.*

INTRODUCTION

In the last few years, microbial resistance has developed due to irrational use of existing antibiotics in the treatment of infectious diseases (Saeed *et al*., 2006). Wound infections dermatitis, tuberculosis, pneumonia, gonorrhea, urinary tract and various fungal infections are now becoming more resistant to currently available antimicrobial therapy (Munazir *et al*., 2012). According to literature study, about 70 percent of the bacteria are resistant to the commonly prescribed antibiotics (CDC, 2005). Some microbes are resistant to all available antibiotics and cause serious infections in human beings. Intestinal infections, for example, are the most common cause of diarrhea, which is a leading cause of death worldwide. Emergence of resistance to multiple antimicrobial agents in pathogenic bacteria has become a significant public health threat as there are increasingly fewer, or even no effective antimicrobial agents available for infections caused by these bacteria. Gram-positive and gram-negative bacteria are both affected by the emergence and rise of antimicrobial resistance. Infections with multidrug and extensively drug-resistant (MDRs and XDRs) can lead to inadequate or delayed antimicrobial therapy, and are associated with poor patient outcomes. This alarming situation needs to search for new plant based antimicrobial drugs. In developing countries, like Pakistan above 50% of the population rely on traditional medicine for their primary health care (Fazli *et al*., 2012). Although plant extracts have a great potential to combat microbial infections but only 5% of the plant species have been screened so far as potential medicine (Munazir *et al*., 2012).

A. aspera commonly known as Prickly-chaff flower is an erect herb with woody roots and stiff unbranched stem found throughout Pakistan and India. The plant contains saponin, betaine and achyranthine as the principal alkaloids. The presence of ecdysterone is also reported (Chakrabarti and Vasudeva, 2006). In traditional medicine the whole plant is reported to be used as an abortifacient (Vasudeva and Sharma, 2006), anti-inflammatory, antirheumatic (Sharma, 2003), leprosy (Gokhale *et al*., 2002) and in treating renal (Rao and Chakrabarti, 2005), skin (Tahiliani and Kar, 2000), and lungs (Gokhale *et al*., 2002) infections. Literature review showed that hot water extract of the plant is used for the treatment of arthritic pain, while the dried leaf powder mixed with honey is useful in the early stages of asthma.

The present study was conducted to evaluate antibacterial and antifungal activities of leaf extract and various fractions of this plant against selected strains of bacterial and fungal pathogens in order to detect new sources of antimicrobial agents.

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MATERIAL AND METHODS

Plant material, preparation of crude extract and fractionation

Leaves of *A. aspera* were collected in Charsadda (Peshawar division), Khyber Pakhtunkhwa, Pakistan. Identification of the plant was done at the department of botany, university of Peshawar by Prof. Dr. Muhammad Ibrar. Voucher specimen 8708-1 (BOT), was deposited in the herbarium of the same department. Air dried, powdered plant material was extracted, using methanol at room temperature for three consecutive days. After filtration the extract was then dried under vacuo at about 40°C using rotary evaporator, until 25g of the crude extract was obtained. Different extracts were then prepared using both polar and non-polar organic solvents (chloroform, *n*-hexane, *n*-butanol, ethyl acetate and water).

Microorganisms

Bacteria, *Salmonella typhi* ATCC 19430, *Shigella flexneri* clinical isolate, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Bacillus subtilus* ATCC 6633 and fungi, *Candida albicans* ATCC 2091, *Trichophyton longifusis* (Clinical isolate), *Aspergillus flavus* ATCC 32611, *Candida glabarata* ATCC 90030, *Microsporum canis* ATCC 11622 and *Fusarium solani* ATCC 11712 were used for the assay.

Antibacterial activity

The effect of different extracts on selected bacterial strains was assayed by 'agar well diffusion method' using a cell suspension (1.5×10^6 CFU/mL), following 'Macfarland turbidity standard No. 0.5'.The Mueller-Hinton agar (MHA) plates (8 mm thick) were seeded with standardized inoculum (0.1ml) of each selected bacterial strains. Uniform wells of about 6 mm diameter were then made**,** in the agar. Plant extracts, standard drug(s) or DMSO (100µL) was filled in the wells. The inoculated plates were incubated at 37±1°C for 24 hours. Zone of inhibition for each extract was then measured and expressed in millimetre (mm) (Saeed and Perween, 2007).

Antifungal activity

Antifungal activity was performed using agar diffusion method. Sabouraud dextrose agar was loaded with test samples (400µg/ml, DMSO) in test tubes. After solidification of media in slanting position, it was inoculated with selected fungal strains, followed by incubation at 29 ^{0}C for 3-7 days. Amphotericin-B, used as standard drug for *A. flavus*, while Miconazole was used as standard for rest of the selected fungal strains. Linear growth inhibition was measured in millimeters (mm), while percent inhibition of growth was calculated with reference to negative controls using formula (Paxton, 1991).

$$\% \text{ Inhibition} = 100 - \frac{\text{Linear growth in test (mm)}}{\text{Linear growth in control (mm)}} \times 100$$

MIC determination using macro dilution method

Crude methanolic extract and their subsequent fractions were dissolved in DMSO, followed by serial dilution with sterile water (to get various dilutions in the range of 0.1 – 300 mg/mL) in microplates, under laminar flow condition. Test growing cultures (1×10^4 CFU) were then added to different wells and incubated for 72 hrs in case of fungi and 24 hrs for bacteria at 37^{0}C. Tetrazolium violet was added to each well and the growth was indicated with the appearance of violet color of the culture. MIC values were then calculated by taking the lowest concentration of the test solution that caused growth inhibition. Amphotericin B, miconazole and Imipenem were used as standard while DMSO was used as a negative control (Atta-ur-Rehman *et al*., 2001).

RESULTS

To determine antifungal and antibacterial activities of different extracts of plant leaves of *A. aspera* a total of 12 isolates belonging to different strains of fungi and bacteria were used in the present study. The antibacterial and antifungal MIC values of *A. aspera* leaf extract are presented in tables 1 and 2, respectively. The MIC values for bacterial strains ranges from 0.29 to 250 mg/mL (table 1), while for fungal strains the range was 0.25 to 200 mg/mL (table 2). The results showed that ethylacetate fraction exhibited the highest antibacterial activity against *S. typhi* with 17 mm mean zone of inhibition, while the MIC value was 0.29 mg/mL. This was followed by chloroform fraction which showed 16 mm zone of inhibition against *S. flexneri* with MIC value as 0.29 mg/mL. *E. coli* and *S. flexneri* remained the most susceptible bacterial strains with MIC values ranges from 0.43 to 0.50 mg/mL and 0.29 to 0.37 mg/mL, respectively. The MIC values in butanol extract were 0.50 and 0.37 mg/mL against *E. coli* and *S. flexneri*, respectively. *P. aeruginosa*, *B. subtilus* and *S. aureus* showed no susceptibility to all tested extracts.

The results of antifungal assay are presented in table 2.The chloroform extract of *A. aspera* displayed the best activity against *M. canis* with an MIC value of 0.25mg/mL. Crude methanolic extract and *n*-hexane fraction depicted similar percent growth inhibition (40%) against *A. flavus.* Among the fungal strains, *F. solani*, *C. glabarata*, *T. longifusis* and *C. albicans* were found resistant against all extracts screened.

DISCUSSION

The selection of *A. aspera* for the present study was based on their traditional use to treat infectious diseases (bacterial & fungal) like dysentery, skin and urinary tract

Table 1: Antibacterial activity of crude extract and various fractions

Bacteria	MIC[a] (mg/mL)							Zone of Inhibition (mm)						
	Cr	Ch	*He*	*Bu*	Et	Aq	St	Cr	Ch	He	Bu	Et	Aq	St
E. coli	>250	0.43	200	0.50	50	200	0.0002	-	13	-	12	-	-	32
S. flexneri	200	0.29	50	0.37	>250	>250	0.0015	-	16	-	14	-	-	34
P. aeruginosa	>250	>250	>250	>250	150	>250	0.0028	-	-	-	-	-	-	30
S. typhi	50	50	150	50	0.29	150	0.0007	-	-	-	-	17	-	36
B. subtilus	>250	>250	>250	200	>250	>250	0.0003	-	-	-	-	-		33
S. aureus	200	150	50	>250	200	>250	0.0004	-	-	-	-	-	-	40

Std. drug: Imipenem, [a]Minimum inhibitory concentration Cr: Crude, Ch: Chloroform, He: *n*-Hexane, Bu: *n*- Butanol, Et: Ethylacetate, Aq: Aqueous, St: Standard

Table 2: Antifungal activity of crude extract and various fractions

Fungi	MIC[a] (mg/mL)							Inhibition (%)						
	Cr	Ch	*He*	*Bu*	Et	Aq	St	Cr	Ch	He	Bu	Et	Aq	St
A. flavus	0.33	>250	0.37	>200	>200	>200	0.0002[1]	40	-	40	-	-	-	100
F. solani	50	100	>200	100	50	>200	0.0015[2]	-	-	-	-	-	-	100
M. canis	>200	0.25	150	0.35	>200	100	0.0001[2]	-	50	-	40	-	-	100
C. glabarata	>200	50	50	>200	150	>200	0.0023[2]	-	-	-	-	-	-	100
T. longifusis	100	>200	>200	50	>200	>200	0.0008[2]	-	-	-	-	-	-	100
C. albicans	>200	150	150	100	>200	50	0.0024[2]	-	-	-	-	-	-	100

[1]Std drug: Amphotericin B, [2]Std drug: Miconazole [a]Minimum inhibitory concentration

infections, enteric fever and venereal diseases. *E. coli* is mainly responsible for causing urinary tract infections in humans (Russo and Johnson, 2003; Pena *et al*., 2008), while *S. flexneri* is responsible for causing Traveler's diarrhea (Mukhopadhaya *et al.,* 2003; Uyttendaele *et al*., 2001). Due to lake of appropriate health facilities, these diseases are commonly treated with local herbs. Our results against the two microbes showed that *A. aspera* leaves have some potential compounds that could provide a scientific base for the treatment of stated infections by the herbal practitioners (Hakims).

A. aspera is also used in treatment of skin and lungs diseases, since *M. canis* is a common dermatophyte (Alteras and Feuerman, 1981; King *et al*., 1996) and *A. flavus* usually affect lungs (Gokhale *et al*., 2002), results of the present study indicates association between traditional therapeutic use of *A. Aspera* and the *in vitro* antifungal screening. These results corroborate the importance of ethnobotanical surveys for screening plants as a potential source for bioactive compounds. Phytochemical screening of *A. aspera* shows the presence of various chemical substances such a phenolic compounds, oils, saponins, flavonoids, alkaloids and tannins (Priya *et al*., 2012). Flavonoids and many phenolic compounds act as antimicrobial agent against many pathogenic bacteria and fungi such as *S. aureus*, *P. aeruginosa, B. subtilus, E. coli, M. canis, A. flavus* and *F. solani* (Ehsan *et al*., 2011). In light of the above-mentioned facts, this preliminary study could result in discovery of novel antimicrobial drugs for the prompt and effective management of infectious diseases.

CONCLUSION

It can be concluded from the present data, that the leaves of *A. aspera* posses considerable antimicrobial activity. Since chloroform and butanol fraction shows promising antifungal activity against M. *canis,* which is a common dermatophyte therefore, further investigation is needed to isolate the specific compound and to make a safe and more effective topical dosage form.

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