

ANTIFUNGAL ACTIVITY OF TAMARIX DIOICA — AN IN VITRO STUDY

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ABSTRACT

Objective: To investigate the antifungal activity of crude extract of leaves of Tamarix dioica tree.

Material and Methods: Antifungal activity of crude extract of T. dioica leaves was studied. The extract was dissolved in Dimethyl Sulfoxide, then six strains of fungi were subjected to crude extract separately by agar tube dilution method. Inhibitory effect of extract in millimeters was determined.

Results: Crude extract possess significant activity against A. flavis and M. canis while moderate activity against F. solani.

Conclusion: From this study it is concluded that T. dioica can be used topically as an antifungal agent.

Key words: Tamarix dioica, antifungal activity, plant extract.

INTRODUCTION

Plants are among the most important and common sources of potentially valuable new drugs. Therefore, there is a need to investigate the biological properties of medicinal plants in order to develop new drugs. Much work has been carried out on herbal treatment of various diseases but there is need to work more^{2,3}

The main objective of this study was to search for medicinal plants with strong antimicrobial activity. In this study we report the antifungal activity of Tamarix dioica plant.

Tamarix dioica is an evergreen tree found in N.W.F.P, Punjab, Balochistan and Sindh provinces of Pakistan.

Family	Tamariceae
English name	Tamarix
Urdu name	Jhao, Tarfa
Local names	Ghaz, Khagal
Parts used	Bark, leaves and twigs are used as herbal medicines

As a herbal medicine it is used as a carminative, diuretic and for the treatment of hepatic and splenic inflammation.

MATERIAL AND METHODS

Collection and preparation of plant material: Fresh leaves of the plant were collected and air-dried. Then ground with a grinder to a powder, the powdered material was 2 kg. Crude extract of air-dried powder was subjected to cold extraction

with 96% ethanol for 8 days and then filtered through filter paper. The ethanol extract was concentrated in a vacuo 40°C rotary evaporator, and air-dried at room temperature to give a final yield of 105 gm.^{10,15}

In vitro Bioassay studies: Cytotoxicity test

The Brine shrimps (*Artemia salina*) larvae are the most commonly used as a rapid and simple preliminary method for screening of biological activity of drugs as a cytotoxic bioassay. Shrimps eggs were sprinkled in one portion of the tray (22× 32×6), which was half filled with filtered brine solution and covered with aluminum foil. After incubation for two days at 28 ± 1°C the hatched shrimps larvae were harvested. Twenty milligram plant extract was dissolved in 2 ml methanol and from this solution 500 µl, 50 µl and 5 µl aliquots were transferred to suitable vials.

Three replicates of each concentration were prepared. Vials were kept for evaporation of organic solvent. Ten larvae of *Artemia salina* were deposited in each vial using pasteur pipette and volume were made upto 5ml by adding sea water which was incubated at 27 ± 1°C for 24 hours under illumination.

After 24 hours, the numbers of surviving larvae were counted using a colony counter and magnifying lens. Vials containing equal volumes of solvent (without extracts) and vials with reference cytotoxic drugs served as a negative and positive control respectively.

Amphotericin B and miconazole were used as reference cytotoxic drugs. Data was analysed

by using Finney computer programme to determine LD50 values with 95% confidence interval.²⁰

Antifungal Bioassay

For antifungal bioassay, agar tube dilution method was used. In the preliminary stage of antifungal bioassay, crude extract of the plant was studied against the following fungi:

- Trichophyton longifusus
- Candida glaberata
- Fusarium solani
- Microsporum canis
- Aspergillus flavus
- Candida albicans

Methodology: Assays measuring inhibition of mycelial growth on agar media were used. Twenty four mg of crude extract was dissolved in 1ml sterile Dimethyl sulfoxide (DMSO) serving as a stock solution. Then transferred 4 ml Sabouraud dextrose agar (SDA) growth media in each screw capped tube, under sterile condition and autoclaved at 121°C for 15 minutes. These tubes were allowed to cool to 50°C and non solidified SDA of each tube was loaded with 66.6 µl of crude drug solution. Tubes then allowed solidifying at room temperature. Then each glass tube was inoculated with 4 mm diameter piece of inoculum removed from 7 days old culture of fungus. In case of non- mycelial growth an agar streak was employed. Other media supplemented with DMSO and reference antifungal drugs used as a negative and positive control respectively.^{9, 11}

Incubation

All these tubes were incubated at 28±1°C for 10 days. A relative humidity was maintained of 40-

50% in the incubation room. Cultures were examined twice weekly during incubation. Growth in the media was determined by measuring linear growth (mm) in the sample, as well as negative control and reference drug, and then percentage inhibition of fungal growth was determined.^{9,13}

RESULTS AND DISCUSSION

Table 1 shows the results of bioactive compounds of the crude extract of Tamarix dioica, subjected to brine shrimps, As is observed in the table that with a dose of 1000ug/ ml the number of dead larvae is only 03 out of 30 shrimps while with a dose of 100ug/ml and 10ug/ml the number of dead larvae is zero. LD50 was determined by Finney computer programme, which shows no lethality.

For antifungal activity the crude extract was subjected to six fungi as shown in Table 2 and two types of standard drugs were used in this study. From the table the linear growth (mm) of the sample and control as well as there inhibition % age shows that inhibition of crude extract against Candida albicans and Candida glaberata is zero percent, while % age inhibition against Trichophyton longifusus, Fusarium solani, Aspergillus flavus, and Microsporum canis is 10, 60 ,70 and 85% respectively. Thus sample shows significant activity against A. flavis and M. canis while moderate activity against F. Solani.

CONCLUSION

From the present study it is concluded that the crude extract of Tamarix dioica tree is significantly effective against two micro organisms (M. canis and A. flavis) and moderately effective against one micro organism (F. solani). Further studies are required to confirm its effectiveness as a topical antifungal agent.

Table 1: Cytotoxic activity of Tamarix dioica (crude extract).

Dose in µl/ml	No of Shrimps	Vial No	No of Survivors	No of deaths	LD50 µg/ml	Upper toxic concentration µg/ml	Lower toxic concentration µg/ml
1000	30	1	09	03	No Lethality	-----	-----
		2	09				
		3	09				
100	30	1	10	0			
		2	10				
		3	10				
10	30	1	10	0			
		2	10				
		3	10				

Table 2: Antifungal activity of Tamarix dioica crude extract.

Name of fungus	Linear growth (mm)		%age Inhibition	Std. Drugs	M I C µg/ml
	Sample	Control			
Trichophyton longifusus	90	100	10	Miconazole	70
Candida albicans	100	100	–	Miconazole	110.8
Aspergillus flavus	30	100	70	Amphotericin B	20
Microsporum canis	15	100	85	Miconazole	98.4
Fusarium solani	40	100	60	Miconazole	78.25
Candida glaberata	100	100	–	Miconazole	110.8

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