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ANTIMICROBIAL ACTIVITY OF THREE MEDICINAL PLANTS (*ARTEMISIA INDICA, MEDICAGO FALCATA* AND *TECOMA STANS*)

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Abstract

Background: *Artemisia indica*, *Medicago falcata* and *Tecoma stans* are traditionally being use for medicinal purposes in Pakistan. Present study was designed to check *in-vitro* efficacy of these plants against selected bacterial and fungal strains.
Methodology: Chloroform, butanol, ethyl acetate and n-hexane extracts of these plants were used for antimicrobial screening. Antibacterial activity was tested against four pathogenic bacterial strains i.e. *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus* while antifungal activity was tested against four fungal strains i.e. *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus* and *Fusarium solani*.
Results: Chloroform, butanol and ethyl acetate extracts of *Artemisia indica*, *Medicago falcata* and *Tecoma stans* showed high inhibitory activities (between 15-20 mm) against *E. coli*, *P. aeruginosa* and *S. aureus.* However, all extracts of *Artemisia indica* showed inhibitory activities (12-14 mm) against *Salmonella typhi.* As antifungal activities, the n-Hexane and chloroform extracts of *Artemisia indica* have completely inhibited the growth of *Aspergillus flavus* and *Fusariun solani*, respectively. Ethyl acetate and butanol extract of *Medicago falcata* completely inhibited *Fusarium solani* and *Aspergillus fumigates*, respectively. The n-hexane extract of *Tecoma stan* completely inhibited *Fusarium solani*, while its ethyl acetate extract shows excellent activity against *Aspergillus niger*.
Conclusions: These findings provide scientific evidence of traditional use of medicinal plants and also indicate the potential of these plants for the development of antimicrobial agents.

Key words: Medicinal Plants, Traditional uses, phytochemical effects, Antimicrobial activity.

Introduction

Medicinal plants are abundant source of antimicrobial molecules. A wide range of medicinal plants extracts are used to treat several infections as they have potential antimicrobial activity. Some of these bioactive molecules are screened and traded in market as raw material for many herbal industries (Renisheya et al., 2011). Experts turned their concentration back towards obtaining advantages from medicinal plants after observing more side effects of synthetic drugs compared to their benefits (Bushra et al., 2012). It is estimated that about 35,000 to 70,000 plants species are used as medicinal plants out of 422127 reported worldwide plant species (Bibi et al., 2011). In Pakistan 80% of the population belonging to the rural areas depends on the traditional medicines (Munir et al., 2013). *Artemisia indica* is a perennial plant species mostly found in Asia (India, China, Japan, Nepal and Pakistan) and Europe. It can grow to a height of 6 feet; contain a stalk of small reddish brown or yellow colour. In Pakistan, its infusion is traditionally used in the treatment of nervous and spasmodic effect, asthma and helpful in increasing appetite (Nadeem et al., 2013). In Nepal, the juice of *Artemisia indica* is used to treat stomachic, diarrhea, dysentery, abdominal pains and relieving burning sensation in conjunctives (Satyal et al., 2012). Moreover, the roots are antiseptic and used as tonic for the kidneys (Sarnim et al., 2013). *Medicago falcata* is also a perennial herb belonging to the family Fabaceae and is extremely cold tolerant and winter hardy. It is native to Mediterranean basin, but has worldwide distribution. It contains widely branched roots, a deep set crown, less upright stems, narrow leaves and yellow flower.

The leaves of *Medicago falcata* contain large source of vitamins and a number of proteins. Its seeds are commonly used salad, sandwiches and cooked in soups. An appetite enhancing tea is also made from the leaves. Its flavour is somewhat slightly laxative (Muthu et al., 2012). *Tecoma stans* known as yellow elder is an erect shrub or small tree belong to family Bignonaniceae. The plant has been used for a variety of purposes in phytomedicines, treating diabetes and digestive problems. Extracts from *Tecoma stans* leaves have been found to inhibit the growth of yeast infections (Jennie et al., 2003). A growing body of literature is available on the medicinal properties of these three medicinal plant species.

However, the literature mining revealed that *Artemisia indica, Medicago falcata and Tecoma stans* have not been studied for antimicrobial activities. Hence, the present study is first systematic attempt to analyse the antibacterial and antifungal potential of these three species on selected bacterial and fungal strains. The objectives of the study were to screen out selected medicinal plants of Kohat for their antimicrobial activities.

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Material and Methods

Collection of plant sample

Fresh samples of *Artemisia indica, Medicago falcata and Tecoma stans* were collected from district Kohat, Khyber Pakhtunkhwa (KP), Pakistan, and authenticated at department of Botany, Kohat University of Science and Technology, Kohat, KP. These plant samples were washed with tap water in order to remove the dust. Collected plant materials were then dried under the shade and mashed with the help of mortar and pestle.

Preparation of extract

About 30g powdered material of each plant species was soaked in 300 mL solvent of n-hexane, chloroform, Ethyl acetate and Butanol for 2 weeks, shaked well twice a day and then filtered. The filtrates were then evaporated under reduce pressure to obtain a gummy residue with the help of rotary evaporator. All extracts were stored in sterile glass bottle at room temperature until screened.

Antibacterial bioassay

The different solvent soluble fractions were subjected to antibacterial evaluation against four bacterial strains *Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa), Salmonella typhi (S. typhi), Staphylococcus aureus (S. aureus)* as described by Usman et al. (2013). Muller Hinton Agar (Oxoid UK) was prepared in conical flask in accordance to the directions provided by the manufacturer. The media along with petri dishes, pipette and metallic borer were sterilized in autoclave for 15 minutes at 121^{o}C and 15 psi pressure. The media was poured into Petri dishes under aseptic condition (Yogeshi et al., 2004). The stock solutions of corresponding fractions were prepared in dimethyl sulfoxide (DMSO) (Perez et al., 1990).

The modified method of Perez et al., (1990) was followed. All of the four bacterial strains were obtained from Department of Microbiology, Kohat University of Science and Technology, Kohat, KPK, Pakistan. Bacterial culture was inoculated on MHA (corresponding to 10^6 CFU/ml). Bacterial strains were spread on the solidified agar media, then 7 mm wells were punched in the agar media by using sterile metallic borer. Stock solutions of crude extract and fractions in DMSO at concentration of 20 mg/ml were prepared and 200 µl from each stock solution was added into respective wells (Rehman et al., 2001). The petri dishes were incubated at 37^{o}C for 24 hours and control wells containing antibiotic (Levofloxacin), which is a positive control, was also run side by side. After 24 hours antibacterial activities were measured as diameter of the zones of inhibition and compared with the zone of inhibition of control (Levofloxacin).

Antifungal bioassay

The antifungal bioassay was determined by agar tube dilution method (Islam et al., 2011). Four fungal strains i.e. *Aspergillus flavus (A. flavus), Fusariun solani (F. solani), Aspergillus fumigatus (A. fumigatus) and Aspergillus niger (A. niger)* were used for antifungal activities. To refresh fungal strains, 13g/L nutrient broth in distilled water was prepared. Sterilized in autoclave and four flasks of 250ml were filled from broth, to each flask fungal colonies were inoculated separately. These flasks were then placed in incubator at 30°C for 3 days for refreshing fungal strains. SDA was used for growth of fungal strains. The flask was sterilized in autoclave at 121°C for 15 minutes at 1.5 pounds pressure. Antibiotic, Clotrimazole (Canesten) an antifungal drug was taken as a positive control and dissolved in distilled water (30µg/ 6µL) while DMSO was incorporated as negative control. About 7ml of medium was added to clean, dry and sterilized test tubes. Solutions of crude extracts and sub fractions were prepared each of 2µg/µL. One ml of sample (2µg/µL) was also added to each test tube, the test tube was kept in inclined position to make a slant. The same process was repeated for all of test tubes.

After cooling and solidifying, the fungi inoculums suspension was spread over the SDA medium uniformly using sterile cotton swabs. After that the test tubes were kept in incubator for 3 days at 30°C. After 3 days the fungal growth was observed in each test tube by the absence and presence of fungal strains.

Results and Discussion

In the present study, the antibacterial and antifungal activities of *A. indica, M. falcata* and *T. stans* were recorded against different bacterial strains including *E. coli, S. typhi, P. aeruginosa* and *S. aureus*, and fungal strains like *A. flavus, F. solani, A. fumigatus* and *A. niger*. The extracts of these plants showed variable activities.

Antimicrobial Activities

Antibacterial activity

The extract of *A. indica* was evaluated for *in vitro* antibacterial activity against, *E. coli, S. typhi, P. aeruginosa* and *S. Aureus* indicating different zones of inhibition (Table 1). Our results revealed that chloroform, butanol, ethyl acetate extract of *A. indica* showed significantly higher inhibitory activity (between 15-19 mm) against *E. coli, P. aeruginosa* and *S. aureus.* All extracts of *A. indica* showed activity (12-14 mm) against *S. typhi* (Table1). The present investigation has shown that the chloroform, butanol, ethyl acetate extract of *A. indica* have active phytochemical, which can inhibit the growth of pathogenic bacteria and fungi. Farhat et al., (2011) investigated that *Artemisia* spp. have great antibacterial and anti allergic potential due to the presence of large amounts of Flavonoids (Table 2).

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It was found that chloroform, butanol, ethyl acetate and n-hexane extract of *M. falcata* showed significantly higher zone of inhibition (17-19 mm) against *E. coli*. While all extracts showed moderate activity (12-13 mm) against strains of *P. aeruginosa*, *S. Typhi* and *S. aureus* (Table 1). According to Baloch *et al*., (2013), the antibacterial activity of the chloroform, butanol, ethyl acetate and n-hexane extracts of *Medicago* spp. possess good antibacterial activity against *Bacillus subtilis, E. coli, P. aeruginosa, S. typhi* and *S. aureus*. The antimicrobial activity of the tested extracts and fractions are comparable with the standard drugs. These activities may be due to strong occurrence of different chemical compounds such as flavonoids, tannins, alkaloids, steroids, phenols and saponins (Table 2)**.**

All four extract of *T. stans* showed significantly highest zone of inhibition (16-20 mm) against *S. aureus* and *E. coli*. Ethyl acetate and n-hexane extract showed no activity against *P. aeruginosa* and *S. typhi*, respectively (Table 1). Our results are supported by the study of Sing et al. (2011), in which he reported that ethanol, methanol and water extracts of *T. stans* can be effective against tested bacteria *(S. aureus, E. coli, P. aeruginosa* and *S. typhi.* Phytochemical analysis revealed the presence of alkaloids, flavonoids, saponins, phenols, steroids, anthraquinones and tannins (Table 2). The flavonoids from plant extracts have been found to possess antimicrobial and antioxidants properties in various studies (Amarlal et al., 2009; Lin et al., 2008). The alkaloids are also effective in their antimicrobial (Erdemoglu et al., 2007) and antioxidant (Benabdesselam et al., 2007) activities.

Antifungal activities

The ethyl acetate and butanol extracts of *A. indica* showed activity against all studied fungal strains. However, n-hexane and chloroform fractions did not show any activity against *A. flavus* and *F. solani*, respectively (Table 3). Similarly, the n-hexane and chloroform extracts of *M. falcata* showed activity against all studied fungal strains. Our results are also in line with the study of Juvatkar et al. (2012), who reported that the aqueous, chloroform and n-hexane extracts of *Artemisia* spp. Baloch et al. (2013) investigated that the antifungal activity of the different extract and different fractions from aerial parts of *Medicago* possess good antifungal activities against *Microsporum, Candida albicans, Aspergillus flavus, Fusarium solani, Aspergillus fumigatus* and *Candida glaberata*. shows significant antibacterial and antifungal activities. More significant antifungal activities of n-hexane and chloroform extract may be due to the combine effect of glycoside, saponins, alkaloid, tannin and flavonoids (Table 2). Furthermore, the phytochemical estimation of crude and its fractions showed the presence of alkaloids, flavonoids, phenols, tannins and diterpenes (Ireland, and Dziedzic, 1986) which contributes action against fungal strains. However, its ethyl acetate and butanol fractions did not show any activity against *F. solani* and *A. fumigatus* (Table 3).

Chloroform fraction of *T. stans* showed activity against all fungal strains, however its n-hexane and butanol fractions did not show activity against *F. solani and A. fumigatus.* Ethyl acetate fraction has only showed activity against *A. flavus*. The results are also supported by Farhat *et al*. (Farhat et al., 2011), who reported that *T. stans* is more active against *F. solani* and *A. niger*. It may be due to the presence of saponins in *T. stans*, which are naturally occurring surface active glycosides. Many pharmacological activities have been reported about saponins such as antibiotic, antifungal, antiviral (Ireland, and Dziedzic, 1986). Farhat et al. (2011) also confirmed the presence of significant saponins amount in *T. stans*.

Table 1: Zone of inhibition (mm) against four bacterial strains.

Plants	**Frictions**	***Escherichia coli***	***Pseudomonas aeruginosa***	***Salmonella typhi***	***Staphylococcus aureus***	**ANOVA**
Artemisia indica. L	n-hexane	18.66±1.15	15.66±0.577	13.33±1.15	18.66±1.15	$p < 0.01$
	Chloroform	17±1	17.33±1.15	14.33±1.15	15.33±0.57	$p < 0.05$
	Ethyl acetate	17.33±1.15	17±1	13±1	15.33±1.15	$p < 0.01$
	Butanol	15±1	17.33±0.57	13.66±0.57	19±1	$p < 0.01$
Medicago falcata. L	n-hexane	19.33±1.15	0	15.33±1.15	18.66±1.15	$p < 0.01$
	Chloroform	18.33±1.52	17.33±1.15	13.33±1.15	12±1	$p < 0.01$
	Ethyl acetate	19.66±0.57	14±0	11.33±0.57	0	$p < 0.01$
	Butanol	17.33±1.154	12.66±0.57	13.33±0.57	13±0.57	$p < 0.01$
Tecoma stans. L	n-hexane	15.66±0.57	0	0	16.33±0.57	$p < 0.01$
	Chloroform	15±1	13.66±0.57	11.66±0.57	18.33±1.52	$p < 0.01$
	Ethyl acetate	17.33±1.154	0	13±1	17.33±1.15	$p < 0.01$
	Butanol	16±2	19.66±0.57	15±1	19±1	$p < 0.01$
DMSO		0	0	0	0	
Standard drug (Levofloxacin)		21	21	22	23	

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Table 2: Review on the ethnomedicinal uses and phytochemical of studied plant species.

Plant Name	**Family**	**Part use/ Ethnomedicinal uses**	**Phytochemicals**	**Citations**
A. indica	Asteraceae	Leaves, stem, flower, seeds/ (i) Plant extract is used against intestinal worms, (ii) Whole plant decoction is used as a tonic, (iii) Leaves powder is used for gastric problems, (iv) Seed powder is taken orally to treat rheumatism, (v) Powdered seed paste is applied on teeth for pain relief, (vi) Good fodder for goats, (vii) Treats ear pain, (viii) The smoke of twigs is considered good for burns, (ix) Its infusion given as a depurative, (x) Used in treating Malaria and respiratory diseases	Flavonoids, Saponins, Essential oils, Terpenoids, Borneol, γ-cadinene, α-cadinol, Camphene, Camphor, Chrysanthenone, Arnesene, Limonene, β-thujone, alcohol borneol, ,1,8-cineole, p-cymene, β-eudesmol, α-gurjunene, α-pinene, terpene-4-ol	Nadeem et al 2013 Farhat et al 2011
M. falcata	Fabaceae	Seeds, leaves, sprouted seeds, root, flowers (i) Above ground parts are grind to prepare paste with water and applied on wounds, (ii) Dry or fresh leaves are used as tonic, (iii) Plant is taken orally for treating anaemia, diabetes, asthma, ulcers, rheumatism, colitis, haemorrhage, menopausal complaints, pre-menstrual tension, fibroids etc, (iv) A poultice of heated leaves is used in earache	Flavonoids, apigenin, luteolin, tricin, apigenin glycosides, luteolin, glycosides, chrysoeriol, Alkaloids, Flavonoids, Saponins, Phenols, Steroids, Anthroquinones, Tannin	Sylwia et al., 2010 Nizam et al 2013
T. stans	Bignoniaceae	Entire plant ,leaves, bark, and roots (i) Bark used as muscle relaxant, mild cardio tonic and lowering chloretic activity, (ii) Root is reported to be a powerful diuretic, vermifuge and tonic, (iii) Decoction of roots with lemon juice is used in small quantities as remedy for snake and rat bites and also externally applied on wounds, (iv) Leaves are given in diabetes	Tecomanine, Tecostanine, Anthranilic acid, Alkaloids, Flavonoids, Saponins, Phenols, Steroids, Anthroquinones Tannin	Singh et.al.2011 Farhat et al 2011

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Table 3: Activities of different plant extracts against four fungal strains.

Plants	**Fractions**	***Aspergillus flavus***	***Aspergillus niger***	***Aspergillus fumigatus***	***Fusarium solani***
A. indica.	n-hexane	-	+	+	+
	Chloroform	+	+	+	-
	Ethyl Acetate	+	+	+	+
	Butanol	+	+	+	+
M. falcata.	n-hexane	+	+	+	+
	Chloroform	+	+	+	+
	Ethyl Acetate	+	+	+	_
	Butanol	+	+	-	+
T. stans	n-hexane	+	+	+	_
	Chloroform	+	+	+	+
	Ethyl Acetate	+	_	_	_
	Butanol	+	+	_	+
Standard drug Clotrimazole (Canesten)		+	+	+	+

+ Presence of antifungal activity
-- Absence of antifungal activity

Conclusions

It can be concluded that most fraction of medicinal plant *A. indica, M. falcata and T. stans* showed potential antimicrobial activities against the tested bacterial and fungal strains. The antimicrobial activities may be due to strong occurrence of active compounds i.e. saponins, tannins, alkaloids, steroids, phenols and flavonoids. Results of our findings confirmed the use of *A. Indica, M. falcata* and *T. stans* as traditional medicine. However, these medicinal plant species may be subjected to detailed phytochemical and pharmacological studies in order to find out new drugs against pathogenic bacterial and fungal strains.

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