INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 18, No. 5, p. 86-99, 2021

RESEARCH PAPER OPEN ACCESS

Antioxidant, Antimicrobial and Cytotoxic Potential of Selected Medicinal Plants Collected from Khanewal Valley, Pakistan

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Key words: Medicinal plant, Antimicrobials, Antioxidants, Cytotoxic, Radical scavenging, Phytochemicals, Phytomedicine.

http://dx.doi.org/10.12692/ijb/18.5.86-99 Article published on May 16, 2021

Abstract

The increasing demand for novel therapeutics as antimicrobial and anti-oxidant agents, renaissance the interest towards medicinal plants. Based on their long-term application, there is a general belief that herbal remedies are safe. The research was conducted to evaluate the antimicrobial, antioxidant, and cytotoxic properties of *Medicago denticulata, Terminalia arjuna, Pyrus pashia and Schinus molle*. These plants were collected from district Khanewal and their extracts were tested for antimicrobial activity against ATCC (American Type Culture Collection) strains of *Bacillus spizizenii*, *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli* and *Salmonella typhimurium* using well diffusion method. Antifungal activity was performed against *Aspergillus niger*, *Aspergillus flavus*, *Fusarium solani*, *Aspergillus fumigatus* and *Mucor* using the agar tube dilution method. The extracts were also tested for their antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay and cytotoxic activity using brine shrimp. Among all the plants, *T. arjuna* fruit extract exhibited the highest antibacterial activity against *S. aureus* and *B. spizizenii* (MICs 82µg/100**µl** and 162µg/100**µl**) and Gram-negative bacteria with MICs >1.5mg/100**µl**. All the tested strains of fungi were inhibited by *T. arjuna*. In the DPPH assay, the extract of *S. molle* showed free radical scavenging at a concentration of 16µg/ml while effective (>80%) scavenging free radical activities of *P. pashia* and *T. arjuna* leaf extract were at a concentration of 161 µg/ml. Cytotoxicity against brine shrimp larvae was reported only for the extract from *T. arjuna* fruit and root. The therapeutic potential of the plants studied, provides scientific evidence for the support of using *M. denticulata*, *T. arjuna*, *P. pashia* and *S. molle* in traditional medicine and these plants have potential applications as bioactive agents for the treatment of various diseases.

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Introduction

Medicinal plants are an effective and potential source of drugs in developing countries for combating different contagious diseases. As medicinal agents are present in the plant metabolites (Sultana and Muhammad Asif, 2017). Many people still rely on the drugs obtained from the plant for primary health care. Therefore, it is important to provide a scientific basis for the possible use of traditional medicine in the prevention and treatment of diseases such as tumors, oxidative stresses and those caused by microorganisms. The antimicrobial properties of medicinal plants have been investigated for many years throughout the world and this knowledge is utilized in phytomedicine (Andrew and Izzo, 2017). Therefore, further research in this field will help in a better understanding of plant properties, their safe use and efficacy in medicine (Manach *et al.*, 2017).

World health organization (2004) reported that 80% of the world's population particularly in developing countries is still using medicinal plants as a traditional remedy for the treatment of different diseases. Due to the emergence of resistance against antibiotics and their numerous side effects, the development of new drugs is required (Rampioni *et al.*, 2017). However, designing new drugs against these resistant microbes is challenging and time-consuming, so attention is diverted to look for herbal medicine and biologically active compounds, their isolation and characterization from plants (Rossiter *et al.*, 2017). Treatment of microbial infections bacterial, fungal, and viral selects for the emergence of resistant organisms that may be rare in the initial population but become increasingly prevalent under selective drug pressure (Gupta and Birdi, 2017; Sommer *et al.*, 2017). Examples of microorganisms that show resistance include *Staphylococcus aureus, Pseudomonas aeruginosa, Shigella dysenteriae, Escherichia coli, Salmonella typhi, Salmonella enteritidis, Streptococcus faecalis, Proteus* sp. and *Candida albicans* (Enioutina *et al.*, 2017). Antimicrobial compounds from higher plants may offer a new mode of action than the antibiotics already in clinical use and thus they may present great clinical significance in the treatment of resistant microbes (Singh and Yeh, 2017; Zarins-Tutt *et al.*, 2017). Many fungal strains developed resistance against various fungicides as a result of continuous usage. It necessitates the search for alternate options to control fungal infections (Niemirowicz *et al.*, 2017). The free radicals in the body such as reactive oxygen and nitrogen species are responsible for heart diseases, cancer and the aging process (Tripathy and Mohanty, 2017). Besides antimicrobial activities, some natural products have abilities to control free radicals, so have anti-cancer and anti-oxidant properties. Natural products have a long history to be used as anti-cancerous agents, started with traditional medicine. Numerous studies reported anticancer agents from plants and many antioxidants and antimutagens have been proposed as a cancer preventive and therapeutic agents (Estrela *et al.*, 2017; Srinivasan, 2017). Pakistan is a rich country for natural products. According to a study conducted by Hussain *et al* (Hussain *et al.*, 2017), there are about 6000 flowering plants present in Pakistan and among them, about 400–600 are medicinally important. Considering the widespread diversity and potential of local flora, samples of selected plants were collected to determine the unexplored properties of the 04 selected plants i.e. *Medicago denticulata, Terminalia arjuna, Pyrus pashia and Schinusmolle* collected from district Khanewal as given in Fig. 1.

T. arjuna is an important medicinal plant and is a well-known cardiotonic (Bhat *et al.*, 2017; Shengule *et al.,* 2019). Bark powder is reported to cure headache, to clean teeth and kill dental worms (Alamgir, 2017). *T. arjuna* is also used for the treatment of fever and also to control high blood pressure. *P. pashia*is used in treatment of digestive and gastric ailments (Bao *et al.*, 2017; Ain and Khan, 2019). *P. pashia leaves* are consumed by some communities in butter tea beverages (Pandey *et al.*, 2017). *S. molle* (Feuereisen *et al.*, 2017; Garzoli *et al.*, 2019) in different pharmacological studies showed hypotensive (DeLima *et al.*, 2017), antibacterial (Eryigit *et al.*, 2017), antifungal, antitumor (Abdel-Hameed and Bazaid, 2017), anti-inflammatory and antidepressant

effects (Al-Zubairi *et al*., 2017; Rabiei and Rabiei, 2017). *Medicago* species are reported as good-quality feed/forage for domestic livestock, phytoremediation and a source of phytochemical agents (Gholami *et al*., 2014). Alfalfa (*Medicago* spp.) is reported to help in human health problems (Avato *et al*., 2017).

Due to the medicinal importance of the selected plants, their extracts were obtained from different parts of the plants using organic solvents and were evaluated for their antimicrobial, antioxidant and cytotoxic properties.

Materials and methods

Four plants used in the study were collected from Khanewal District, Pakistan. Different parts of the plant extracts were obtained with different solvents like Methanol, Dichloromethane and Methanol: Chloroform (1:1). Table 1 shows the botanical name, plant part used extraction solvent and abbreviation used for the plants under study for their antimicrobial, antioxidant, and cytotoxic properties. The study was carried out at the Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan.

Antibacterial assay

Antibacterial assay with various fractions of plant extracts was carried using the agar well diffusion method. Stock solutions of all the samples were prepared by dissolving 30mg of plant extract in 1ml of Dimethyl sulfoxide (DMSO). The stock solutions were further diluted, and 4 dilutions of each extract were prepared using the two-fold dilution method. The volume of each extract used was 100µl per well with concentration of 30 mg/ml, 15mg/ml, 7.5mg/ml and 3.75mg/ml. Nutrient agar medium (MERCK USA) was used for the growth of five different bacterial strains used in the study including G +ve strains *Bacillus spizizenii* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538) and *Staphylococcus epidermidis* (ATCC 12228) and two G -ve strains i.e. *Escherichia coli* (ATCC 10536) and *Salmonella typhimurium* (ATCC 14028). These bacterial strains were streaked on the agar plates, incubated overnight and were used for antibacterial assay by agar well diffusion method. McFarland 0.5 was prepared by adding 0.5ml of 0.048M $BaCl_2$ to 99.5ml of 0.36N H_2SO_4. This standard was used to compare the turbidity of bacterial culture. McFarland turbidity standard (4 to 6ml) was taken in the screw-capped test tube and bacterial culture turbidity was compared. The test strains from nutrient agar medium were diluted with sterilized normal saline solution (0.9% NaCl w/v) and its turbidity was corrected by using McFarland 0.5 turbidity standard [10^6 (CFU) per ml density]. The freshly prepared bacterial strains were used to make bacterial lawn on Mueller Hinton agar containing Petri plates. The plates were seeded with inoculums of different freshly prepared strains of bacteria with the help of sterile cotton swabs. Ten wells per plate were made using a sterile steel borer (8mm). Aliquots of 100µl of each plant extract (prepared in DMSO) with different dilutions were poured in respective well, then labeled. Erythromycin (1mg/ml) was used as positive control and DMSO was used as a negative control. After 24 hours of incubation at 37°C zone of inhibition (clear zones) was measured. Bactericidal and bacteriostatic activity of respective plant extracts was determined by measuring the zone of inhibition. Finally, minimum inhibitory concentration (MIC) was determined for all the extracts with antibacterial activities. The assay was performed in triplicate.

Antifungal assay

For the antifungal assay of the plant extracts, the agar tube dilution method was used (Favre-Godal *et al*., 2019). The fungal strains obtained from the First Fungal Culture Bank of Pakistan (FCBP) used in this study were *Aspergillus niger*198, *Aspergillus flavus* 198, *Fusarium solani* 291, *Aspergillus fumigatus* 66 and *Mucor* 300. Fungal strains were grown on potato dextrose agar media (MERCK) and the same medium was used for inoculum preparation.

The extracts were diluted from an initial stock solution of 15mg/ml to get a final concentration of 250µg/ml for antifungal assay. Nystatin 100000 Units/ml and DMSO (67µl/ml) were used as positive and negative control respectively.

Potato dextrose agar PDA (4ml) was added to autoclaved cotton plugged test tubes and these tubes were marked to 10cm height these test tubes were cooled up to 50°C after autoclaving and then seeded with plant extracts to get a final concentration of 0.25mg/ml of the extract in each tube. The test tubes were placed in a slanting position and allowed to solidify at room temperature. Inoculation was done at the center of each tube by taking a 4mm piece of each fungal mycelium. The mycelium block was prepared with the help of a cork borer from the growing area of a 7 days old culture of the test fungi on PDA. The inoculated plates were incubated at 30°C for 48 hours. Proper negative control (PDA without extract only DMSO) and positive control (PDA Nystatin 50µl) were also maintained. After 48 hours linear growth of the fungus was calculated in the slant. Growth inhibition was also calculated with reference to negative control of DMSO. The experiments were performed in triplicate against each fungal strain. The percentage inhibition of mycelial growth of the test fungus was calculated by the following formula:

$$\text{Inhibition of fungal growth } (\%) = \frac{(10 - \text{Linear growth in test (cm)})}{\text{Linear growth in negative control (cm)}} \times 100$$

Determination of Minimum Inhibitory Concentration (MIC): The MIC for each test organism was determined by following the modified agar well diffusion method.

The MIC, taken as the lowest concentration of the test extract that completely inhibited the growth of the microorganism, shown by a clear zone of inhibition (>8 mm), was recorded for each test organism.

Antioxidant assay

The free radical scavenging activity was measured by using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay. Different concentrations of test samples were prepared from the stock solution (10mg/ml in 95%, methanol) (see Table 2). Ascorbic acid was used as a reference for comparison in the assay.

The DPPH stock solution absorbance was adjusted to about 0.980 (±0.02) at wavelength 517nm using the spectrophotometer. An aliquot (3ml) of this working solution was mixed with 100µl of the plant samples at five different varying concentrations (4-322µg/ml). The solution in test tubes was shaken well and put in the dark for 15 minutes at room temperature. Then again, the absorbance was measured at 517nm. The percent radical scavenging activity was determined based on the percentage of DPPH radical scavenged by using the following equation;

$$\text{Scavenging effect } (\%) = \frac{(\text{control absorbance} - \text{sample absorbance})}{\text{control absorbance}} \times 100$$

Brine shrimp cytotoxicity assay

The brine shrimp lethality bioassay was used to determine the preliminary cytotoxic potential of the plant extracts (Naher, Aziz *et al.* 2019). Each plant extract was dissolved in DMSO to prepare the stock solution (10mg/ml). Cytotoxicity was measured by transferring of 100, 10 and 1µl from the stock solution to vials corresponding to 200µg/ml, 20µg/ml and 2µg/ml respectively. In each vial 2ml of saline was added and then with the help of Pasteur pipette, 10 shrimps were transferred in each vial. Raised the volume of saline in each vial to 5ml and incubated at 25°C. After 24 and 48hour of incubation number of survivors was counted using a magnifying glass. Then by using Abbot's formula calculations were made as below:

$$\text{Death } (\%) = \frac{(\text{number of survivors in control} - \text{number of survivors in sample})}{\text{number of survivors in control}} \times 100$$

Statistical analysis-

Data analysis was performed with Microsoft Excel 2010. All experiments were conducted in triplicates. Values were expressed as means ± SE.

Results

The work describes antimicrobial, antioxidant and cytotoxic properties of extracts from *M. denticulata, T. arjuna, P. pashia*and *S. molle* collected from District Khanewal.

Antibacterial activity

The antibacterial activity of the plant extracts under study was determined. Bactericidal activity, shown as

a zone of inhibition and MIC values of different extracts from different parts of the selected plants at a concentration of 3 mg/100µl is summarized in Tables 3 and 4 respectively. MTAF i.e. methanol extract of *T. arjuna* fruit showed antibacterial activity in the agar well diffusion assay against all tested strains and the lowest MIC value was found against *S. aureus* **(**82µg/100µl). But from overall MIC values, it is clear that Gram-positive bacteria are more sensitive than Gram-negative bacteria to this plant extract. Plant extracts MMD, DMD and MTAL, MTAR and MCPPS showed antibacterial activity against at least one strain of bacteria and their respective MIC value are given in Table 4. The extracts MPPS, MPPL and MPPF, MSML and MCSML showed no antibacterial activity. Strong bacteriostatic activities were observed in the case of MMD and DMD while no activity was observed in the case of MPPL, MPPF and MCPPS.

In the case of bacteriostatic activity re-growth of bacteria started after 24hour of incubation as shown in Table 5.

Table 1. Name of the plants and their extracts used.

Plant	Plant part	Extraction solvent	Abbreviation used
Medicago denticulate	Whole Plant	Methanol	MMD
		Dichloromethane	DMD
Terminalia arjuna	Leaf	Methanol	MTAL
	Fruit	Methanol	MTAF
	Root	Methanol	MTAR
Pyrus pashia	Stem	Methanol	MPPS
		Methanol + Chloroform	MCPPS
	Leaf	Methanol	MPPL
	Fruit	Methanol	MPPF
Schinusmolle	Leaf	Methanol	MSML
		Methanol + Chloroform	MCSML

Antifungal assay

The antifungal activity of the plant extracts was determined against five fungal strains. The results shown here indicate the decrease in the growth of fungus in the presence of plant extract. This inhibition in the growth of fungal strains was compared with the standard drug nystatin and to the growth of fungus in a control tube containing no extract. Inhibition in the growth pattern is shown in Table 6 and Table 7 respectively.

Table 2. Concentrations of test sample used for the antioxidant assay.

Concentration (µg/ml)	Weight of extract (mg)	Volume of solvent (ml)
322	10mg	1ml
161	5mg	1ml
16	500µg	1ml
8	250µg	1ml
4	125µg	1ml

All the tested plant extracts were active against at least one strain of fungi. Detail of the growth inhibition pattern described against each strain is given below. *Aspergillus niger*: Significant growth inhibition of *A. niger* was seen against plant extracts MTAF, MPPS and MSML and the growth inhibition after 48hour of incubation was 100%, 95% and 96% respectively. All other extracts showed less than 80%

growth inhibition. The same plant extracts MTAF, MPPS and MSML showed 77%, 55% and 70% growth inhibition after 72hour while plant extracts MTAL and MCSML showed no growth inhibition. Other extracts showed less than 50% growth inhibition. *Aspergillus flavus*: Incubating the fungal strains with plant extracts MMD, MTAF, MTAR, MCPPS and MPPL showed fungal growth inhibition to a significant level and recorded as 93%, 100%, 86%, 78% and 80% respectively. Other extracts also showed growth inhibition except for MCSML which did not show any inhibition. After 72 hours the growth inhibition of the same extracts given above is 68%, 71%, 60%, 61% and 59% respectively.

Table 3. Zone of inhibition of plant extracts at a concentration (3 mg/100µl).

	Zone of inhibition (mm)				
Extract	*E. coli*	*S. typhimurm*	*B. spizizenii*	*S.aureus*	*S. epidermidis*
MMD	-	-	-	16	-
DMD	-	-	-	14	-
MTAL	10	-	-	-	-
MTAF	11	12	16	16	11
MTAR	-	13	-	-	-
MPPS	-	-	-	-	-
MCPPS	-	11	-	-	9.5
MPPL	-	-	-	-	-
MPPF	-	-	-	-	-
MSML	-	-	-	-	-
MCSML	-	-	-	-	-
Erythouromycin	13.5	12.7	20	16.4	15.1

- = no zone of inhibition.

Fusarium solani

All the plant extracts showed growth inhibition to *F. solani* but a low level compared to *Aspergillus niger* and *Aspergillus flavus*. Plant extracts MTAF, MCPPS and MPPL showed better growth inhibition after 48-hour and were 77%, 73% and 84% respectively. After 72-hour only MPPL showed 46% growth inhibition while the growth inhibitions by the remaining extracts were lower.

Table 4. MICs values of plant extracts used.

	Minimal inhibitory concentration (µg/100µl)				
Extracts	*E. coli*	*S. typhimurm*	*B. spizizenii*	*S. aureus*	*S. epidermidis*
MMD	-	-	-	750	-
DMD	-	-	-	1500	-
MTAL	3000	-	-	-	-
MTAF	1500	1500	162	82	1500
MTAR	-	750	-	-	-
MPPS	-	-	-	-	-
MCPPS	-	1500	-	-	1500

- = no MIC found.

Aspergillus fumigatus

The growth inhibition by plant extracts MTAF and MPPF against *A. fumigatus* was 100% and 67% respectively while MMD, DMD, MTAR and MPPS did not inhibit the growth after 48 hours of incubation. But after 72 hours interestingly MTAF and MPPF showed 66% and 47% fungal growth inhibition.

Mucor

At 48 hours of incubation only extract MTAF showed 70% growth inhibition against *Mucor*. All other extracts showed less than 50% growth inhibition. Plant extract MTAF showed 51% growth inhibition after 72 hours.

Antioxidant assay

The antioxidant activity of all eleven plant extracts was assessed by DPPH free radical scavenging assay and the percent radical scavenging values are summarized in Table 8.

The antioxidant activity depends on the dose of plant extract and by increasing the concentration the percent scavenging power increased. At low conc. 8µg/ml only MSML and MCSML showed 74% and 53% scavenging activity respectively while at 322µg/ml both extracts showed higher antioxidant potential than ascorbic acid used as a reference standard. At conc. of 161µg/ml the remaining extracts i.e. MTAL, MPPL, MSML and MCSML showed more than 80% radical scavenging potential.

Table 5. Bacteriostatic activity of various plant extracts (3mg / 100µl).

	Bacteriostatic zones (mm)				
Extracts	*E. coli*	*S. typhimurm*	*B. spizizenii*	*S. aureus*	*S. epidermidis*
MMD	-	41	45	-	-
DMD	-	39	43	-	-
MTAL	-	9	-	-	-
MTAF	-	-	-	-	-
MTAR	-	-	-	12	-
MPPS	-	-	-	-	-
MCPPS	-	-	-	13	-
MPPL	-	-	-	-	-
MPPF	-	-	-	-	-
MSML	32	25	35	26	-
MCSML	33	26	33	26	-

- = no zone of inhibition.

Brine shrimp cytotoxicity assay

The cytotoxic effect of the extracts was determined by performing a brine shrimp cytotoxic assay. The % mortality of the extracts after 24hour and 48hours of incubation are shown in Table 9. Cytotoxicity was reported for only two plant extracts MTAF and MTAR which indicate 37% and 50% killing of brine shrimp larvae after 24 hours respectively. When the cytotoxic behavior of the extracts was observed for another 24 hours, an increased rate of mortality i.e. 45% and 55% was found for the plant extracts MTAF and MTAR respectively.

Table 6. Growth inhibition (%) of fungal strains in the presence of different plant extracts after 48 hours of incubation.

	Inhibition (%) of fungal growth against				
Extract	*A. niger*	*A. flavus*	*F. solani*	*A. fumigatus*	*Mucor*
MMD	66.06	93.30	40.85	3.47	31.41
DMD	69.85	66.14	43.84	3.24	26.98
MTAL	25.52	70.99	29.13	42.79	42.66
MTAF	107.98	100.67	76.66	100.29	69.84
MTAR	33.18	86.32	50.93	4.61	24.28
MPPS	94.97	62.33	35.95	0.00	46.12
MCPPS	49.54	78.05	73.16	39.94	0.00
MPPL	68.72	79.69	83.92	46.60	22.65
MPPF	73.09	33.81	62.71	66.88	0.00
MSML	96.42	59.37	28.09	52.83	38.98
MCSML	0.00	0.00	43.11	13.02	0.00
Nystatin	117.45	113.88	102.67	109.42	106.45
DMSO	27.61	21.70	12.35	19.82	14.65

Discussion

Plants produce a large number of secondary metabolites for natural protection against microorganisms and insect attacks (Mauch-Mani *et al.*, 2017). Plants are a valuable source of antimicrobial (Schieber, 2017), anticancer agents analgesics, anti-diarrheal antifungal (Yue *et al.*, 2017) anti-inflammatory and other therapeutic agents (Chaudhari *et al.*, 2017) which could be used as drugs. Plants have been in the extensive study for controlled and beneficent use in pharmaceutical, food and cosmetics industries, etc.

Table 7. Growth inhibition (%) of fungal strains in the presence of different plant extracts after 72 hours of incubation.

	Inhibition (%) of fungal growth against				
Extracts	*A. niger*	*A. flavus*	*F. solani*	*A. fumigatus*	*Mucor*
MMD	44.50	67.84	10.39	0.00	0.00
DMD	48.26	33.18	8.54	0.00	0.00
MTAL	0.00	53.88	19.75	37.85	34.47
MTAF	76.84	71.47	41.18	65.54	51.12
MTAR	27.51	59.98	14.67	0.00	9.97
MPPS	54.65	47.50	28.00	0.00	23.12
MCPPS	43.15	60.64	20.93	32.16	0.00
MPPL	54.16	58.90	45.78	7.31	0.00
MPPF	40.64	46.78	29.07	47.17	0.00
MSML	70.05	44.93	18.75	30.65	32.82
MCSML	0.00	0.00	27.91	0.00	0.00
Nystatin	102.16	96.74	89.03	97.15	96.40
DMSO	14.63	5.26	0.00	9.64	5.88

The therapeutic effects of different extracts obtained from plants have been studied. Therefore, in this study, efforts were made to explore the natural flora of district Khanewal which could be a valuable source of antimicrobial and other therapeutic agents. In this study, eleven extracts from four different plants were used. These plant extracts were investigated for their antimicrobial (antibacterial, antifungal), cytotoxic and antioxidant activities. In our study interesting results were found from different extracts which are the focus of our discussion.

Table 8. DPPH radical scavenging (%) activity of plant extracts.

	Percent radical scavenging at conc.				
Plant Extracts	322µg/ml	161µg/ml	16µg/ml	8µg/ml	4µg/ml
MMD	-	29.79	12.38	10.34	8.90
DMD	-	16.17	10.85	8.19	6.76
MTAL	89.74	81.81	17.68	7.52	-
MTAF	20.73	17.07	1.22	0.20	-
MTAR	58.03	40.14	0.71	0.10	-
MPPS	37.30	20.73	1.93	-0.10	-
MCPPS	66.36	53.05	3.25	1.42	-
MPPL	90.35	89.94	44.51	18.70	-
MPPF	24.29	19.51	0.61	-0.61	-
MSML	92.28	85.77	81.30	73.58	-
MCSML	93.90	90.04	84.76	53.05	-
Ascorbic acid	-	-	93.70	89.13	56.10

Antibacterial activity of different plant extracts was determined against Gram-positive and Gram-negative bacteria. The MIC value for *M. denticulata* Methanol and Dichloromethane (DCM) extract was 750µg/100µl and ≥1500µg/100µl respectively against *S. aureus* while previously there is no report for antimicrobial activity of this plant. Extracts of *M. denticulata* did not show any activity against *E. coli* and *S. epidermidis* while large bacteriostatic zones were observed for *S. typhi* and *B. spizizenii*. Other *Medicago* species are reported for their antimicrobial properties against *S. aureus, E. coli* and *S. epidermidis* and MICs values were found in the range of 31.3–500µg/ml (Ali *et al.*, 2017). In our study little, the bacteriostatic activity of *M. denticulata* was found against *S. typhi* and *B. spizizenii* but there was no activity against *E. coli* and *S. epidermidis* even at the highest concentration used in this study (3mg/100µl). Clinical isolates of *E. coli* and *S. aureus* were found resistant to *Medicago* extracts. The antimicrobial activity of *Medicago* species was due to its triterpenoids content (Avato *et al.*, 2006).

Table 9. Death (%) of larvae in the cytotoxic assay.

	After 24 hours			After 48 hours		
Extract	Conc. 1	Conc. 2	Conc. 3	Conc. 1	Conc. 2	Conc. 3
MMD	3.333	0	0	0	0	0
DMD	3.333	0	0	7.143	0	0
MTAL	0	0	0	0	0	0
MTAF	36.667	0	0	44.827	0	0
MTAR	50	0	0	55.172	10	0
MPPS	0	0	0	0	0	0
MCPPS	0	0	0	0	0	0
MPPL	0	0	0	0	0	0
MPPF	3.333	0	0	0	0	0
MSML	0	0	0	0	0	0
MCSML	3.333	0	0	0	0	0
DMSO	0	0	0	3.333	0	0

Methanol extract of the fruit of *T. arjuna* (MTAF) used in the present study was active against all five tested strains of bacteria. The MICs of MTAF against *E. coli, S. typhi and S. epidermidis* was 1500µg/100µl, for *B. spizizenii*, 162µg/100µl and *S. aureus* MIC was 82µg/100µl. These results indicate that extracts are more active against Gram-positive microbes compare to Gram-negative. In another study conducted (Rani and Khullar, 2004) different plant parts were active against two different strains of *S. typhi* (MTCC 531 and B 320). A related species from the same genus *T. chebula* showed zones of 13-16mm against *Salmonella* and 10-14mm against *E. coli* (Li *et al.*, 2019). According to the best of our knowledge, there are no or very few reports for antifungal activity of the plant extracts against fungal species, however, the activity of *S. mole* extract was reported against *Candida* species (Van Vuuren and Holl, 2017). Antifungal activity of triterpenoid saponins from certain families is reported against different fungus. Therefore, the interest was to look for the antifungal activity of the selected plants. Our results show that *T. arjuna* fruit (MTAF) was more toxic to the growth of fungi. Among the species tested *A. niger* and *A. flavus* were more sensitive to all the tested fungal strains. The minimum inhibitory effect was observed in the case of *Schinusmolle* leaf and *Pyrus pashia* stem extracts. Acetonic leaf extracts of *T. arjuna* were tested but no antifungal activity was reported for these extracts (Mbosso *et al.*, 2019).

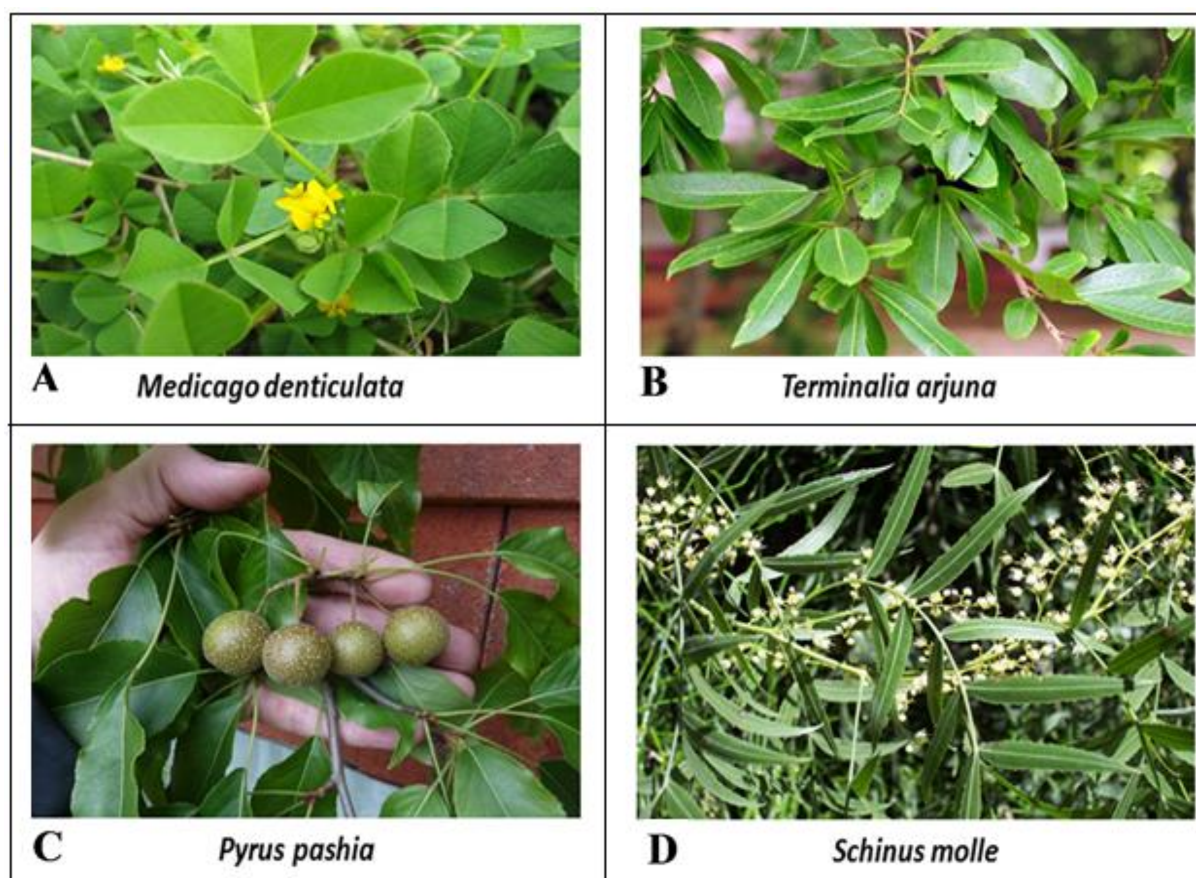

Fig. 1. Medicinal plants used in the study (A) *Medicago denticulate* (B) *Terminallia arjuna* (C) *Pyrus pashia* (D) *Schinus molle*.

In our results, we report antifungal activity of *T. arjuna* leaves against different species of fungi and fruit extracts of the same plant showed greater inhibition than leave extracts. Our results show that the few of the plant extracts are toxic for limited species of fungi but others have a broad range of toxicity which could be explained by nature of the compounds present in the different extracts and each plant has a specific capacity for biosynthesis of these inhibitory compounds. A similar trend was observed by Bonzi (Aneja *et al.*, 2012) testing different plant extracts.

Due to oxidative stress, free radicals are produced which are involved in many disorders such as neurodegenerative diseases and cancer (Poprac *et al.*, 2017). Plants due to the presence of antioxidants attracted interest recently as they can scavenge free radicals produced in the living system and are useful for the management of those diseases (Mondal, 2017). In the present study, four different plants showed significant antioxidant activity using DPPH free radical scavenging assay.

The DPPH radical scavenging activity is not reported for *M. denticulata* earlier and we report it for the first time. MMD extract at concentration 161µg/ml has 30% scavenging ability to free radical. The scavenging power was found to be high with methanol extract. *T. arjuna* leaf extract showed 82% scavenging activity at concentration 161µg/ml. Maximum antioxidant activity was for leaf extract MTAL followed root, which could be explained by concentration difference of the bioactive compounds in different plant parts. *P. pashia* is known for its antioxidant activity and its stem extract MCPPS and leaf extract MPPL offered 53% and 90% radical scavenging activity at concentration 161µg/ml respectively. DPPH free radical scavenging activity of methanol and acetone extract of *P. pashia* was also reported elsewhere (Saini *et al.*, 2014) and was found to be 62.11±21.08 and 10.72±2.15 (mg catechin equivalents per 100g of

fruit weight) (Saini *et al.*, 2014). In another study, the IC_{50} value for the antioxidant activity of *P. pashia* leaves was >300µg/mL and its methanol and water extract shown IC_{50} less than 12µg/mL. Thus different studies confirm the antioxidant potential of this plant and its different parts or extracts could be used in different food and pharmaceutical products. Another plant used in this study was *S. molle* and its methanolic and MCSML extracts showed scavenging activity of 73% and 53% respectively at concentration 8µg/ml higher than ascorbic acid used as a control in this study. Antioxidant and free radical scavenging activity for essential oils of *S. molle* were reported by (Bendaoud *et al.*, 2010).

The research in the development of new anticancer drugs is one of the most prominent areas in natural products. Therefore, the cytotoxic potential of different plants collected from Khanewal was investigated for possible cytotoxic activity using brine shrimp cytotoxic assay. Among the four medicinal plants used in the study higher mortality rate was observed for root and fruit extracts of *T. arjuna* which was 50% and 37% respectively. These plants are not well assessed for their *in-vitro* cytotoxic activities and it will be interesting to evaluate their cytotoxic potential against cell lines.

Conclusion

Our preliminary results showed that the extracts obtained from different plants exhibit several biological activities including antimicrobial, antioxidant and cytotoxic.

They are a source for many bioactive compounds and therefore this study provides an important basis for further investigation into the isolation, characterization and mechanism of antimicrobial and cytotoxic compounds from the plants under study.

Acknowledgements

The work was supported by internal University Research Funds of Qauid-i-Azam University Islamabad. The authors also thank Dr. Khurram Afzal for help in the collection of the plant samples used in the study.

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