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In Vitro Antioxidant, Antifungal and Cytotoxic Activities of Selected Medicinal Plants of District Bannu and Lakki Marwat

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ABSTRACT In the present study, the researchers selected four plants from District Lakki Marwat and Bannu. The purpose of the study was evaluate the methanol extracts of these medicinal plants, namely, *Tamarix aphylla, Euphorbia helioscopia, Coriandrum sativum* and *Peganum harmala* for antimicrobial, antioxidant, and cytotoxic activities. Plants dried in shade, were ground and obtained their methanol extracts. The extracts were evaluated for biological assays using the standard protocol for in vitro study. For the study of cytotoxic properties of plants methanol extracts were used against brine shrimps and their antimicrobial activities were also measured. Antioxidant activities against different free radicals were also checked. Brine shrimps showed seventy percent mortality rate to *Tamarix aphylla* extract having concentration of 500 µg/ml. The scavenging activity for free radicals was also significant at 500µg/ml. The present study revealed that these activities may be owing to the presence of bioactive constituents, isolation and purification of which is necessary.

INTRODUCTION

Medicinal plants play an important role in prevention of many long -lasting diseases and improvement of human health. Since long, they have helped humanity in maintaining their health and have assisted them in everyday life including cosmetics, dyes, beverages, medicines and oils. Medicinal plants have bioactive constituents like steroids, flavonoids, ascorbic acid, saponins and phenolic compounds (Nabavia et al. 2015; Khan et al. 2016). Products of plants' origin have an important role in obtaining antibiotics and drugs used against cancer (Shameem et al. 2015; Shaikh et al. 2016). According to Khan et al. (2015) medicinal plants and their bioactive compounds yield seventy-eight percent drugs. These evidences suggest screening of different plants for bioactive constituents and important metabolites (Bushra et al. 2013). In developing countries various infectious diseases are the main cause of death (Ambrus 2004). Nowadays, various bacterial species are resistant against antibiotics and spread various diseases (Sakoulas and Moellering 2008; Howden et al. 2010). The development of resistance by certain microorganisms against antibiotics, the side effects of these antibiotics and the reappearance of previous less frequent infections, it is necessary to have new antimicrobial drugs of plants origin (Ruiz-Bolivar et al. 2008). Oxidation of lipids, in many food products, can be prevented by using plant extracts like green tea and grape seeds (Rababah et al. 2004). The study was carried out for evaluation of antimicrobial, antioxidant and cytotoxic activities of *Tamarix aphylla*, *Euphorbia helioscopia*, *Coriandrum sativum* leaves and *Peganum harmala* seeds methanol extracts.

Aim of Study

The aim of this work was to evaluate some of the selected plants of the area for their antimicrobial, antioxidant and cytotoxic activities.

METHODOLOGY

Plant Collection

Tamarix aphylla, *Euphorbia helioscopia*, *Coriandrum sativum* leaves and *Peganum har-*

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mala seeds were collected from District Bannu and Lakki Marwat during March to May 2013. Collected plants were shade dried, at ordinary room temperature, for 5 days and finely ground into powder.

Plant Extract Preparation

For this purpose 200 g powder of each sample was mixed with seventy percent (70%) methanol and thoroughly stirred. Qualitative Whatman filter papers were used to filter the extracts after 72 hours. The filtrate thus obtained was kept at 40 °C in vise bath to evaporate the whole methanol. For future use the extracts were preserved at 4 °C in refrigerator.

Cytotoxic Brine Shrimp Lethality Test

The test was conducted using the protocol of Meyer- Alber et al. (1992). Four (04) fractions of each of the four plant extracts, *Tamarix aphylla* (TAME), *Euphorbia helioscopia* (EHME), *Coriandrum sativum* (CSME) and *Peganum harmala* (PHME), were prepared from stock solution having concentrations of 500, 250, 150 and 100µg/ml using the formula M1V1 =M2V2. For preparation of culture medium for shrimp hatching 5 g of sea salt was dissolved in 250 ml distilled water and stirred thoroughly for 2 hours using magnetic stirrer. Brine shrimps were allowed to hatch in trays containing two compartments having sea salt saline. The eggs were sprayed in the dark portion and after 24 hours of hatching larvae were collected from the illuminated portion of the tray. The test tubes samples were marked as 50, 100, 250 and 500 µg/ml. For this purpose half ml of each of the solution (1 mg/ml, 0.5 mg/ml, and 0. 25 mg /ml) were placed in vial and allowed to evaporate the solvent. The remaining extract was dissolved in 2 ml saline. Healthy shrimps were taken in each vial; increased their final volume to 5 ml and incubated at temperature of 27 °C. Survival rates were counted after the incubation of 24 hours, 48 hours and 72 hours using magnifying glasses and percentage mortality rate was calculated using Abbot's formula: Percentage of death = (Sample-control/control) × 100.

Antibacterial Assay

Different fractions of the sample extracts that range from 0.86, 1.875, 3.75, 7.5, 15 and 30 mg/ml were made in distilled water. The antibacterial potentiality of different concentrations of *Tamarix aphylla*, *Euphorbia helioscopia*, *Coriandrum sativum* leaves and *Peganum harmala* seeds methanol extracts were determined using the standard bioassay of Bagamboula et al. (2003) against *Escherichia coli* and *Staphylococcus aureus*.

Antifungal Bioassay

The plant extracts were screened for antifungal activity through dilution of agar tubes according to the protocol used by Duraipandiyan and Lgnacimuthu (2009).

DPPH Radical Scavenging Activity

Duraipandiyan and Lgnacimuthu (2009) protocol was followed to prepare stock solution by dissolving 2.4 mg DPPH in 100 ml methanol. Dilution of stock solution with methanol for obtaining absorbance density less than 1.00 was done using spectrophotometer at 517 nm wavelength. Hundred microliter (100 µl) sample solution was added to 3 ml prepared solution (1:100 µg/ml) and measured its optical density at wavelength of 517 nm. Percentage of Inhibition of free radicles was measured using the formula: Scavenging property (expressed in percentage) = [(control O.D - sample O.D) / (control O.D)] ×100. Graph Prism Pad Software was used to obtain IC50 value.

ABTS Radical Scavenging Assay

For preparation of stock solution Equal volumes of ABTS (7 mM) solution and potassium per sulfate (2.45 mM) solution were mixed together for preparation of stock solution (Re R et al. 1999). To obtain ABTS•+ radicals containing dark solution, the stock solution was kept in darkness at room temperature for twelve hours. For preparation of solution for working, having an initial absorbance of 0.700 (± 0.02) at 745 nm, stock solution and 50% methanol were mixed together at controlled temperature of 30°C. For determination of free radical scavenging activity 300 µl of different fractions (50 to 500 µg /ml in methanol) were added to 3.0 ml of ABTS one by one and absorbance decrease was measured after 1 minute and 6 minutes. In the experiment ascorbic acid was used as a positive control. Based on the percentage of ABTS radicals scavenged the scavenging effect was measured by the formula given below: Scavenging activity (%) = [(control absorption " sample absorption) / control absorption)] × 100.

Determination of Superoxide Radical Scavenging Assay

For preparation of working solution 1 ml of solution of Nitro Blue Tetrazolium (l M NBT in 100 mM buffer of phosphate, pH 7.4), concentrations of various samples and ascorbic acid o.1 ml (50 mM buffer of phosphate, pH 7.4) and NADH solution (l M NADH in 100 mM buffer of phosphate, pH 7.4) 1 ml (Nishiki et al. 1972). Hundred micro litters (100ìl) of PMS solution (60ìM PMS in 100 mM buffer of phosphate, pH7.4) were mixed together and allowed to react. Bright light was used for 15 minutes to illuminate all the tubes. Optical density of the mixture was recorded before and after illumination at wavelength of 530 nm. Superoxide release inhibition was measured by comparing the optical densities of experimental to the control. The scavenging property of superoxide radical was calculated by using the formula: Percentage scavenging = (1– absorbance with sample / absorbance without sample) x 100

Statistical Analysis

To determine various parameters of the plant extract treatment effects, Microsoft Excel and Graph Prism Pad were used to calculate the mean and standard deviation.

RESULTS

Cytotoxic Activity (Survival %)

The cytotoxic activity of the extracts was recorded after 24, 48 and 72 hours of incubation and observed that the survival percentage was negatively related to the extract concentrations and the duration of incubations. These results show that methanol extracts of *Tamarix aphylla* (TAME), *Coriandrum sativum* (CSME), *Euphorbia helioscopia* (EHME) and *Peganum harmala* (PHME) possess significant cytotoxic activities against the growth of brine shrimps as shown in Table 1. The data revealed that at concentration of 01mg/ml, zero percentage survival rates were found after 72 hours in *Tamarix aphylla* methanolic extract, *Coriandrum sativum* methanolic extract and *Euphorbia helioscopia* and 1.2 percent survival rate was found in *Peganum harmala* extract. The order of percentage of survival rates of *Tamarix aphylla*, *Coriandrum sativum*, *Euphorbia helioscopia* and *Peganum harmala* methanolic extract is shown in Table 1.

Antibacterial Assay

Tamarix aphylla, *Coriandrum sativum*, *Peganum harmala* and *Euphorbia helioscopia* methanol extracts were also used for screening of antibacterial activity as tabulated in Table 5. Growth of *Staphylococcus aureus* (Gram-positive bacteria) and *Escherichia coli* (Gram-negative bacteria) was significantly retarded by *Peganum harmala* followed by *Euphorbia helioscopia* and Coriandrum sativum. Growth inhibition in *S. aureus* was recorded for all concentrations in case of *Peganum harmala*, from 30 mg/ml to 3.7 mg/ml in *Euphorbia helioscopia* and from 30 mg/ml to 7.5 mg/ml in *Coriandrum sativum* methanolic extracts. Similarly, inhibitory activities against Gram-negative bacteria *E. coli* were recorded from 30 mg/ml to 1.8 mg/ml in case of *Peganum harmala*, 30 mg/ml to 3.7 mg/ml in *Euphorbia helioscopia* and 30 mg/ml to 15 mg/ml in *Coriandrum sativum* methanolic extracts. The methanolic extracts of *Tamarix aphylla* showed no inhibitory activity against the growth of the two test bacteria at all concentrations. This data shows the presence of bioactive metabolites, in the methanol extracts of the three plants, having inhibitory efficacy against the two test bacteria (Table 2).

Table 1: Survival and mortality of brine shrimps in the presence of various concentrations of plant extracts

Concentration	*Tamarix aphylla*		*Euphorbia helioscopia*		*Coriandrum sativum*		*Peganum harmala*	
	% Survival	% Death	% Survival	% Death	% Survival	% Death	% Survival	% Death
50 ìg/ml	90	10	90	10	100	0	100	0
100 ìg/ml	70	30	80	20	90	10	80	20
250 ìg/ml	50	50	60	40	70	30	60	40
500 ìg/ml	30	70	40	60	50	50	30	70

Table 2: Antibacterial activity of methanolic extract (mm)

	E. coli						*S. aureus*					
	30mg/ml	*15mg/ml*	*7.5mg/ml*	*3.75mg/ml*	*1.875mg/ml*	*0.86mg/ml*	*30mg/ml*	*15mg/ml*	*7.5mg/ml*	*3.75mg/ml*	*1.875 mg/ml*	*0.86 mg/ml*
Tamarix aphylla	-	-	-	-	-	-	-	-	-	-	-	-
Euphorbia helioscopia	15.2±1.5	13.5±1.2	12.4±0.8	9.4±0.5	-	-	16.5±0.5	13.5±0.4	10.4±0.5	9.4±0.6	-	-
Coriandrum sativum	13.5±2.4	10.1±1.5	-	-	-	-	16.3±0.8	11.4±0.9	9.4±1.0	-	-	-
Peganum harmala	30.5±2.7	25.4±1.4	18.4±1.0	11.1±0.8	9.1±0.9	-	30.5±1.8	25.4±0.8	20.4±0.8	17.4±0.9	13.1±1.0	9.1±1.0

Mean± SEM

Antifungal Activities

For screening of the plant extracts for antifungal activities *Tamarix aphylla*, *Coriandrum sativum*, *Peganum harmala* and *Euphorbia helioscopia*, 67 µl (200 µg/ml) of each of the plant extract CSME, PHME, EMME and TAME, 67 µl of the DMSO (99.9%) and 367 µl (200 µg/ml) of the terbinofine were used. The *Tamarix aphylla* methanol extract (TAME) showed significant antifungal activity, that is, seventy, fifty-five and fifty-four percent against *Aspergillus flavius*, *Aspergillus niger*, *Aspergillus fumigatus* respectively. *Euphorbia helioscopia* methanol extract (EHME) showed significant antifungal activity, that is, 60.2, 42.1 and 55.2 percent against *Aspergillus flavius*, *Aspergillus niger*, *Aspergillus fumigatus* respectively. *Coriandrum sativum* methanol extract (CSME) showed significant antifungal activity, that is, seventy-five, 65.2 and forty percent against *Aspergillus flavius*, *Aspergillus niger*, *Aspergillus fumigatus* respectively. The Methanol extract of *Peganum harmala* (PHME) showed significant antifungal activity, that is, forty-five, 55.2 and seventy percent against *Aspergillus flavius*, *Aspergillus niger*, *Aspergillus fumigatus* strains, respectively. Likewise, terbinofine, used as positive control indicated high activity against all these fungi and the DMSO used as negative control indicated lowest growth retardation potency against all the experimental fungal strains (Table 3).

DPPH Radicle Scavenging Activity

The researchers used DPPH radical scavenging assay for comparison of the antioxidant activity. The free radical scavenging activity of the standard ascorbic acid and 1,1- diphenyl 2-picryl-hydrazyl (DPPH) of the samples extracts *Tamarix aphylla*, *Coriandrum sativum*, *Peganum harmala* and *Euphorbia helioscopia* were recorded. It was observed that the scavenging property of the samples extracts was always slightly less as compared to the ascorbic acid (standard) as given in Table 4.

ABTS Scavenging Activity

The researchers used ABTS radical scavenging assay for comparison of the antioxidant activity which can be equally applied to lipophilic and hydrophilic antioxidants. The samples' extracts and the standard ascorbic acid ABTS (2,2,

Table 3: Antifungal activity of TA, EH, CS and PH methanol extract % inhibition:

Strain	*Terbinafine*	*Tamarix aphylla*	*Euphorbia helioscopia*	*Coriandrum sativum*	*Peganum harmala*	*DMSO*
Aspergillus niger	99.40±5.5	70.0	60.2	75.0	45.0	2.0±0.6
Aspergillus flavius	98.07±3.7	55.0	42.1	65.2	55.2	10.0±0.5
Aspergillus fumegatus	99.02±2.0	54.0	55.2	40.0	70.0	10.0±0.8

Mean±SD

azo-bis (3-ethyl benzothiazoline-6-sulphonic acid)) free radicals scavenging activities were recorded and observed that the scavenging activity of the standard ascorbic acid was always slightly greater than the sample extracts as given in Table 5.

Superoxide Radical Scavenging Activity

It is the reactive oxygen species (ROS) that is toxic and causes harm to many cell constituents and may causes many fetal diseases. Table 6 shows the scavenging activities of various con-

Table 4: Comparison b/w *Tamarix aphylla, Euphorbia helioscopia, Coriandrum sativum and Peganum harmala methanolic* extracts and ascorbic acid scavenging activity for DPPH free radicals

Concentration (in µg/ml)	*% TA Scavenging*	*% EH Scavenging*	*% CS Scavenging*	*% PH Scavenging*	*% As corbic acid Scavenging*
50	24.0±0.1	26.0±0.2	22.2±0.0	28.1±0.3	58.0± 0.03
100	45.1±0.2	47.2±0.2	42.0±0.0	48.2±0.0	78.1± 0.06
150	54.1±0.3	56.1±0.3	50.2±0.2	55.1±1.0	80.1± 0.05
200	64.0±0.4	66.2±1.0	55.3±0.5	65.3±2.0	82.04± 0.02
250	70.5±1.0	71.1±2.0	64.0±0.1	71.2±3.0	85.13± 0.1
500	75.5±1.0	74.0±3.0	70.2±3.0	77.0±3.0	94.6± 0.08

Mean± SEM

Table 5: Comparison b/w *Tamarix aphylla, Euphorbia helioscopia, Coriandrum sativum and Peganum harmala* methanolic extracts and ascorbic acid scavenging activity for ABTS free radicals

Concentration (in µg/ml)	*% TA Scavenging*	*% EH Scavenging*	*% CS Scavenging*	*% PH Scavenging*	*% As corbic acid Scavenging*
50	25.1±0.1	27.0±0.2	24.2±0.0	24.1±0.3	58.0± 0.03
100	46.1±0.2	48.2±0.2	44.0±0.0	46.2±0.0	62.1± 0.06
150	53.0±0.3	55.1±0.3	50.2±0.2	57.1±1.0	70.1± 0.05
200	62.2±0.4	65.2±1.0	57.3±0.5	62.3±2.0	75.04± 0.02
250	68.4±1.0	72.1±2.0	65.0±0.1	71.2±3.0	83.13± 0.1
500	71.4±1.0	76.4±3.0	72.2±3.0	74.0±3.0	91.6± 0.08

Mean± SEM

Table 6: Comparison b/w *Tamarix aphylla, Euphorbia helioscopia, Coriandrum sativum and Peganum harmala methanolic* extracts and ascorbic acid scavenging activity for hydrogen per oxide free radicals

Concentration (in µg/ml)	*% TA Scavenging*	*% EH Scavenging*	*% CS Scavenging*	*% PH Scavenging*	*% As corbic acid Scavenging*
50	20.1±0.1	25.0±0.2	26.0±0.0	26.1±0.3	59.0± 0.03
100	33.1±0.2	35.0±0.2	45.0±0.0	47.2±0.0	63.1± 0.06
150	41.0±0.3	40.0±0.3	49.0±0.2	58.1±1.0	72.1± 0.05
200	55.2±0.4	55.2±1.0	56.2±0.5	63.3±2.0	77.04± 0.02
250	61.4±1.0	60.2±2.0	60.3±0.1	72.2±3.0	84.13± 0.1
500	65.4±1.0	66.1±3.0	71.2±3.0	77.0±3.0	94.6± 0.08

Mean± SEM

centrations of *Tamarix aphylla*, *Coriandrum sativum*, *Peganum harmala* and *Euphorbia helioscopia* methanolic extracts that demonstrated highest superoxide radical scavenging potentials respectively.

DISCUSSION

Medicinal plants have fundamental contributions in curing of various human diseases owing to the possession of various biologically active constituents such as heart diseases, oxidative stress, inflammation and cancer (Shameem et al. 2015; Shaikh et al. 2016). Due to fewer side effects as compared to synthetic drugs medicinal plants are being frequently used. Traditional medicines may be used as an alternative throughout the world. Pakistan having abundant medicinal plants, diseases of heart, skin and digestive system are frequently treated through folk medicines by local healers (Abou-Donia et al. 2009). Due to the inhibition of growth of microorganism medicinal plants are also very important. The results obtained from methanol extracts showed inhibition of bacterial growth up to a significant level. *Tamarix aphylla*, *Coriandrum sativum*, *Peganum harmala* and *Euphorbia helioscopia* methanol extracts showed significant antioxidant and antifungal activities. Such significant outcomes were also found by Khan et al (2015). *Datura alba* and *Withania somnifera* possess bioactive components, which have significant antibacterial activities (Abou-Donia et al. 2009) that agrees with our findings. The data revealed the survival rate of brine shrimps as shown in the Table 1. The outcomes presented shows that the rate of survival is negatively related to the concentration of the extract used and duration of the incubation. Rabash et al. (2004) also reported similar results from the organic fractions of *Rubus imperialis*. Likewise Zaidi et al. (2006) reported that high doses of methanol extract of *Arceuthobium oxycedri* exhibited one hundred percent lethality for brine shrimps; these findings are in accordance to our results. The result obtained shows that the methanol extracts of these plants have also free radicals scavenging activities which are similar with the work already done by Re R et al. (1999), Rababah et al. (2004) and Khan et al. (2010).

CONCLUSION

Results of the current project revealed that medicinal plant extracts of *Tamarix aphylla*, *Euphorbia helioscopia*, *Coriandrum sativum* and *Peganum harmala* have significant antioxidant, cytotoxic and antimicrobial activities. They are source for many biological constituents and thus provide important basis for further investigations leading to isolation, purification, characterization and mechanism of antimicrobial and cytotoxic compounds from the plants under study.

RECOMMENDATIONS

The result of the study shows that the plants under study have significant antimicrobial, antioxidant and cytotoxic activities which can be further processed for the extraction, isolation and purification of potential antibiotics, fungicides and insecticides.

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