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Antibacterial Action of *Tamarix indica* Wild against Human Pathogen

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ABSTRACT
Tamarix indica Wild generally found as shrub or a small tree is a common plant in Sindh and is distributed in various Asian countries including Bangladesh, India, Srilanka, Afghanistan and Andaman Island. Sometimes it is invasive and become a harmful weed. Various parts of this plant are used in eastern medicine. This led us to attempt evaluation of its antibiotic potential. The antibacterial potential was tested in ethanol, chloroform and sterile distilled water extracts of leaves of Tamarixindica. Chloroform extracts of Tamarix indica showed maximum antibacterial activity towards E.Coli followed by Salmonella typhi at all concentrations (10, 50, 100, 250 and 500 mg/ml) except 1mg/ml. At 1mg/ml Staphylococcus aureus was least affected. Ethanol extract disclosed antibacterial activity against bacteria (E.Coli>Salmonella typhi>Bacillus subtilis>Pseudomonas>Staphylococcus aureus). Likewise aqueous extract also greatly suppressed the growth of gram negative bacteria among which Staphylococcus aureus was least affected.
Keywords: *Tamarix Indica, Sindh, Anti bacterial, Human pathogens*

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INTRODUCTION

Owing to infectious bacteriological ailments most of the humans die worldwide [1]. The infectious organisms including *Staphylococcus, Pseudomonas* and *Bacillus* are the foremost source to cause severe ailments in humans. These microorganisms have the ability to withstand in harsh environment due to their severe adaptability to environment [2]. A new source of antibacterial agents with probably innovative mechanism of action derived from natural products of plants [3, 4]. The examination of naturally occurring substances derived from plants has always been of great curiosity to researchers observing for new sources of beneficial remedies against pathogens. Infections have increased and antibiotic opposition becomes an ever increasing therapeutic problem in the recent years [5].

Tamarix indica Willd (*Tamaricaceae*), locally known as 'Jhau' in Pakistan. It is also recognized by the names of (synonyms)*Tamarix gallica* and *Tamarix troupii*. This plant is primarily found as green shrubs or low tree. It is distributed in the coastal forests of Bengal, India, Pakistan, Bangladesh, Srilanka, Afghanistan and Andaman Island.. It exists in several areas as an invasive species, often becoming a harmful weed[6]. Particularly from the leaf, flower and bark many different chemical constituents have been reported in the plant[7-9]. This plant is likewise used for fuel wood and timber in certain countries in the world[6, 8-10]. The bark is bitter and a severe tonic; leaves and fruit are useful for chronic diarrhea and dysentery [8, 9, 11] as well as the roots are beneficial for sore throat and ulcerating piles[9].

Tamarix prefers alluvial soil, but matures well in alkaline and saline land. It is a relatively long-standing plant that can withstand a broad range of environmental situations and resist stresses such as salt, high temperature and drought conditions. It is mostly found in the saline areas and in between interdual regions of the desert [8, 10, 12].Presently, it is mainly grown up gregariously in Pakistan on newly formed alluvial land, rivers and the coastal areas of Sindh (Keti Bunder and Shah Bunder) which is vulnerable to climate change.Sindh coastal area is facing severe drought situations in the form of lengthy dry curses. Due to decrease in precipitation, sea water intrusion occurs and temperature is elevated resulting in increased evaporation from the soil surface. Soil salinity is increasing that encourages the intrusion of salt loving plants (halophytes)that are increasing and attaining greater prominence or dominance in the flora. The rising level of the sea is intruding the fertile lands of the area[13, 14].

The present investigation attempts to evaluate the antimicrobialaction of leaf extracts of *Tamarix indica* with aqueous, chloroform and ethanol extracts against human pathogenic bacterial strains comprising of *Staphylococcus aureus* and *Bacillus subtilis* (Gram positive)and *Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginosa*(Gram negative).

MATERIALS AND METHODS

SAMPLING

The plant material selected in this study consisted of mature leaves of *Tamarixindica*Willd. They were collected in October, 2013 from the coastal areas of Sindh (Shah Bunder, Thatta District) which is highly affected byseawater intrusion and also due to climate change. They were transported to the Institute of Environmental Studies, University of Karachi.The Plant leaves were initially washed with distilled water and then dried while being enveloped in paper towels in the hot air oven for 1 hour until they were crisp and moisture free.

TEST MICROORGANISMS

Five bacterial strains were used for this study. Gram positive bacteria include *Staphylococcus aureus* and *Bacillus subtilis* while gram-negative bacteria include *Escherichia coli,Salmonella typhi* and *Pseudomonas aeruginosa*. All the tested strains were collected from the Department of Microbiology University Of Karachi. The microbial cultures were sustained in nutrient agar slants at 4 C and maintained on a nutrient broth at 37 C. Each culture was further identified through Gram Staining[15]. Whereas *E. coli* was further purified on EMB agar and *Pseudomonas aeruginosa* on cetrimide agar respectively [16].

PREPARATION AND PRESERVATION OF PLANT EXTRACT

Leaves of *Tamarixindica Wild* were grounded in mortar and pestle to get dried powdered material. The powder was then added in different solvents that is, ethanol, chloroform and distilled water to make extracts in order to determine the antibacterial activity of the leaves. The dried powder material of leaves was weighed out 10 mg, 50 mg, 100mg, 250mg and 500mg and soaked separately in 10 ml of sterilized distilled water, Chloroform and Ethanol to make 1mg/ml, 5mg/ml, 10mg/ml, 25mg/ml and 50mg/ml aqueous, Chloroform and Ethanol extract of different concentrations respectively. They were contained in screw capped test tubes and left undisturbed for 24 hours and subsequently filtered using sterile filter papers (Whattman No. 1) into sterilized screw capped test tubes. The standard extracts thereby obtained were then stored at 4 C for further use.

Sterile Discs of Whatmann No. 1 filter paper of 6.35 mm diameter were prepared by taking 0.5ml of 1mg/ml, 5mg/ml, 10mg/ml, 25mg/ml and 50mg/ml stock concentrations of aqueous, chloroform and ethanol extract individually in another petri plate and soaking the Whatmann No. 1 filter discs in them separately.

PREPARATION OF CONTROL

The control was prepared by separately dipping the sterile discs of Whatmann No. 1 filter paper of 6.35 mm diameter in 0.5ml autoclaved distilled water, pure ethanol and pure chloroform solvents.

STANDARD ANTIBACTERIAL AGENT

Antibiotic Cefixime of 100mg was used as the Standard Antibacterial agent against all gram negative and gram positive bacterial strains used in this work.

ANTIBACTERIAL ASSAY

Antibacterial assay was done by the disc diffusion susceptibility technique using plant extracts and commonly used antibiotics i.e. Cefixime.

Nutrient Agar media were poured onto sterile Petri dishes and allowed to be solidified and then nutrient agar media plates were seeded with 18 to 24 h grown cultures of *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhi, Pseudomonas and Escherichia coli* by spread plate technique. Filter paper discs of aqueous, ethanol and chloroform extracts and antibiotic Cefixime were placed by sterile forceps on the media on the corresponding quadrants of the flooded nutrient agar plates marked on the back with the same concentration. This was done both for the test compounds as well as for antibiotic Cefixime. The plates were incubated overnight at 37 C. After incubation, the diameter of the zone of inhibition around each disc was calculated in millimeters (mm) and the results tabulated.

RESULTS AND DISCUSSION

Plants are considered to be an important source of medicine in a variety of traditional medications [8, 9, 17-19]. Tables 1, 2 and 3 shows the antibacterial activities of leaves of *Tamarixindica* Willd against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli,Salmonella typhi* and *Pseudomonas aeruginosa*in Ethanol, chloroform and aqueous extracts.

CHLOROFORM EXTRACT

It is noticeably seen from the results presented in Table 1 that the Chloroform extracted samples of *Tamarix indica* Willd leaves showed maximum antibacterial activities toward*E. coli* followed by *Salmonella typhi, Bacillus subtilis* and *Pseudomonas aeruginosa* at all concentrations of Chloroform except for 1mg/ml concentration in which *E. coli* has been succeeded by *Bacillus subtilis* while the minimum zone of inhibition was recorded for *Staphylococcus aureus*.

As the concentrations of the chloroform extract increased, the zone of inhibition also increased (Table 1). The zones of inhibition obtained on the Petri plates of chloroform extract of *E. coli* ranged from 1.5mm in 1mg/ml to 59.4mm in50mg/ml while the zones of inhibitions for *Salmonella typhi* varied from 1 mm in 1mg/ml to 55.2 mm in 50mg/ml .The lowest reduction in the growth of *E. coli* and *Salmonella typhi* was noted from chloroform extracted samples at 1 mg/ml concentration with 1.5mm and 1.0mm of zones of inhibition respectively.Chloroform yielded minimum results in all cases as compared to the other two solvent extracts.

Table 1.Antibacterial action of Chloroform extract of *Tamarix indica* Wild

Test Microorganism	Gram Reaction	Zone of inhibition (mm) (Mean ± Standard Error)					
		Standard	**Chloroform Extract (mg/ml)**				
		Cefixime (100 mg)	**1**	**5**	**10**	**25**	**50**
Bacillus subtilis	Gram positive	3.8±0.3	1.2±0.5	6.1±0.1	16.1±0.18	23.2±0.22	50.2±0.54
Salmonella typhi	Gram negative	4.7±0.07	1.0±0.21	6.6±0.15	17.1±0.38	25.4±0.31	55.2±0.63
Staphylococcus aureus	Gram positive	7.1±0.5	0.6±0.26	4.4±0.22	14.4±0.08	17.5±0.38	40.2±0.13
Pseudomonas aeruginosa	Gram negative	4.4±0.15	0.9±0.43	5.9±0.29	15.9±0.14	21.4±0.36	44.7±0.19
Escherichia coli	Gram negative	2.9±0.10	1.5 ±0.39	6.9±0.41	17±0.26	27.3±0.48	59.4±0.21

ETHANOL EXTRACT

Table 2 shows the antibacterial action of Ethanol extracted samples of *Tamarix indica* Willd leaves which have shown maximum antibacterial activity toward *E. coli* succeeded by *Salmonella typhi, Bacillus subtilis* and *Pseudomonas aeruginosa* at all concentrations of Ethanol extracts while the minimum antibacterial activity was observed against *Staphylococcus aureus*. The zones of inhibition obtained on the Petri plates of an ethanol extract of *E. coli* ranged from 1.7 mm in 1mg/mlto 61.8 mm in50 mg/ml to while the zones of inhibitions for *Salmonella typhi* varied from 1.5 mm in 1mg/ml to 57.9 mm in 50 mg/ml .

The inhibition of these microbes increased with increasing concentration of ethanol as this is rather clear from Table 2 that as the concentrations of the ethanol extract increased so the zone of inhibition increase.

AQUEOUS EXTRACT

Antibacterial activity of aqueous extracted samples of *Tamarixindica* Willd leaves are presented in Table 3 which have shown maximum antibacterial activity toward *E. coli* followed by *Salmonella typhi, Bacillus subtilis*and *Pseudomonas aeruginosa* at all concentrations of aqueous extracts. However, *Staphylococcus aureus* showed minimum inhibition zone similar to above mentioned extracts. The zones of inhibition obtained on the Petri plates of an aqueous extract of *E. coli* ranged from 2.1 mm in 1mg/mlto 63.4 mm in50mg/ml while the zones of inhibition for *Salmonella typhi* varied from 1.8 mm in 1mg/ml to 60.9mm in 50mg/ml .

The inhibition zone of these human pathogens increased with increasing concentration of aqueous as this is rather clear from Table 3 that as the concentrations of the aqueous extract is increasing so is the zone of inhibition increasing.

The data evidently suggested that aqueous extracted samples at all five concentrations exhibited maximum inhibition zone against *Escherichia coli, Salmonella typhi,Bacillus subtilis* and *Pseudomonas aeruginosa* after that next in line approximately similar results were obtained by ethanol extracted samples at all concentrations while chloroform has yielded minimum results in all cases. It can be concluded that in our study *E. coli*, *Salmonella typhi, Bacillus subtilis* and *Pseudomonas aeruginosa* are most susceptible to the leaf extracted samples of *Tamarixindica* Willd as previously reported[9] and this plant exhibits successful antidiarrheal activity same as reported [8, 20].

Lowest inhibitory against *Staphylococcus aures* was found in chloroform 1mg/ml extracted sample producing a 0.6mm zone of inhibition. However, the inhibition zone of these samples augmented with increasing concentration as this is rather obvious from the results that as the concentrations of the solvents are increased so the zone of inhibition increase. Aqueous and ethanol 50mg/ml (extracted samples) were little effective to control the *Staphylococcus aures* growth as the zones of inhibition varied from 45.2mm to 48.6mm. It can be concluded that *Staphylococcus aures* is resistant to the leaf extracted samples of *Tamarix indica* in contrast with other bacterial strains[9].

Moreover, It is also apparent from the information presented in Table 1, 2 and 3 that chloroform, ethanol and aqueous extracts respectively were strongly effective in inhibiting the growth of all bacterial strains when compared with standard cefixime antibiotic. The chloroform, ethanol and aqueous solvent extracts of the leaves of *Tamarixindica* Wild exhibited more effective results than those of pure solvents taken as controls against all bacterial strains.

The antimicrobial agent found in *Tamarix indica* may help as an inexpensive and newer source for the treatment of infectious ailments. Further inquiry is indispensable to assess the sensitivity of the plant extract againstvirus, fungi and other microorganisms.

Table 2.Antibacterial action ofEthanol extract of *Tamarix indica* Willd

Test Microorganism	Gram Reaction	Zone of inhibition (mm) (Mean ± Standard Error)					
		Standard	**Ethanol Extract (mg/ml)**				
		Cefixime (100 mg)	**1**	**5**	**10**	**25**	**50**
Bacillus subtilis	Gram positive	3.8±0.3	1.3±0.1	6.4±0.18	16.8±0.14	25.4±0.54	53.4±0.47
Salmonella typhi	Gram negative	4.7±0.07	1.5±0.15	6.9±0.11	17.5±0.22	27.7±0.41	57.9±0.58
Staphylococcus aureus	Gram positive	7.1±0.5	0.9±0.15	4.8±0.23	14.8±0.16	19.8±0.21	45.2±0.51
Pseudomonas aeruginosa	Gram negative	4.4±0.15	1.1±0.20	6±0.31	16.1±0.35	23.2±0.36	48.7±0.22
Escherichia coli	Gram negative	2.9±0.10	1.7±0.24	7.3±0.41	18.6±0.22	29.1±0.45	61.8±0.31

Table 3. Antibacterial action of Aqueous extract of *Tamarix indica* Wild

Test Microorganism	Gram Reaction	Zone of inhibition (mm) (Mean ± Standard Error)					
		Standard	**Aqueous Extract (mg/ml)**				
		Cefixime (100 mg)	**1**	**5**	**10**	**25**	**50**
Bacillus subtilis	Gram positive	3.8±0.3	1.5±0.17	6.8±0.33	17.2±0.56	26.7±0.58	55.8±0.38
Salmonella typhi	Gram negative	4.7±0.07	1.8±0.14	7.2±0.28	17.9±0.51	29.2±0.45	60.9±0.35
Staphylococcus aureus	Gram positive	7.1±0.5	1±0.18	5.1±0.41	15.2±0.37	20.1±0.14	48.6±0.33
Pseudomonas aeruginosa	Gram negative	4.4±0.15	1.3±0.10	6.5±0.46	16.5±0.29	23.8±0.16	50.3±0.25
Escherichia coli	Gram negative	2.9±0.10	2.1 ±0.21	7.7±0.68	19.4±0.24	31.4±0.41	63.4±0.45

CONCLUSION

The present study indicates that *Tamarix indica* is an extremely beneficial antibacterial plant and its application in a wide range of remedies to cure sore throat and ulcerating piles, dysentery and chronic diarrhea to mention a few. The antimicrobial agent found in *Tamarix indica* Wild may serve as an inexpensive and new source for the treatment of infectious ailments. It is recommended that *Tamarix indica* may be suggested as a beneficial source to prepare natural bioactive products from which we can develop new antimicrobial drugs which will be profitable because the plants are generously found free of cost. Screening of such various natural organic compounds and identification of active agents must be considered as a rewarding approach.

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