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VALIDATION OF SOME OF THE ETHNOPHARMACOLOGICAL USES OF *XANTHIUM STRUMARIUM* AND *DUCHESNEA INDICA*

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Abstract

The antimicrobial potential of alcoholic extract and various fractions of *Xanthium strumarium* (XS) and *Duchesnea indica* (DI) against different strains of bacteria and fungi was investigated. The chloroform fraction from *Xanthium strumarium* was found to be the most active among the fractions, showing good activity against *Escherichia coli, Shigella flexneri, Bacillus subtilus* and *Staphylococcus aureus.* Interestingly, most of the activity detected was against gram-positive (*Staphylococcus aureus*) bacteria. The chloroform fraction exhibited significant antibacterial activity (19 mm zone of inhibition) against gram negative (*Shigella flexneri*), bacteria. Promising level of antifungal activity was observed in all fractions against *Aspergillus flavus, Fusarium solani* and *Microsporum canis.* Looking to these results it may be concluded that *Xanthium strumarium* and *Duchesnea indica* can be a potential source for activity guided isolation of lead compounds with antimicrobial properties.

Introduction

Duchesnea indica (Rosaceae), a perennial, trifoliate creeping herb commonly occurs on shady, moist grassy slopes (up to 2400 meters) in Pakistan, India, and China (Qiao *et al.,* 2009). *D. indica* contain flavonoids, tri-terpenes and sterols as the major constituents (Peng *et al.,* 2008). In folk medicine, it has been used for treatment of cough, wound healing, throat swelling, pharyngitis, pyelitis and diarrhea (Zuoa *et al.,* 2008; Qiao *et al.,* 2009; Lee *et al.,* 2008). The anticancer and anti-inflammatory properties of *D.indica* have also been reported (Choudhary *et al.,* 1995; Purohit & Vyas 2004; Sharma, 2003; Peng *et al.,* 2008).

Xanthium strumarium (Compositae), an annual herb with stout, short and hairy stem, is mainly distributed in china and Europe. The principal compounds isolated from *X.strumarium* include xanthanol, isoxanthanol, hydroquinone, caffeyolquinic acids, alkaloids, and thiazinedione (Han *et al*., 2007; Ying-Tsun *et al*., 1998). In traditional medicine, *X. strumarium* has been used for urticaria, headache, sinusitis, arthritis and emphysema (Qin *et al*., 2006; Han *et al*., 2007; Yoon *et al*., 2003). All parts of plant possess sedative, diaphoretic and diuretic properties. The plant also shows its efficacy in mitigating longstanding cases of malarial fever (Sharma, 2003). The genus *Xanthium* also possess, antibacterial (Talakal, *et al*., 1995), antiviral (Tsankova *et al*., 1994), antimalarial (Joshi *et al*., 1997), fungicidal (Ginesta-Peris *et al*., 1994), insecticidal, (Kamboj & Saluja 2010), and cytotoxic activities against cancer cell lines (Kinghorn *et al*., 1999). Commercially the plant is used in yellow dye manufacturing (Shah *et al*., 2010).

The use of *D. indica* and *X. strumarium* for treatment of various ailments has been reported in folk medicine. The present paper has elucidated antimicrobial activities of the ethanolic extract and its fractions obtained from these plants in order to validate their ethnopharmacological uses.

Materials and Methods

Plant material, preparation of crude extract and fractionation: Leaves of *X. strumarium* (XS) were collected from Charsadda (Peshawar Division), while roots of *D. indica* (DI) were collected from Bara Gali (Hazara Division), Khyber Pakhtunkhwa, Pakistan. Voucher specimens bearing No: 8708-BOT and 10708-BOT identified by Prof. Dr. Muhammad Ibrar, Department of Botany, University of Peshawar and specimens x were deposited in the herbarium of the same department.

The shade dried and powdered plant material (1 kg each plant) was extracted using methanol at room temperature for three days. After filtration the dark green extract was concentrated to dryness under vacuum at low temperature (40°C) using rotary evaporator, until 25g of the crude extract of each plant was obtained. The extracts were then dissolved in distilled water and sequentially partitioned with various solvents to obtained n-hexane, chloroform, ethyl acetate, n-butanol and aqueous fractions. Crude extract and its fractions were then screened for antibacterial and antifungal activities.

Antibacterial activity: The antibacterial activity was determined by the 'agar-well diffusion method' using a cell suspension of about 1.5 × 106 CFU/mL obtained following 'Macfarland turbidity standard No. 0.5'. Standardization of the suspension concentration was achieved by adjusting the 'optical density' to 0.1 at 600nm (Shimadzu, UV-VIS Spectrophotometer). Holes of 6 mm diameter were then made on the MHA plate (6mm thick) and filled with 100 µL of methanolic extract, fractions or standard drug(s), followed by incubation at 37 ± 1°C for 24 hrs. Zone of growth inhibition around the hole was then measured to evaluate antimicrobial activity. The procedure was repeated in triplicate and the mean diameter was recorded (Nisar *et al*., 2010).

Antifungal activity: Antifungal activity was determined by the agar diffusion method. Test samples (400 µg/ml, DMSO) were diluted in Sabouraud dextrose agar followed by solidification in slanting positions. Test fungal cultures were inoculated on the slant and were incubated at 29°C for 3-7 days. Test tubes were observed for linear growth inhibition of fungi in mm upon completion of the incubation period. Percentage inhibition was calculated with reference to negative and positive controls by applying the formula (Paxton, 1991; Nisar *et al*., 2011).

$$\% \text{ Inhibition of fungal growth} = 100 - \frac{\text{Linear growth in test}}{\text{Linear growth in control}} \times 100$$

Results and Discussions

Most extracts obtained from roots of *Duchesnea indica* showed activity against different strains of bacteria used in assay. Chloroform and ethylacetate fractions revealed highest antibacterial activity (14 mm zone of inhibition each) against *Shigella flexneri* and *Staphylococcus aureus* (Table 1). The results support the ethnopharmacological uses of this plant and make it interesting for further evaluation. Among dermatophytes, the most susceptible strain was *Microsporum canis* which showed 50% and 60% inhibition against crude extract and ethylacetate fraction, respectively, as compared to standard (Table 2). *Aspergillus flavus* was the second most susceptible fungi, exhibited 50% inhibition against *n*-hexane fraction. This study suggests the possibility of using these extracts as starting points for finding antifungal agents that selectively inhibit its growth, which can help in the treatment of dermatomycoses.

Table 1. Antibacterial activity of crude extract and various fractions of *Duchesnea indica*.

	Zone of inhibition (mm)						
Bacteria	**Std. drug**	**Crude**	**$CHCl_3$**	**n-Hexane**	**n-Butanol**	**EtOAc**	**H_2O**
E. coli	32	12	-	-	-	-	-
S. flexneri	34	-	14	-	-	-	-
P. aeruginosa	30	-	-	-	13	-	-
S. typhi	36	-	-	-	-	-	-
B. subtilus	33	-	-	-	-	-	-
S. aureus	40	-	-	13	-	14	-

Std. drug: Imipenem

Table 2. Antifungal activity of crude extract and various fractions of *Duchesnea indica*.

	Inhibition (%)						
Fungi	**Std. drug**	**Crude**	**$CHCl_3$**	**n-Hexane**	**n-Butanol**	**EtOAc**	**H_2O**
A. flavus	100[2]	-	-	50	-	-	40
F. solani	100[1]	40	-	-	-	-	-
M. canis	100[1]	50	-	30	-	60	-
C. glabarata	100[1]	-	-	-	-	-	-
T. longifusis	100[1]	-	-	-	-	-	-
C. albicans	100[2]	-	-	-	-	-	-

[1]Std drug: Miconazole, [2]Std. drug: Amphotericin B

In Western herbalism, *Xanthium strumarium* is used for treatment of yellow diarrhea, caused by various species of *Shigella*. Results obtained in this study indicate that chloroform fraction from *Xanthium strumarium* showed significant activity (19 mm zone of inhibition) against *Shigella flexneri*. The same fraction revealed good antibacterial activity against *Bacillus subtilus* and *Staphylococcus aureus*, the main causative agents for skin related disorders (Table 3).

Regarding antifungal activity the chloroform and *n*-hexane fractions from *Xanthium strumarium* exhibited 60% and 50% inhibition activity against the major dermatophyte fungi, *Microsporum canis* (Table 4). These results are quite interesting which authenticate the traditional use of this plant in the treatment of yellow diarrhea and skin related infections. However, it is important to isolate the specific compounds exhibiting antimicrobial activity, as well as, to establish the mechanism of action of the extract to come to a definite conclusion.

Table 3. Antibacterial activity of crude extract and various fractions of *Xanthium strumarium*.

	Zone of inhibition (mm)						
Bacteria	**Std. drug**	**Crude**	**$CHCI_3$**	**n-Hexane**	**n-Butanol**	**EtOAc**	**H_2O**
E. coli	32	-	15	-	-	-	-
S. flexneri	34	-	19	13	-	-	-
P. aeruginosa	30	-	-	-	-	-	-
S. typhi	36	-	-	-	-	-	-
B. subtilus	33	-	16	-	-	-	-
S. aureus	40	-	16	-	-	16	-

Std. drug: Imipenem

Table 4. Antifungal activity of crude extract and various fractions of *Xanthium strumarium*.

	Inhibition (%)						
Fungi	**Std. drug**	**Crude**	**$CHCl_3$**	**n-Hexane**	**n-Butanol**	**EtOAc**	**H_2O**
A. flavus	100[2]	-	-	-	-	-	-
F. solani	100[1]	40	30	30	-	20	-
M. canis	100[1]	-	60	50	-	-	30
C. glabarata	100[1]	-	-	-	-	-	-
T. longifusis	100[1]	-	-	-	-	-	-
C. albicans	100[2]	-	-	-	-	-	-

[1]Std drug: Miconazole, [2]Std. drug: Amphotericin B

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