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ANTIMICROBIAL POTENTIAL OF ALKALOIDS AND FLAVONOIDS EXTRACTED FROM *TAMARIX APHYLLA* LEAVES AGAINST COMMON HUMAN PATHOGENIC BACTERIA

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Abstract

Background: Alkaloids and flavonoids are secondary metabolites extracted from different medicinal plants. *Tamarix aphylla* a traditionally valuable medicinal plant; was used for the extraction of alkaloids and flavonoids in order to evaluate their antibacterial activity.
Methodology: The leaves of the plant were collected from district Kohat, Pakistan, and their alkaloids and flavonoids were extracted with ethanol and methanol, respectively. Four bacteria i.e. Gram positive (*Staphylococcus aureus*) and Gram negative (*Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginosa*) were selected for the biological screening of these phyto-constituents.
Results: The concentration of alkaloids was found to be more in the leaves of *Tamarix aphylla* than flavonoids. The extracted phytochemicals showed varied inhibition zones against tested bacterial isolates. Alkaloids showed highest inhibitory activity against *Staphylococcus aureus* (14±0.6 mm) followed by *Pseudomonas aeruginosa* (13±0.7 mm). Conversely, flavonoids showed the highest inhibitory affect against *Salmonella typhi* (17±0.7 mm) followed by *Staphylococcus aureus* (14±0.7 mm). However, both extracts showed the lowest inhibitory effects against *Escherichia coli*.
Conclusions: It was concluded that the alkaloids and flavonoids from *Tamarix aphylla* leaves have antimicrobial potential against common human bacterial pathogens. However, flavonoids were found to be more active phytochemical against tested bacterial strains as compared to alkaloids.

Keywords: Kohat; Medicinal plants; Phytochemical screening; *In-vitro* activity; Bacterial strains.

Introduction

Tamarix aphylla (L.) Karst., locally referred to as Khagal (Urdu) and Ghaz (Pashto) in Pakistan, is used as medicinal plant all over the world (Lefahal et al., 2010; Emad and Gamal, 2013). *Tamarix aphylla* (Family, *Tamaricaceae*) is the largest and most common species of Pakistan and considered as ornamental tree, planted on road side within the country (Marwat et al., 2008). Ethno-botanically its bark, leaves, stem and twigs are preferred for different diseases with no side effects by local practitioners in the country. They have needle leaves which are boiled in water and tied to the affected areas of body. Thus, leaves were considered a source for curing different infectious diseases (Marwat et al., 2008; Marwat et al., 2009; Panhwar & Abro, 2007). According to Nisar et al. (2010) effective medicinal agents from plants being safe and cheap may also appear as potential alternatives for controlling microbial infections.

The good antimicrobial activities of medicinal plants are due to the presence of secondary metabolites in them. Previously different phyto-constituents like alkaloids, tannins and phenolic contents have been found in the leaves of *Tamarix aphylla* (Achakzai et al., 2009).

And literature proved that phyto-constituents extracted from medicinal plants had strong inhibitory effects on disease causing pathogens (Adnan et al., 2014). According to a research study; the extracts of *Tamaricaceae* plants showed good antibacterial activities against *Escherichia coli, Pseudomonas, Salmonella* and *Staphylococcus species* due to the presence of secondary metabolites in them (Lefahal et al., 2010; Emad and Gamal, 2013). *Escherichia coli* are bacterial pathogens that causes diarrhea; similarly, *Pseudomonas aeruginosa* causes serious progressive pulmonary infection and *Staphylococcus aureus* causes pneumonia, enteric infections and skin infections (Abid et al., 2010; De Victorica and Galván, 2001; Antai, 1987). According to Ahmad et al. (2012).

The extracts of *Tamarix aphylla* showed antimicrobial activity against *S. aureus* (4 mm, zone of inhibition) while no activity was observed in *E. coli* and *Salmonella* species.

Based on the previous researches, the present study was designed to isolate and identify alkaloids and flavonoids from the leaves of *Tamarix aphylla.* The study was expected to show good antibacterial activities of these phyto-constituents against *E. coli, P. aeruginosa, S. typhi* and *S. aureus.*

Materials and Methods

Plant collection and extraction

Tamarix aphylla leaves were collected in the vicinity of Kohat University of Science and Technology (KUST), Kohat. It was identified by expert taxonomist and Voucher specimen was deposited in the departmental herbarium. The collected plants were shade dried and powered afterwards their alkaloids and flavonoids were extracted with ethanol and methanol, respectively.

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Alkaloids determination

5 gm of plant sample was taken in 200 ml of 20 % acetic acid in the ethanol and kept for 4 hours. After 4 hours these was filtered and the extract was concentrated using water bath to decrease the volume to one quarter of the original volume. Then concentrated ammonia hydroxide was added drop wise to the extract, until the precipitation was complete. The whole solution was allowed to settle and the precipitation was collected by filtration and weighed (Harborne, 1973).

Flavonoids determination

10 gm of plant powder was put into 100 ml of 80 % aqueous methanol at room temperature for 2-4 hours. The whole solution was filtered through Whatman paper No1. After filtration, the filtrate was put into a petri dish and evaporated into dryness over a water bath and weighed to a constant weight (Boham and Kocipai, 1974).

Antibacterial activity

For antibacterial activity, four bacterial isolates were provided by the department of Microbiology, KUST. The studied bacterial isolates were *Staphylococcus aureus* (Gram positive), *Salmonella typhi, Escherichia coli* and *Pseudomonas aeruginosa* (Gram negative), as these are the most common disease causing bacteria.

Agar well diffusion method

Antibacterial activity was determined by this method. A loopful bacterial culture was immersed in sterile distilled water to form the dilution of the inoculums. The nutrient agar plates were prepared and left for solidification. Then wells were formed by using sterile cork borer. The plates were then inoculated by the bacterial culture using the sterile swabs. The wells were filled with the plant extracts. For negative control Dimethyl sulfoxide (DMSO) was added to one of the well. The petri plates were incubated overnight at 37 ^{0}C. After incubation the petri-plates were checked for different zone of inhibition (mm) formed by the plant extracts (Boyanova et al., 2008). This experiment was repeated three times.

Results

Tamarix aphylla was considered one of the important medicinal plant species. Ethno-botanically in Pakistan it is traditionally used for 9 major disease categories like fever, gastrointestinal, dermatological etc. (Table 1). Previous studies showed that traditional healers mostly used leaves among other aerial parts for the treatment of variety of diseases in the country (Figure 1). Furthermore, literature review showed that different parts of *Tamarix aphylla* contain a number of phytochemical compounds like alkaloids, saponins, tannins and phenolic compounds etc. Due to the presence of these compounds; pharmacologically this species was used for antiseptic, antileishmanial, antibacterial, antitumor and antifungal activities (Table 1).

In the present study it was observed that phytochemical constituents present in the *T. aphylla* extract include alkaloids and flavonoids. Both alkaloids and flavonoids were found in different concentrations. The alkaloids were 0.12 grams in 5 gram sample while flavonoids were 0.1 gram in 10 gram sample of plant extract. Thus, alkaloids and flavonoids were 2.4 and 1 percent in 5 and 10 grams of samples, respectively (Table 2). The alkaloids and flavonoids showed varied activity against four different bacterial pathogens. The alkaloids showed highest inhibitory activity against *S. aureus* (14±0.6 mm) followed by *P. aeruginosa* (13±0.7 mm) and *S. typhi* (12±2 mm). However, it showed lowest inhibitory effects against *E. coli* (7±2 mm) (Table 3). In addition, the flavonoids showed highest inhibitory effect against *S. typhi* (17±0.7 mm) followed by *S. aureus* (14±0.7 mm). Moreover, against *P. aeruginosa* the zone of inhibition was (13±0.33 mm). Like alkaloids, the flavonoids showed comparatively low activity against *E. coli* (11±1 mm) (Table 3).

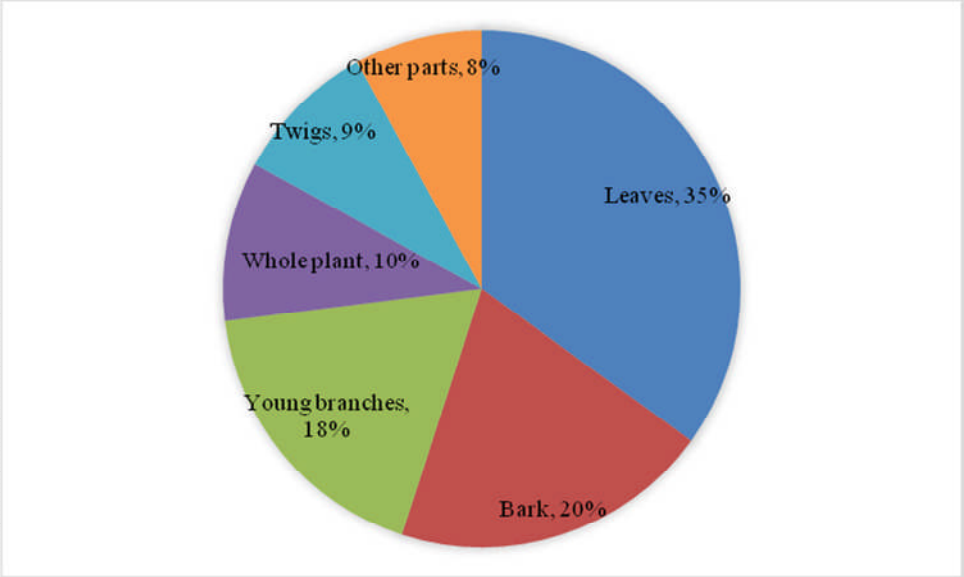

Figure 1: Percentage of parts of *Tamarix aphylla* used ethnobotanically in Pakistan.

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Table 1: General ethnobotany, phytochemistry and pharmacology of *Tamarix aphylla.*

Category	Parts used	Details	References
Ethnobotany	Leaves, barks, twigs, young branches, galls,	Antipyretic, Gastrointestinal, Dermatological, Dental, Pain killer, Wound healing, Joint pain, Jaundice, Astringent.	(Marwat et al., 2009; Saheen et al., 2012; Badshah and Hussain, 2011;Shah et al., 2013).
Phytochemistry	Bark, galls, wood of tree, leaves, stem	Alkaloids, saponins, tannins, phenols, glucose, alkenes, aromatic hydrocarbons, benzofuranose dextrin and moisture.	(Iqbal et al., 2012; Mughal, 2008; Said, 1984; Achakzai et al., 2009; Mughal et al., 2011).
Pharmacology	Bark, leaves, whole plant.	Antiseptic, Antileishmanial, Antibacterial, Antitumor activity, Antifungal	(Hamid et al., 2012 Iqbal et al., 2012; Ahmad et al., 2012; Mughal et al., 2011).

Table 2: Determination of concentrations of alkaloids and flavonoids in the leaves of *Tamarix aphylla*.

Phytochemicals	Sample Quantity	Concentration found in the sample quantity	% age concentration in the sample quantity
Alkaloids	5 g	0.12 g	2.4
Flavonoids	10 g	0.10 g	1

Table 3: Antimicrobial activities of alkaloids and flavonoids of *Tamarix aphylla* leaves against four bacterial pathogens. SE denotes the standard error.

Extracts of *Tamarix aphylla*	*E. coli* (mm) (Mean ± SE)	*S. typhi* (mm) (Mean ± SE)	*P. aeruginosa* (mm) (Mean ± SE)	*S. aureus* (mm) (Mean ± SE)	Control of DMSO (mm)
Alkaloids	7±2	12±2	13±0.7	14±0.6	0
Flavonoids	11±1	17±0.7	13±0.33	14±0.7	0

Discussion

Literature overview of *Tamarix aphylla*

From the very beginning of time human beings have used plants as medicines for the treatment of various ailments. They were identified as important source of medicine after various observations and experimentations (Malik, 2001). The medicinal plants are preferentially used by people because they are cheaper than allopathic medicines, culturally acceptable and are least harmful with little or no side effects (Marwat et al., 2009; Hameed et al., 2011). Scientifically it has been proved that *Tamarix aphylla* is a medicinal plant species used for the treatment of a number of disease categories like pyretic, gastrointestinal, dermatological, wound treatment etc. in Pakistan (Table 1). But the plant is not only valuable within a specific country; this species is used well in other countries of the world. For instance, the leaves of *Tamarix aphylla* were used for the treatment of inflammation and wound healing in Saudi Arabia (Emad and Gamal, 2013). As leaves are the commonest parts of medicinal plants used ethno-botanically in the remedy preparation. In Pakistan traditional healers used aerial parts but mostly leave of *Tamarix aphylla* for the treatment of variety of diseases (Figure 1). Since leaves are expanded parts of the plant and are commonly available throughout the year (Shaheen et al., 2012).

Furthermore, literature review showed that different phytochemical compounds like alkaloids, saponins, tannins and phenolic compounds etc. have been isolated from different parts of *Tamarix aphylla* and due to the presence of these secondary metabolites this plant has been shown to have antibacterial, antifungal, antiseptic, and antileishmanial activities (Table 1). According to Rochfort et al. (2008); plant bioactives of almost every chemical class were extracted and showed antimicrobial activities. Similarly a study was conducted in 1984, according

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to which the phytochemical compounds were considered to produce biological activities in both human and animal and considered to be used as drugs (Sofowora, 1984).

Concentration of phytochemicals

This study showed that *Tamarix aphylla* contains alkaloids in high amount as compared to flavonoids. It might be due to the fact that alkaloids are protective for the plant. Generally plants being sessile in nature are unable to avoid their predators. Therefore, producer plants could protect themselves against their enemies by using these alkaloids. According to Achakzai et al. (2009), the alkaloids functions as a natural source of insecticides and fungicides. Furthermore, the increased ratio of alkaloids might be due to the fact that they are biologically used in storage of waste nitrogen, cationic balancing and protection against parasites (Ting, 1982).

Our findings were contradictory with a study conducted on the Saudi folk medicine by Emad and Gamal, (2013) where they concluded that flavonoids were absent in the *Tamarix aphylla;* and this might be due to difference in geographical distribution of two countries.

Alkaloids activity against bacterial pathogens

The results of the present study are in line with Ahmad et al. (2012), where *S. aureus* showed the highest activity against methanolic extracts of *Tamarix aphylla* as compared to *E. coli*, and *S. typhi*. Further literature also proved that Gram-positive bacteria are more sensitive to plant extract than Gram-negative. As Gram negative bacteria contain hydrophobic lipo-polysaccharide in the outer membrane. And this lipo-polysaccharide in membrane provides protection against different agents (Cosentino et al., 1999; Karaman et al., 2003). Thus, *S. aureus* being Gram positive bacteria showed the highest inhibition zone. However, the activity against Gram negative bacteria like *Salmonella* and *Pseudomonas* also cannot be ignored.

This might be due to some type of penetration power of alkaloids in the outer membrane of *S. typhi* and *P. aeruginosa*. Our results were contradictory with Adnan et al. (2014) where alkaloids of *Justicia adathoda* showed good activity against *E. coli*; although in the present study alkaloids of *Tamarix aphylla* showed very less activity against the bacterium due to some unknown reasons.

Flavonoids activity against bacterial pathogens

The flavonoids showed almost good antibacterial activity against tested pathogens. However, the observed activity was low when compared to other bacterial strains in *E. coli*. Contradictory to the present results it has been proved previously that; flavonoids extracted from medicinal plants showed good antibacterial activity against *E. coli* (Aslam et al., 2009). Our findings are in line with Yahaya et al. (2011) that flavonoids are more active against *S. typhi* than other microbes. The good antibacterial activity of this phyto-constituents might be linked with its capacity to become complex with bacterial cell walls and the lipophilic flavonoids may also disrupt microbial membranes (Cowan et al., 1999).

Conclusions

In conclusion, the people of Pakistan traditionally employ *Tamarix aphylla* in curing different ailments like gastrointestinal, antipyretic, dermatological problems etc. This species contain alkaloids, saponins, tannins, phenols, glucose and alkenes etc. in their different parts, although further studies are required for the isolation and screening of secondary metabolites due to the limited available literature on this aspect. From the present study it can be concluded that alkaloids are more abundantly present in *T. aphylla* than flavonoids. However, flavonoids were found to be more active phytochemical against tested bacterial strains as compared to alkaloids.

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