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ANTIMICROBIAL EFFICACY OF TAMARIX DIOCA (L.) LEAVES AND FLOWERS

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ABSTRACT

Tamarix dioca (L.) belongs to family Tamaricaceae is traditionally a vital plant used for treatment of various diseases. Efforts were made to investigate the antimicrobial efficiency of T. dioca.There was three concentrations of crude methanolic extracts of 200µg/ml, 100µg/ml and 50 µg/ml were checked against six pathogenic fungi Aspergillus fumigatus, aspergillus flavus, Fusarium solani, Aspergillus niger, Penicillium digitatum and Penicillium tubesulum and six bacterial strains Bacillus subtilis, Enterobactor aerogenes, Escherichia coli, Kliebsiella pneumoniae, Salmonella typhi and Staphylococcus aureus. It was noted that Percentage inhibition in the growth of fungi and bacteria was dosage dependent. Terbinafine a standard antifungal drug, 10mg/ml and Cefexime 10mg/ml (antibacterial) were used as a positive control. The results were compared with control and most of the results were found significant. Maximum inhibition was showed by T. dioca against fungal strain A. niger (74%) and bacterial strain K. pneumoniae (48%). It can be use as powerful antimicrobial agent in near fture.

KEYWORDS

T. dioca, Methanol extract, antifungal, antibacterial efficacy

1. INTRODUCTION

T. dioica belongs to family, Tamaricaceae and is commonly known as Ghaz (Pashto) or Khagal and Tamarix (Eng). It is an evergreen tree, with green slack twiglets and thin leaves. The leaves are minute, tips subjugated greenish with whitish border [1]. The flowers are androgynous, purplish-blue or pinkish and strongly attached, spikes, 2-4.3 cm long. Stamens are six. Fruit capsular. They are growing up to 8 meters in height and making an impenetrable shade. They are highly salt tolerating plants and therefore they are also called salt cedars [2]. It is well adapted to alkaline environment [3]. The T. dioica has main chemical constituents are Tamarecine, Isoferulic acid, Kampferol and Quercetin. It is conventionally used from early time as antimicrobial mediator [4]. It is grown as decorative plant. T. dioica is used for domestic needs like fire, furniture and construction. It is a potential agro-forestry plant species [5]. The present research work was conducted to assess the extract of T. dioca flowers and leaves against a few pathogenic bacteria and fungi.

2. MATERIALS AND METHODS

2.1 Plant Collection

T. dioica leaves and flowers were collected from Lakki Marwat, KPK, Pakistan. Professor Abdur-Rehman, Chairman, Botany Department, Govt. Post Graguate, College Bannu identified the plants species. The Tamarix dioica specimen (voucher No. 3684), was submitted to Herbarium of Hazara University.

2.2 Preparation of Plants Extracts

T. dioica powder two hundred (200 G) was dissolved in 1± 0.02 liter of (80%) methanol to obtain crude methanolic extract. The extract was kept for 72 hours at room temperature, filtered and then the extract was kept openly to evaporate the methanol. The extract was stored in refrigerator at 4oC for future study.

2.3 Antibacterial Assay

The microorganism used in this study was obtained from microbiology Department of Quaid Azam University Islamabad and selected mostly based on their significance as opportunistic human pathogens. For antibacterial assay, procedure of Mc Laughlin (1998) was used with some modification.

2.4 Solution Preparation

The solutions were made by dissolving 10mg/10ml crude methanolic extract in dimethyl sulfoxide (DMSO). The stock solution was further diluted up to 5µg/ml and 1µg/ml.

2.5 Media for Bacteria

The growing medium was set for antibacterial activity by dissolving 2 G of nutrient agar (Merck) in 100 ml of distal water (pH 7.0) and then sterilized in autoclave.

2.6 Mc. Farland Turbidity Standard

McFarland (0.5 BaSO4) was used to compare the turbidity of bacterial culture. The standard was obtained by addition of 0.5 ml of (0.048) MBaCl2 to 99.5ml of (0.36) ammonium sulphate. Barium sulfate (turbidity standard 4 to 6ml) was taken in test tube and compare with the turbidity of bacterial culture.

2.7 Microorganisms

Six bacterial strains were obtained from Quaid-e-Azam University, Islamabad, namely Enterobactor aerogenes (ATCC 13048), Bacillus subtilis (ATCC 6633), Kliebsiella pneumoniae (MTCC 618), Salmonella typhi (ATCC 0650) Escherichia coli (ATCC 15224) and Staphylococcus aureus (ATCC 6538).

2.8 Assay Procedure (Agar Diffusion Method)

The growing medium was prepared by addition of nutrient agar (Merck) 2.3G in 100 ml distil water (pH 7.0). The medium was sterilized at 121°C for 20 min. It was cooled up to 45 °C. By pouring 25 ml of nutrient agar in Petri plates (9 cm) were prepared and was to solidify. Now the inoculum was seeded on the marked petri plates by sterile cotton swabs in a criss- cross manner. Using (9mm) sterile cork borer five wells were made on each petri plates. By micropipette, 67.0µl of sample solutions was added in respective wells in which three were for sample solutions, one for negative control and one for positive control. A synthetic drug cefixime (10 mg/ml) was used for positive control. For negative control DMSO was used.

As a final point, the petri plates were kept at 37 °C for 24H. As a result, clear (inhibition) zones were noted around each well. DMSO alone (10 µl /ml) was used as a negative control by measuring the diameter of inhibition zone with the help of transparent scale antibacterial efficacy was calculated as a mean of three replicates.

2.9 Assay Procedure for antifungal activity

Microorganisms Used: Six fungal strains Aspergillus fumigatus (66), Aspergillus flavus (0064), Aspergillus niger (0198), Penicillium digitatum (0218), Fusarium solani (0300), and Penicillium tubesulum (128) were used for antifungal activity.

2.10 Medium for Antifungal Assay

SDA (Sabouraud dextrose agar) (Merk) was used to grow the fungi. The composition was peptone complex 10g /l, agar, 15g /l and glucose, 40g /l.

2.11 Solution Preparation

The Stoc solutions was prepared by dissolving 10 mg /10ml and then sub diluted to 5µg/ml and 01µg/ml in DMSO. Terbinafine (2 mg/ml) was used as a positive control. DMSO was used as a negative control.

2.12 Assay Procedure

By dissolving 6.5g /100 ml in distil water media for fungi was made. 10cm slants were made by pouring SDA in 10ml/ test tubes and closed it with cotton pluggs. For 20 min the tubes were autoclaved at 121 °C. Tubes were cooled up to 45 °C and non-solidified SDA was poured to test tubes up to 5 cm. Then 67 µl of each sample solution (10µg/ ml, 5µg/ml and 1µg/ml) were added to test tubes. The tubes were then solidified in slanting position at room temperature and thus, the slants of 10 cm were prepared.

Now the tubes were inoculated with 4mm diameter piece of inocula, taken from one-week old culture of fungus. Positive and negative control test tubes were also inoculated. All the test tubes were kept at 28 °C for 7 days. During the incubation period cultures were supervise twice weekly. By measuring the linear growth of fungus, Inhibition was calculated with respect to negative control. The average readings were taken in triplicate for each fungal species.

Inhibition in the growth of every fungus was computed according to the given formula:

$$(C\text{-}T/C)\ x100$$

Where, C is for linear growth in control tube (mm), T is the linear growth observed in experimental test tubes. At the end calculated data was evaluated statistically by SPSS 13.0.

3. RESULT AND DISCUSSION

The T. dioica was evaluated against six bacterial strains, Enterobactor aerogenes, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Bacillus subtilis, and Salmonella typhi. The present studies revealed the encouraging results. T. dioica proved maximum inhibition in K. pneumoniae (48.0%±0.03) followed by S. typhi (22.0%±0.07), E. coli (26.0%±0.03), S. aureus (18.0%±0.03) and E. aerogenes (13.0%±0.03). However, B. subtilis showed resistance. Our present results in accordance with a study, they have reported that T. dioica can be used as ant protozoans and antimicrobial [6]. From the ancient time T. dioica conventionally used as antimicrobial agent. In the present study aerial parts especially, leaves and flowers were used, which are rich with alkaloids against the pathogenic microorganisms Table 1 [7].

Table 1: In Vitro, Antibacterial activity of Tamarix dioica.

Plants extracts	Conc. (µg /ml)	%Zones of inhibition					
		S. Aureus	*K. Pneumonia*	*S. Typhi*	*E. aerogenes*	*B. subtilis*	*E. Coli*
DMSO	99.9%	_	_	_	_	_	_
Cefix.	10	35±0.08	41.4±0.02	45±0.04	25±0.05	100±0.05	26.±04
TDME	10	18±0.03	48 ±0.03	22±0.07	13±0.02	_	20±0.09
	05	11±0.05	12±0.09	10±0.02	8.0±0.00	_	11±0.02
	01	03±0.09	3.2±0.04	2.1±0.04	0.5±0.06	_	5.0±0.04

Mean ± SD: at P < 0.01, as compared to control

Key: TDME, Tamarix dioca methanolic extract. (-), Means No activity, Cefix, Cefixime.

The in-vitro antifungal activity of T. dioica was also momentous. T. dioica showed notable inhibition i.e. (74.11±2.03%) against A. niger as compared to positive control. T. dioica proved significant inhibition against A. niger (74.11%±0.03) followed by A. fumigatus (57.14%±0.06), A. flavus (30.77% ±0.04) and P. tubesulum (28.00%±0.04) but P. digitatum showed resistance and no inhibitory zones were detected.

Further moreover, T. dioica was very effective against fungi and bacteria in the present course of work. Previously, different workers have also reported the high antimicrobial capability of this plant [8, 9].

Table 2: In Vitro, Antifungal efficacy of Tamarix dioica.

Plant extract	Conc.(µg/ml)	% Zone of inhibition					
		A. flavus	*A. Fumigates*	*A. Niger*	*F. solani*	*P. Digitatum*	*P. tubesulum*
DMSO		_	_	_	_	_	_
TB	10	92.05±2.03	80±00±0.02	100.0±0.04	90.5±0.03	78.3±0.03	68.90±0.04
TDME	10	30.77±0.04	57.14±0.06	74.11±2.03	18.0±0.04	_	28.00±0.05
	05	16.62±0.05	34.57±0.02	53.00±3.09	05.7±0.08	_	16.67±0.06
	01	04.31±3.07	6.4.71±0.08	08.45±0.02	0.86±0.02	_	13.33±0.06

Mean ± SD: at P < 0.01, as compared to control

Key: TDME, Tamarix dioca methanolic extract.TB, Terbinafine. (-), Means No activity.

Interestingly, with respect to antimicrobial activity our results are in conformity with a study by different group researchers [10-13]. However, the results of present study concerning the antimicrobial assay, are analogous with [14-15]. The plant studied during the present course of work, can be used as powerful antimicrobial agents in future.

4. CONCLUSION

The authors conclude that Tamarix dioca leaves and flowers have a considerable antibacterial and antifungal efficiency against pathogenic microorganisms and can be used as a successful antimicrobial agent in the near future.

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