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Studies on Antifungal Activity and Elemental Composition of the Medicinal Plant *Trianthema pentendra* Linn.

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Abstract. Antifungal activity of crude solvent and aqueous extracts of the medicinal plant, *Trianthema pentendra* Linn., against the dermatophytic fungi, *Aspergillus niger*, *Aspergillus flavus*, *Paecilomyces varioti*, *Microsporum gypseum and Trichophyton rubrum* revealed that ethanol and aqueous extracts were the most effective antifungal agents as compared to methanol, chloroform and ethyl acetate extracts. Some basic elements, Al, Ca, Cu, Fe, Mg, Mn, P, S and Zn were also determined in the medicinal plant, *T. pentendra*, using atomic absorption spectrophotometry and U.V spectrophotometry. *T. pentendra* contained considerable amount of elements which have therapeutic effects in skin diseases.

Keywords: *Trianthema pentendra*, antifungal activity, essential elements

Introduction

Plants are the best source of active secondary metabolites which are beneficial to mankind. Many plant origin drugs have been reported with biological properties like analgesic, anti-inflammatory, antioxidant, hypoglycemic and antifungal agents (Sindhu, 2009). Skin diseases, diarrhoea, diabetes, malaria, respiratory infection, fungal and bacterial infections are the common health problems in developing countries and numerous medicinal plants are used traditionally which are remedial against these diseases (Pinn, 2000).

Trianthema pentendra Linn., commonly know as waho, is a traditional medicinal plant which is utilized in many parts of Pakistan for the treatment of various fungal skin diseases like tinea capitis, tinea pedis, tinea manuum and tinea corporis etc. The root of plant is irritant and cathartic. Leaves of the plant are used as astringent and abortifacient and as remedy in abdominal diseases and bladder pain, for snake bite etc. (Baquar, 1989; Shahani and Memon, 1988; Kirtikar and Basu, 1935).

Elements play essential role in the maintenance of the skin health. Aluminum acetate solution, copper sulphate and zinc lotions are used as skin disinfectant, cleansing agents, antiseptic and soothing and cooling agents. Calcium, magnesium and manganese are used in the formation of the collagen and connective tissue. Phosphorus and sulphur are used for the treatment of scabies and leprosy. (Sahito *et al*., 2003; Soderberge and Halimans, 1982; Underwood, 1981). Skin diseases are usually caused by fungi and are one of the main problems of Sindh province. The present paper describes the antifungal potential of different solvent extracts of *T. pentendra* and is also its elemental study.

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Materials and Methods

Plant material. The leaves and shoots of *T. pentendra* were collected from different areas of Kohistan regions, District Dadu and reference sample was identified by referring to Flora of Pakistan (Nasir and Ali, 1990). The collected plant materials were washed with distilled water and placed in shade at room temperature for two weeks. One kg of dried plant material was dipped in five litre ethanol in a bottle for 20 days for cold percolation. The extract was filtered and concentrated under reduced pressure below 40 °C using rotary evaporator. The residue was completely dried and from it five different extracts *viz*., ethanol, ethylacetate, chloroform, methanol and aqueous extracts were prepared using separating funnel. The extracts were left at room temperature, so that the solvents were completely evaporated and organic compounds remained in dry form. The extracts so obtained were mixed with the sterilize water (1 g, 5 ml) and each extract sample was applied for antifungal activity.

Collection of dermatophytes. The dermatophytic fungi namely: *Aspergillus niger, Aspergillus flavus, Paecilomyces varioti, Microsporum gypseum, Trichophyton rubrum* were scraped from the skin of different body parts at out patient departments of Liaquat University Hospital, Jamshoro and Hyderabad.

Treatment of different solvent extracts. The human skin pathogens were treated with different extracts and results were taken after 72 h at 30 °C. The percentage of mycelial

inhibition was calculated as follows (Ali Shtayeh and Abu Ghdeib, 1999, Usmanghani and Shameel, 1986).

$$\text{Mycelial inhibition (\%)} = [(dc\text{-}d1)/dc] \times 100$$

where:

dc = colony diameter in control
d1 = colony diameter in treatment

Methodology for element determination. A suitable dissolution method for biological sample to yield homogenous solution is the crucial first step in elemental determination with atomic absorption spectrophotometric and UV techniques. The decomposition of organic matter must be completed to avoid interference by organic residue. Samples were digested with nitric acid: hydrogen peroxide (30%), for determination of mineral elements. Appropriate working standard solution of aluminum (Al), calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), phosphorus (P), sulphur (S) and zinc (Zn) were prepared from stock standard solution (1000 ppm), in 2 N nitric acid. Calibration curves were drawn for each element using atomic absorption spectrophotometer (Hitachi, model 180-50) and UV-spectrophotometer. The calibration curves obtained for concentration *vs*., absorbance data were statistically analyzed using fitting of straight line by least square method. A blank reading was also taken and necessary correction was made during the calculation of percentage concentration of various elements. The efficiency of extraction method was checked by standard addition method. The sample was spiked with known standards and digested with nitric acid and hydrogen peroxide mixture. The matrix of the standard and the sample solution was the same. The percentage recovery test for different elements by the digestion method adopted was 98.5-99% in range.

Results and Discussion

All the crude extracts had significant antifungal activities against most of the fungi, but the activity of inhibition varied for the fungi with respect to the type of plant extract (Table 1).

Ethanol extract. The highest inhibition was observed against *T. rubrum*, *A. niger* and *A. flavus* being 100%, 95% and 95.2%, respectively, while moderate inhibition activity against *P. varioti* (72.73%) and minimum inhibition activity against *M. gypseum* (56.67%) was recorded.

Ethyl acetate extract. The highest inhibition was observed against *P. varioti* and *M. gypseum*, 54.55% and 50%, respectively while moderate inhibition of *A. niger* (45%) and minimum inhibition of *A. flavus* and *T. rubrum* (42.86% and 40%, respectively) was recorded.

Chloroform extract. The highest inhibition was observed against *P. varioti* 72.73% while moderate inhibition against *A. niger*, *T. rubrum* and *A. flavus* being 62.5%, 60% and 57.15%, respectively, and minimum inhibition activity against *M. gypseum* (50%) was noticed.

Methanol extract. The highest inhibition was observed against *P. varioti* 63.64%, while moderate inhibition against *T. rubrum*, *M. gypseum* and *A. niger* (60%, 50% and 50%, respectively) and minimum inhibition against *A. flavus* (42.86%) was determined.

Aqueous extract. The inhibition observed against *T. rubrum* and *A. niger* was 92% and 87.5%, respectively, while

Table 1. Antifungal activity of solvent extracts of *T. pentendra* Linn.

	Colony diameter (mm)*				
	Aspergillus niger	*Aspergillus flavus*	*Paecilomyces varioti*	*Microsporum gypseum*	*Trichophyton rubrum*
Control	40	35	55	30	25
Ethanol extract	02	05	15	13	00
Inhibition (%)	95	85.72	72.73	56.67	100
Methanol extract	20	20	20	15	10
Inhibition (%)	50	42.86	63.64	50	60
Chloroform extract	15	16	15	15	10
Inhibition (%)	62.5	57.15	72.73	50	60
Ethyl acetate extract	22	20	25	15	15
Inhibition (%)	45	42.86	54.55	50	40
Aqueous extract	05	14	15	15	02
Inhibition (%)	87.50	60	72.73	50	92

* = colony diameter readings taken at 30 °C after 72 h.

moderate inhibition activity against *P. varioti* and *A. flavus* (72.73% and 60%, respectively) and minimum inhibition against *M. gypseum* (50%) was measured.

Elements. Considerable amounts of various elements were found in the medicinal plant *T. pentendra* such as aluminum, calcium, copper, iron, magnesium, manganese, phosphorus, sulphur and zinc (Table 2). These elements are biologically very important in the treatment of different skin diseases.

Table 2. Quantity of different elements in *T. pentendra* Linn.

Elements	Amount (mg/kg)
Aluminum	6.93-7.95
Calcium	6491.09-7603.85
Copper	12.18-12.89
Iron	156.31-174.43
Magnesium	5722.16-6015.41
Manganese	67.70-81.00
Phosphorous	87.94-104.96
Sulphur	213.66-233.85
Zinc	53.64-66.91

In the study, it was observed that all the crude extracts showed significant antifungal activity against most of the fungi, but ethanol and aqueous extract had comparatively maximum inhibition activity being 50% and 100%, respectively. In comparison, methanol, ethyl acetate and chloroform extracts had inhibition activity in the range of 42-72% against test dermatophytes. Although many scientists (Pirzada *et al*., 2007; Bajwa *et al*., 2006; Anjum and Khan, 2003; Natarjan *et al*., 2003; Ficker *et al*., 2003; Adedotum and Okoli, 2002; Thebo and Abro, 2000; Sakharkar and Patil, 1999; Skaikh *et al*., 1990; Usmanghani and Shameel, 1986), had screened the antifungal activity of medicinal plants against dermatophytes, but in this study, first attempt was made to investigate the antifungal activity of medicinal plant *T. pentendra* against dermatophytic fungi *viz*., *Aspergillus flavus*, *A. niger*, *M. gypseum*, *P. varioti*, *T. rubrum* which cause different skin diseases like tinea capitis, tinea pedis, tinea manuum and tinea corporis.

Furthermore, some basic elements *viz*., aluminum, calcium, copper, iron, magnesium, manganese, phosphorous, sulphar and zinc were found in variable range in the medicinal plant *T. pentendra*. But the concentration levels of the elements sulphur and zinc were found to be sufficient in the range of (213.66-233.85) and (53.64-66.91) mg/kg, respectively. All these elements play essential role in the treatment of skin diseases (Saily *et al*., 1994; Janjua, 1990).

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