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In vitro Antimicrobial Study of *Tamarix aphylla* in View of Phytochemical Constituents

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ABSTRACT

Background and Objective: The present study was investigated to aim with the screening of selected medicinal plant to substantiate *in vitro* antimicrobial activity of methanolic extract of *Tamarix aphylla* bark against microbial strains and designed to provide scientific evidence for its use as a folk remedy in view of phytochemical constituents. **Materials and Methods:** Bacterial strains viz. *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and fungal strains including *Aspergillus flavus* and *Candida albicans* were tested using dilutions of 25, 50, 75 and 100 mg mL^{-1} of the stock solution prepared in dimethyl sulfoxide (DMSO) via disc diffusion assay. Inhibitory effect of extract in millimeters was determined by measuring the zone of growth inhibition surrounding the discs and compared with the standard drug. **Results:** Investigation revealed that extract of *Tamarix aphylla* bark was found to exhibit marked zone of inhibition at higher concentration in most of the bacterial and fungal strains examined. **Conclusion:** The present study suggests the presence of great potential of bioactive and phytochemical compounds rationalizing the use of this plant in primary health care.

Key words: Medicinal plant, antibacterial, antifungal activities, zone of inhibition

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INTRODUCTION

The fascination and use of natural products for the treatment of various ailments based on traditional practice from plant extracts have been used for centuries. Higher plants turn out hundreds to thousands of miscellaneous chemical compounds with different pharmacological and biological activities[1]. Plants are medicinally used in several countries and are a source of many potent drugs. The use of plant extracts in medicine, for example against microbial infections is still very widespread which rationale its uses in folk medicine[2]. *Tamarix* belonging to family *Tamaricaceae* with common names of Taramisk, Atheltrep, Salt cedar, ghaz, Athel and Athel pine is composed of 54 species with major center of distribution in Pakistan, Afghanistan, Iran, Turkmenistan, Southern Kazakhstan and Western China area[3]. Many preliminary studies on the use of *Tamarix* have been reported and proved to have important medicinal role for centuries. *Tamarix* species are ornamental bushes or trees with feathery foliage, mostly evergreen relatively long-lived that can tolerate a wide range of environmental conditions and resist a biotic stresses such as high temperature, salt and drought stresses. *Tamarix* prefer alluvial soil but grow well on saline and alkaline soil[4]. Literature revealed that most of the *Tamarix* species have enormous therapeutic potentials with fewer side effects as compared to synthetic drugs and so could be considered as good alternatives for these types of drugs. Amongst its therapeutic properties, most of *Tamarix* species are widely used in traditional practices as astringent, aperitif, stimulus of perspiration, diuretic, anthelmintic, antihaemorrhoid, antidiarrhoeal and gingivitis[5]. *Tamarix aphylla* is an astringent and tonic and is commonly used for the treatment of hepatitis, dysentery, eczema and skin diseases like capititis, syphilis and scaly skin conditions[6]. Its bark powder is used by the local people as a poultice on minor wounds[7]. *Tamarix gallica* has been reported to be useful in leucoderma, spleen trouble and eye diseases

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especially effective in fighting lung and esophageal cancers[8,9]. *Tamarix dioca*, an evergreen tree found in sandy areas of Pakistan is known to have potent anti oxidant and antimicrobial agent[10]. Keeping in view the importance of diverse medicinal flora of this plant species, the present study was conducted to investigate the antimicrobial activities of *Tamarix aphylla* along with the preliminary phytochemical investigation based on ethno botanical uses.

MATERIALS AND METHODS

Collection and extraction of *Tamarix aphylla*: Fresh plant sample of *Tamarix aphylla* was collected from District Kohat, (Khyber Pakhtunkhwa) Pakistan. Taxonomic identity of the plant was authenticated by the herbarium staff of Botany department, Kohat University of Science and Technology. The plant was cleaned from extra weeds and seeds and washed with distilled water; air-dried and chopped into fine homogenized powder in a grinder, passed through 0.5 mm mesh screen and were kept in clean polythene bags in the sterile environment of the laboratory[11]. The respective powdered plant part was soaked in 70% methanol (1 g 10 mL^{-1}) for at least 2-4 days at room temperature in a conical flask and kept on rotary shaker at for occasional stirring. The methanolic extract of powdered drug was filtered through whatman filter paper No. 42 and evaporated in a rotary flask apparatus under reduced pressure and temperature less than 42°C leaving behind brownish syrup residue and air dried again to get the powder form. The values of methanolic plant extract were analyzed according to previous method[12].

Antimicrobial assay: The antibacterial activity was performed against Gram-positive bacteria including *Bacillus subtilis* and *Staphylococcus aureus*, Gram-negative bacteria including *Escherichia coli* and *Salmonella typhi*. Antifungal activity against fungal strains using filamentous fungi *Aspergillus flavus* and unicellular fungi *Candida albicans* were investigated using disc diffusion assay. Kanamycin was used as a standard drug[13]. Reference microorganisms were streaked onto nutrient agar plates and the inoculated plates were incubated overnight at 37°C. Using a sterile loop, small portion of the subculture was transferred into petri-dishes containing nutrient agar and incubated (2-4 h) at 37°C until the growth reached log phase. Dilutions of the stock solution containing 25, 50, 75 and 100 mg mL^{-1} were prepared in Dimethyl Sulfoxide (DMSO) and 100 μL of each dilution was added in the respective wells. Nutrient agar media seeded with standard inoculums suspension was poured in petri-dishes and allowed to solidify. Discs impregnated with standard antibiotic disc (Kanamycin 30 μg $disc^{-1}$, Oxoid Ltd, UK) were placed on the petri-dishes with sterile forceps and gently pressed to ensure contact with the inoculated agar surface. Finally, the inoculated plates were incubated at 37°C for 24 h and antimicrobial activity was assessed by measuring the zone of growth inhibition (mm) surrounding the discs and compared with the control. Each experiment was carried out in duplicate[14].

RESULTS AND DISCUSSION

Antiparasitic activity of plant extract against different bacterial isolates comprising of *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* and fungal strains using *Aspergillus flavus* and *Candida albicans* causing microbial infections were used for evaluation of antimicrobial activity exhibited by the plant sample using different concentrations of 25, 50, 75 and 100 mg mL^{-1} methanol extract (70%) as shown in Table 1. Mainly, the solvent employed in such studies accounts for the complexity and diversity of the compounds being extracted. For this purpose, preferred

Table 1: *In vitro* antibacterial and antifungal activity of *Tamarix aphylla*

Microorganisms tested against *Tamarix aphylla* bark using methanolic extract	Zone of inhibition (mm)				
	Sample drug (mg mL^{-1})				Control drug (μg mL^{-1})
	T_1 (25)	T_2 (50)	T_3 (75)	T_4 (100)	T_5 (30)
Bacterial strains					
Bacillus subtilis	NGI	NGI	2.7±0.4	11.60	20.0
Staphylococcus aureus	NGI	2.4	2.4	13.4±0.3	23.0
Escherichia coli	NGI	NGI	2.2±0.3	6.2±0.9	23.0
Salmonella typhi	NGI	NGI	NGI	2.0±0.3	27.0
Fungal strains					
Aspergillus flavus	NGI	2.3	4.7	13.0±0.7	25.0
Candida albicans	NGI	NGI	NGI	2.0±0.3	17.8

Zone of Inhibition (mm) analyzed as Mean±Standard Deviation (SD) in sample and control drug against bacterial and fungal isolates after application of extract in various concentrations. NGI: No Growth Inhibited. Concentrations used of sample drug T_1: 25 mg mL^{-1}, T_2: 50 mg mL^{-1}, T_3: 75 mg mL^{-1}, T_4: 100 mg mL^{-1} and concentration used of controlled drug T_5: 30 μg mL^{-1}

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is methanol, since it is the most commonly used solvent for preliminary studies of antimicrobial activities in plants[15]. Highly significant degree of activity was observed against *Staphylococcus aureus* having the zone size with 13.4 mm in diameter followed by *B. subtilis* 11.60 mm and then *E. coli* with 6.2 mm in diameter at 100 mg mL^{-1} as compared to standard drug. Although 75 mg mL^{-1} of methanol extract of bark did not show antimicrobial activity against most of the pathogens except for *Aspergillus flavus* (4.7 mm) while the concentration of 50 mg mL^{-1} was considerable ineffective for all strains except for *Staphylococcus aureus* and *Aspergillus flavus*, whereas the lowest concentration of 25 mg mL^{-1} fail to produce any inhibitory effect in medicinal plant. In general, plant sample showed significant degree of antimicrobial activity against tested microorganisms. All antimicrobial activities were observed to be concentration dependent. The activity of extract was less than that of standard antibiotic i.e., kanamycin. This may be due to the fact that at higher concentrations, the rate of diffusion may perhaps be varied and hence, it might not be available to react with the microorganisms[16]. Therefore, concentration may play a role for the observed activity in experiment. Similar fluctuating trend of inhibition zone was reported against same pathogens in the analysis[17]. In this study, the zone of inhibition displayed by microorganisms was in agreement with the results reported by some investigators discussed below. Screening of methanol extract of *Tamarix aphylla* against microbial strains at a concentration of 100 mg mL^{-1} and standard drug (Chloramphenicol 5 mg mL^{-1}) was conducted by analyzing Mean zone of inhibition (mm) of microorganisms ± Standard error of means (Mean±SEM)[18]. Results showed that *Staphylococcus aureus* (15.5±0.5) was highly susceptible to *Tamarix aphylla* followed by *Bacillus cereus* (12.0±2.0), *Salmonella typhi* (11.0±0.0), *Proteus vulgar* (11.0±2.0) and *Pseudomonas aeruginosa* (10.5±1.5) whereas *Escherichia coli* (9.0±1.0) and *Klebsiella pneumonia* (8.5±0.5) were found least susceptible when compared to standard drug chloramphenicol (13.0±1.0 mm). Zain *et al*.,[19] screened out the antimicrobial activities of methanolic extract of *Tamarix aphylla* plant by determining their zone of Inhibition (mm) and showed activity against all bacterial strains namely; *Acinitobacter baumanni*, *Escherichia coli*, *Moraxella lacunata*, *Proteus merabiles*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Micrococcus kristinae*, *Micrococcus luteus*, *Sarcina ventricull*, *Staphylococcus aureus*, *Staphylococcus haemolyticus* and *Streptococcus byogenes* with the exception of *Salmonella typhi* and fungal strains including; *Candida albicans, Saccharomyces cerevisiae*, *Aspergillus flavus*, *Aspergillus fumigatus* and *Penicillium chrysogenum*[19]. The highest activity of *Tamarix aphylla* was obtained against *Aspergillus flavus* followed by *Escherichia coli* and *Streptococcus pyogenes*.

Tamarix macrocarpa was evaluated for its *in vitro* antimicrobial activities using ethanolic extract by an agar dilution-streak method against different bacterial and fungal isolates. The results showed that it is active against *Staphylococcus aureus* and *Bacillus subtilis* at the level of 1000 μg mL^{-1} and *Candida albicans* at the level of 500 μg mL^{-1}. *Tamarix dioca*, caused significant percent inhibition against *Fusarium solani* (60%), *Aspergillus flavus* (70%) and *Microsporum canis* (85%) while low activity against *Trichophyton longifusis* and almost no activity against *Candida albicans* and *Candida glabrata* using crude 96% ethanolic extract. Antimicrobial activity tests on n-butanolic extract and two isolated flavonoids: 5-Hydroxy-4,3,7-trimethoxyflavone and 3,5,7-Trihydroxy-4-methoxyflavone isolated from *Tamarix gallica* has been reported by Kendour and inhibition zone exhibited by micro-organisms was observed against the bacterial strains as *E. coli* (15), *P. aeruginosa* (14), *K. Pneumoniae* (16), *E. aerogenes* (14), *S. aureus* (10) and the fungal strain *C. Albicans* (11) mm in diameter using disk diffusion method[20].

Thorough screening of literature available on *Tamarix aphylla* depicted the fact that it is a popular remedy among various ethnic groups and traditional practitioners for treatment of ailments.

CONCLUSION

According to the phytochemical components found in these plants, it can be proposed that *Tamarix* species are known to have antiparasitic or disease preventive properties as observed in different studies. Result of the present study revealed that *Tamarix aphylla* is an ethnomedicinal plant extensively used for its application in a wide array of medicines and contain active compounds which serve as an alternative agents as the drug therapy in the control of parasitic diseases. The plant should be subjected to intensive studies such as toxicity against animal and human cells using experimental animal models to understand further the medicinal value and exploiting hidden potentials of the plant.

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