Antibacterial, Antifungal and antioxidant activities of some medicinal plants

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Abstract: The purpose of this study was to evaluate the antibacterial, antifungal and antioxidant activities of medicinal plants. The antibacterial activity of methanolic extracts of three medicinal plants (*Swertia chirata, Terminalia bellerica* and *Zanthoxylum armatum*) were tested against Gentamicin (standard drug) on eleven gram positive and seventeen gram negative bacteria by agar well method. It was revealed that seven-gram negative and six gram positive bacterial species were inhibited by these plant extracts. Minimum inhibitory concentrations (MIC) of the extracts were determined by broth micro-dilution method. The significant MIC value of *Swertia chirata* was 20mg/ml against *Serratia marcesens, Zanthoxylum armatum* was 10 mg/ml against *Aeromonas hydrophila* and *Terminali bellerica was* 20mg/ml against *Acinetobacter baumanii* as well as *Serratia marcesens.* Antifungal screening was done for methanolic extracts of these plants by agar well method with the 6 saprophytic, 5 dermatophytic and 6 yeasts. In this case Griseofulvin was used as a standard. All saprophytes and dermatophytes were showed resistance by these plants extracts except *Microsporum canis, which* was inhibited by *Z. armatum* and *S. chirata* extracts. The significant MIC value of *Zanthoxylum armatum* was 10mg/ml against *Microsporum canis* and *Swertia chirata* was 10mg/ml against *Candida tropicalis.* The anti-oxidant study was performed by DPPH free radical scavenging assay using ascorbic acid as a reference standard. Significant antioxidant activities were observed by *Swertia chirata* and *Zanthoxylum armatum* at concentration 200µg/ml was 70% DPPH scavenging activity (EC_{50}=937.5µg/ml) while *Terminalia bellerica* showed 55.6% DPPH scavenging activity ($EC_{50=}$100ug/ml). This study has shown that these plants could provide potent antibacterial compounds and may possible preventive agents in ROS related ailments.

Keywords: *Swertia chirata, Terminalia bellerica, Zanthoxylum armatum,* anti-bacterial activity, antifungal activity, Antioxidant.

INTRODUCTION

Plants have been used as a source of medicines for chronic and infectious disorders since time immemorial. In present time, herbal medicines are so important because of the side effects of synthetic pharmaceutical products and the safety, efficiency and promising potential of plant derived medicine. Researchers are focused on screening methods used for identification of potential antimicrobial plants. In this study diffusion methods as qualitative technique give an idea of the presence or absence of substances of antimicrobial activity in plant extracts. On the other hand, dilution methods are considered quantitative assays once they determine the minimal inhibitory concentration. Tumors as well as age dependent diseases such as atherosclerosis, arthritis, neuro-degenerative disorders associated with generation of oxygen free radical, reactive oxygen species (ROS), hydrogen peroxide. The chemo-preventive role present in consumable fruits, vegetables and beverages so it is essential to discover and characterize antioxidants from natural origin. Three following plant extracts has been used for the assessment of antioxidant potential based on DPPH free radical scavenging method (Lee *et al*., 1998).

Zanthoxylum armatum DC. (Fam. Rutaceae), known as tajbal in Hindi. This herb used as hepatic tonic, anthelmintics, antiviral and mosquito repellent etc. (Gogte, 2000; Kokate, 2001; Kumar & Müller, 1999). *Swertia chirata* Buch. & Ham. (Gentianaceae) has important medicinal and pharmaceutical values. The roots of Chirata possesses significant antipyretic, analgesic properties and a high rise therapeutic clue (Tabassum *et al*., 2012). *Terminalia bellerica* Roxb. (Combretaceae) seed oil is used to cure skin diseases, premature graying of hair and can be applied on painful swollen parts. The fruits of *T. bellerica* can be used to treat cough, cold, hoarseness of voice, asthma, arrest bleeding, boost hair growth, impart black colour to hair, cure conjunctivitis, astringent and anti-diarrheal agent. It also helps in loss of appetite, piles, lowering cholesterol, blood pressure, boosts immunity and prevents ageing (Devi *et al*., 2014).

MATERIALS AND METHODS

Collection and preparation of plant's extracts

The medicinal plants, *S. chirata, T. bellerica and Z. armatum,* were purchased from local market. The plant samples were identified by Prof. Dr. Mansoor Ahmad,

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Faculty of Pharmacy, University of Karachi (voucher no. AW2011-3). The air-dried plant samples were into uniform powder using milling machine separately. Powder materials were percolated with methanol for 15 days at room temperature, then filtered and concentrated on Buchi rotary evaporator under vacuum at 40 C. These extracts were kept in cool and dry place for further studies.

Tested bacteria

The pathogenic bacteria for the sake were obtained from the Department of Microbiology, Federal Urdu University of Arts, Science and Technology, Karachi-Pakistan. The antibacterial activity of plant extracts were checked against eleven gram positive (*Bacillus cereus, Bacillus subtilis, Bacillus thruingiensis, Corynebacterium diptheriae, Corynebacterium hofmanii, Corynebacterium xerosis, Staphylococcus epidermidis, Streptococcus saprophyticus, M. smegmatis, Streptococcus fecalis. Streptococcus pyogenes)* and seventeen gram negative (*Enterobacteraerogenes, Escherichia coli ATCC 8739, Escherichia coli multi drug resistance, Klebsiella pneumonia, Salmonella typhi, Salmonella paratyphi A, Salmonella paratyphi B, Shigella dysenteriae, Serratia marcesens, Acinetobacter baumanii, Campylobacter jejuni, Campylobacter coli, Helicobacter pylori, Hemophilus influenzae, Vibrio cholerae, Aeromonas hydrophila)* bacteria in this study.

Tested pathogenic fungi

The test organisms for this study were members of the 6 saprophytic fungi *Penicillium sp., Aspergilus flavus, Aspergillus niger, Fusarium sp, Rhizopu and Helminthosporum* , 5 dermatophytes *Microsporum canis, Microsporum gypseum, Trichophyton rubrum, Trichophyton mentagrophytes, Trichophytonton surans* and 6 yeast including *Candida albicans, Candida albicans* ATCC 0383, *Saccharomyces cerevisiae, Candida galbrata, Candida tropicalis, Candida kruzei.*

Chemicals and media

Muller Hinton broth, Gentamicin, DPPH (1, 1-diphenyl, 2-picrylhydrazyl), Ascorbic acid, DMSO (Dimethylsulfoxide) Merck, Germany were used during experiments.

Screening of antibacterial activity

All the bacterial isolates were checked and identified on the basis of conventional methods for purity and maintained on nutrient agar at 4°C in the refrigerator for further work. Antibacterial activity of compounds on the test organisms were determined by using agar-well method. Autoclaved Muller Hinton broth was used to keep the bacterial culture in log phase for 2 hrs with constant agitation and subsequently wells were dug onto Muller Hinton Agar. Later, 10 micro-liters of culture were poured into the wells (Perez *et al*., 1990). All plates were incubated at 28±2°C for 24-48 hours. After incubation the zone of inhibition (diameter) was measured in millimeter. Each well contains 30µl of test extract prepared from 5g/100ml. Gentamicin antibiotic was used as a control (Vaghasiya *et al.*, 2009).

Determination of minimum inhibitory concentration (MIC)

Minimum Inhibitory Concentration (MIC) of extracts was determined by Micro broth dilution method using 96-well micro-titer plate (Noor *et al*., 2011). Stock solution of 100 mg/ml of crude extract was prepared in distilled water. Two fold serial dilutions of extracts was made in 100µl broth and subsequently 10µl of two hours old culture perfectly matched the *innocula* of each with 0.5 Mac Farland index later was added in all wells. One well served as antibiotic control while other served as culture control. Micro-titer plate was incubated for 24 hours at 37ºC. The MIC was read as the well showing no visible growth.

Screening of antifungal activity

The fungal isolates were obtained from the Department of Microbiology, Federal Urdu University of Arts, Science and Technology, Karachi-Pakistan. All the fungal isolates were checked for purity and maintained on Sabourd Dextrose agar (SDA) at 4ºC in the refrigerator until required for use. Antifungal activity of 1:1 was tested using agar-well method. Autoclaved distilled water was used for the preparation of fungal spore suspension and transferred aseptically into each SDA plates. The doses of test extracts were prepared from 5g/100ml stock solution from which 30 µl was added to each well. All plates were incubated at 28±2°C for 24 -48 hours and after incubation then diameter of zone of inhibition was measured.

Determination of Minimum inhibitory concentration (MIC)

Minimum inhibitory Concentration (MIC) of was determined by Micro broth dilution method using 96-well microtitre plates. Stock solution of 100mg/ml of was prepared in distilled water. Two fold serial dilutions of extracts was made in 100µl broth and subsequently 10µl of two hour refreshed culture matched with 0.5 Mac Farland index was added to each well. One well served as antifungal agent control while other served as culture control. Microtitre plate was incubated for 24 hours at 37ºC. The MIC was read as the well showing no visible growth.

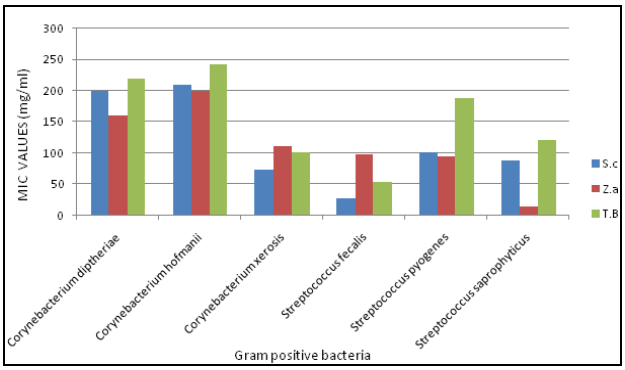
Anti-oxidant activity

Anti-oxidant activity of the test sample was determined by using the method described by Lee *et al*., (1998). 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was prepared in ethanol (300µM). Briefly, 10 µl of test sample and 90µl solution of stable radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH) so that final concentration of test sample was

200µg/ml was added in 96- well micro titer plates and incubated at 37ºC for 30 minutes. Absorbance was measured at 515nm by using a spectrophotometer. Percent inhibition of radicals by treatment of test sample was determined by comparison with a DMSO treated control group.

$$\% \text{ Inhibition} = \frac{(\text{absorbance of the control} - \text{absorbance of the test sample})}{\text{Absorbance of the control}} \times 100$$

Ascorbic acid (100µg/l) was used as standard control. The EC_{50} value calculated denotes the concentration (in µg/ml) of sample required to scavenge 50% of DPPH.

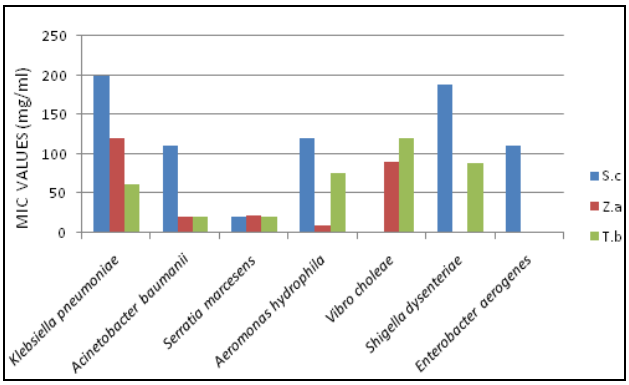


S. c=Methanolic extract of *S. chirata*; Z. a=Methanolic extract of *Z. armatum*; T. b=Methanolic extract of *T. bellerica*

Fig. 1: Comparison between MIC values of methanolic extracts of plants against tested gram-positive bacteria.

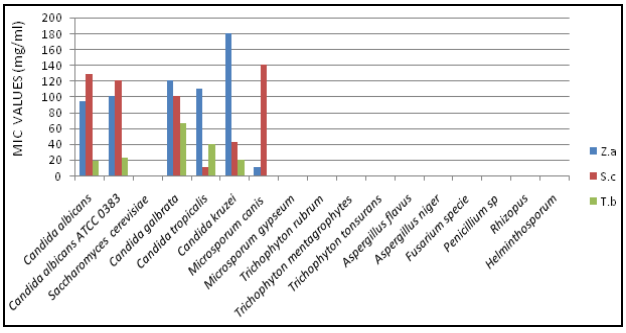
RESULTS

Three plants were evaluated for their antibacterial potential against eleven-gram positive and seventeen-gram negative bacteria in this study by using agar well method. table 1 summarizes the results obtained in terms of diameter of zone of inhibition (mm). Seven-gram negative and six-gram positive bacterial species were inhibited by experimental extracts. The extract of *S. chirata* showed the zone of inhibition in the range of 10±2 to 21±2 mm for gram negative and 15±1 to 29±1mm for gram-positive bacteria. The extract of *Z. armatum* showed the zone of inhibition in the range of 15±0 to 20±0 mm for gram negative and 15±2 to 29±0 mm for gram-positive bacteria. The extract of *T. bellerica* showed the zone of inhibition in the range of 10±2 to 30±2 mm for gram negative and 12±2 to 25±0mm for gram-positive bacteria. table 2 summarizes the MIC values of plant extracts showed significant results. The extract of *S. chirata* has significant MIC values 20mg/ml and 26 mg/ml against *Serratia marcesens* and *Streptococcus fecalis* respectively. The valuable MIC result of the extract *of T. bellerica* was 20mg/ml against both *Acinetobacter baumanii* and *Serratia marcesens* while MIC values of the extract of *Z. armatum* was 10mg/ml against *Aeromonas hydrophila*. Fig. 1 and 2 explain comparison of MIC values of extracts against gram positive and gram negative bacteria respectively.



S. c=Methanolic extract of *S. chirata*; Z. a=Methanolic extract of *Z. armatum*; T. b=Methanolic extract of *T. bellerica*

Fig. 2: Comparison between MIC values of methanolic extracts of plants against tested gram-negative bacteria.



S. c=Methanolic extract of *S. chirata* Z. a=Methanolic extract of *Z. armatum* T. b=Methanolic extract of *T. bellerica*

Fig. 3: Comparison between MIC values of methanolic extracts of plants against tested pathogenic fungi.

Table 3 explains that all saprophytes and dermatophytes fungi are resistant against these plants extracts except *Microsporum canis*, which was inhibited by *Z. armatum* and *S. chirata* extracts. The only one yeast named *Saccharomyces cerevisiae* was resistant against all plant extracts used in experiments. The results recorded in terms of diameter of zone of inhibition (mm). The extracts of *S. chirata and Z. armatum* showed the zone of inhibition in the range of 15±1 to 32±0 mm and the extract of *T. bellerica* showed the zone of inhibition in the range of 10±1 to 32±0 against sensitive pathogenic fungi. Table 4 summarizes the MIC values of plant extracts. The significant MIC value was 10 mg/mlfor *S.chirata* and *Z.armatum* against *Candida tropicalis* and *Microsporum canis* respectively. The valuable MIC result of the extract *of T. bellerica* was 18mg/ml against *Candida albicans.* Fig. 3 showed comparison of MIC values of these plants extracts against tested pathogenic fungi.

Table 5 expresses percentage of the promising scavenging of DPPH free radical of three plants extracts. Plant extracts at 200µg/ml concentration were showed significant inhibition of DPPH free radical i.e.70±0.01, 70±0.02 and 55.6±0.02% for *S. chirata*, *Z. armatum* and

Table 1: Anti-bacterial activity of plant extracts in terms of zone of inhibition (mean ±S.D) in mm

Gram negative bacteria	S.c	Z.a	T.b	Gram positive bacteria	S.c	Z.a	T.b
Enterobacter aerogenes	12±2	-	-	*Bacillus cereus*	-	-	-
Escherichia coli ATCC 8739	-	-	-	*Bacillus subtilis*	-	-	-
Escherichia coli	-	-	-	*Bacillus thruingiensis*	-	-	-
E. coli multi drug resistance	-	-	-	*Corynebacterium diptheriae*	15±1	15±2	17±2
Klebsiella pneumoniae	19±0	19±0	19±0	*Corynebacterium hofmanii*	15±2	19±2	15±1
Salmonella typhi	-	-	-	*Corynebacterium xerosis*	19±1	24±1	24±1
Salmonella paratyphi A	-	-	-	*Staphylococcus epidermidis*	-	-	-
Salmonella paratyphi B	-	-	-	*Streptococcus saprophyticus*	29±1	21±2	12±2
Shigella dysenteriae	21±2		30±2	*M. smegmatis*	-	-	-
Serratia marcesens	19±0	20±0	13±2	*Streptococcus fecalis*	19±2	29±0	24±1
Acinetobacter baumanii	13±2	19±0	14±0	*Streptococcus pyogenes*	24±1	24±1	25±2
Campylobacter jejuni	-	-	-				
Campylobacter coli	-	-	-				
Helicobacter pylori	-	-	-				
Hemophilus influenzae	-	-	-				
Vibrio cholerae	10±2	18±1	11±1				
Aeromonas hydrophila	11±0	15±0	10±2				

S.c =Methanolic extract of *S. chirata;* Z.a = Methanolic extract of *Z. armatum;* T.b = Methanolic extract of *T. bellerica*, whereas Gentamycin 10mg disc (Zone of inhibition 12-15mm) was used as a reference drug for antibacterial activity.

Table 2: Minimum Inhibitory Concentration (MIC) in mg/ml of plant extracts

	Bacteria	S.c	Z.a	T.b
Gram positive	*Corynebacterium diptheriae*	200	160	220
	Corynebacterium hofmanii	210	200	242
	Corynebacterium xerosis	72	110	100
	Streptococcus fecalis	26	98	52
	Streptococcus pyogenes	100	94	188
	Streptococcus saprophyticus	88	14	120
Gram negative	*Klebsiella pneumoniae*	200	120	62
	Acinetobacter baumanii	110	20	20
	Serratia marcesens	20	22	20
	Aeromonas hydrophila	120	10	76
	Vibro choleae	-	90	120
	Shigella dysenteriae	188	-	88
	Enterobacter aerogenes	110	-	-

S.c =Methanolic extract of *S. chirata;* Z.a = Methanolic extract of *Z. armatum;* T.b = Methanolic extract of *T. bellerica.*

T. bellerica respectively. Data in the table 5 reveals that the extract EC_{50} is the amount of antioxidant required to deplete the initial DPPH by 50%. The EC_{50} values of 937.5µg/ml for both *Swertia chirata* and *Zanthoxylum armatum* while the EC_{50} for *T. bellerica* is 100µg /ml.

DISCUSSION

The study was conducted to evaluate antibacterial property of *S. chirata*, *T. bellerica* and *Z. armatum* methonolic extracts against some gram positive and gram negative bacteria. Although the results of each plant extract varied with other against same pathogen but all plant extracts effective against *Corynebacterium diptheriae, Corynebacterium hofmanii, Corynebacterium xerosis*, *Streptococcus saprophyticus*, *Streptococcus fecalis* and *Streptococcus pyogenes* with different zones of inhibition (mm) in case of gram positive bacteria. The antibacterial results of plant extracts against gram-negative bacteria showed some differences. These plant extracts exhibited sensitivity against *Klebsiella pneumonia*, *Serratia marcesens*, *Acinetobacter baumanii*, *Vibrio cholera* and *Aeromonas hydrophila. S. chirata* and *T. bellerica* extracts showed effectiveness against *Shigella dysenteriae* and *Enterobacter aerogenes* showed sensitivity against *only S. chirata* extract. Minimum inhibitory concentration of the crude extract was determined by serial dilution technique, which showed some significant results.

Table 3: Antifungal activity of plant extracts in terms of zone of inhibition (mean±S.D) in mm

	Pathogenic Fungi	Z.a	S.c	T.b
Yeasts	*Candida albicans*	20±1	20±1	32±0
	Candida albicans ATCC 0383	24±0	24±0	28±1
	Saccharomyces cerevisiae	-	-	-
	Candida galbrata	15±1	15±1	10±1
	Candida tropicalis	20±1	20±1	29±2
	Candida kruzei	32±0	32±0	22±1
Dermatophytes	*Microsporum canis*	26±1	26±1	-
	Microsporum gypseum	-	-	-
	Trichophyton rubrum	-	-	-
	Trichophyton mentagrophytes	-	-	-
	Trichophyton tonsurans	-	-	-
Saprophytes	*Aspergillus flavus*	-	-	-
	Aspergillus niger	-	-	-
	Fusarium species	-	-	-
	Penicillium sp	-	-	-
	Rhizopus	-	-	-
	Helminthosporum	-	-	-

S.c =Methanolic extract of *S. chirata;* Z.a = Methanolic extract of *Z. armatum;* T.b = Methanolic extract of *T. bellerica*, whereas Griseofulvin (30 mg disc) was used as a reference drug (zone of inhibition 10-12 mm).

Table 4: Minimum Inhibitory Concentration (MIC) in mg/ml of plant extracts

	Pathogenic Fungi	Z.a	S.c	T.b
Yeasts	*Candida albicans*	94	128	18
	Candida albicans ATCC 0383	100	120	22
	Saccharomyces cerevisiae	-	-	-
	Candida galbrata	120	100	66
	Candida tropicalis	110	10	40
	Candida kruzei	180	42	20
Dermatophytes	*Microsporum canis*	10	140	-
	Microsporum gypseum	-	-	-
	Trichophyton rubrum	-	-	-
	Trichophyton mentagrophytes	-	-	-
	Trichophyton tonsurans	-	-	-
Saprophytes	*Aspergillus flavus*	-	-	-
	Aspergillus niger	-	-	-
	Fusarium sp	-	-	-
	Penicillium sp	-	-	-
	Rhizopus	-	-	-
	Helminthosporum	-	-	-

S.c =Methanolic extract of S. chirata; Z.a = Methanolic extract of *Z. armatum*; T.b = Methanolic extract of *T. bellerica*

Conclusively methanolic extract of *S. chirata* showed significant antibacterial results may be due to presence of flavonoids and tannins (Haslam, 1996; Scalbert, 1991; Dixon *et al*., 1983).

According to Laxmi *et al*., 2011 the MIC values of *S. chirata* extract were 400µg, 800µg and 100µg against *E. coli, V. cholera and B. subtilis* respectively. These significant MIC values were not satisfied with the results observed in this study and exhibited resistance.

According to Safiullah *et al*. (2011) Triphala, an Ayurvedic herb and available in India. Its one component is *T. bellerica* and has been used for the treatment of variety of diseases. In the study of Safiullah *et al*. (2011) the ethanolic and aqueous extracts of *T. bellerica* were used against several bacterial isolates. Ethanolic extract exhibited more antibacterial effect than aqueous extracts. This might be due to the less solubility of the active constituents in aqueous solutions, which results in less or no antibacterial effect on the bacterial isolates at lower

concentration (Safiullah *et al*., 2011). According to Safiullah *et al*. (2011) the aqueous extract and ethanolic extracts of *T. bellerica* showed significant MIC values against *S. typhi, S. paratyphi-B* and *E. coli*, which showed resistance in this study. The MIC values against *Klebsiella pneumoniae and V. cholera* were10µg/ml and 0.1µg/ml by ethanolic extract of *T. bellerica* respectively. The aqueous extract of *T. bellerica* was ineffective against *Klebsiella pneumonia* and MIC value against *V. cholera was* 10µg/ml (Safiullah *et al*., 2011. As in this study the MIC values were 62µg/ml and 120µg/ml for *Klebsiella pneumoniae* and *V. cholera* respectively).

Table 5: Percentage scavenging activity and EC_{50} of methanolic plant extracts

Plant extracts	% inhibition ± SD	EC_{50} µg/ml
Zanthoxulum armatum	70±0.02**	937.5
Swertia chirata	70±0.01**	937.5
Terminalia bellerica	55.6±0.02	100
Ascorbic acid	96.5±0.04**	

Results were expressed as ±SEM

Results of assessment of antibacterial activity revealed variability in different extracts against the test bacterial pathogen. In case of methanolic extract of *Z. armatum*, the significant values of zone of inhibition were observed against *Streptococcus fecalis* whereas the significant MIC value was 10mg/ml against *Aeromonas hydrophil.* According to Akbar *et al*., 2014 thezone of inhibition of ethanolic of *Z. armatum* leaves against *E. coli* and *Salmonella typhi* were15±1mm and 28±1mm respectively whereas the zone of inhibition of aqueous extract *Z. armatum* leaves against *E. coli* and *Salmonella typhi* were2±2 mm and 2±2 mm. Both bacterial strains showed resistance in this study. According to Akbar *et al*., 2014 the zone of inhibition of ethanolic and aqueous extracts of *Z. armatum* leaves against *Klebsiella pneumonia* were *28*±1mm and 3±2mm respectively whereas in this study the observed zone of inhibition against *Klebsiella pneumonia* was19±0mm.

Flavonoids and phenolic compounds from plant extracts and flavors are responsible for biological activities like antimicrobial, anticancer etc. (Kale *et al*., 2010; Greenberg *et al*., 2008). Antifungal screening of *Z. armatum* fruit ethanolic and *n*-hexane extracts was conducted by Barkatullah *et al*. (2012) and showed percentage inhibition of fungal growth in dose dependent manner. According to Barkatullah *et al*. (2012) ethanolic extract of *Z. armatum* fruit showed significant antifungal activity against *Aspergillus flavus.* In this study A. *flavus*was showed resistance against methanolic extracts of *Z. armatum* fruit. Prakash *et al*. (2012) showed that the essential oil of *Z. armatum* completely inhibited the growth of a toxigenic strain of *A. flavus.* The study conducted by Devi *et al*. (2014) and observed significant activity of aqueous extract of *T. bellerica* fruit against fungal isolates compared with chloroform and petroleum ether extracts. The aqueous extract was found to be more effective to control fungal growth. According to Devi *et al*. (2014) *A. flavus, A. niger* and *Rhizopus* were inhibited by all mentioned extracts but experimental results obtained from the present study showed that these fungal isolates were resistant against methanolic extract of *T. bellerica* fruit. According to Hagerman & Butler (1981) that presence of tannins in fruit extract of *T. bellerica* might have found to form irreversible complexes with prolein rich protein resulting in inhibition of cell protein synthesis.

The study of Laxmi *et al*. (2011) conducted to evaluated antifungal activity of aqueous and methanolic extracts *S. chirata* against three fungi. Both the extracts showed concentration dependent activity and observed presence of tannins and glycosides in both extracts. According to Laxmi *et al.* (2011) t hat the antimicrobial potential of the plant is due to the presence of these phytochemicals. In the study of Laxmi *et al*. (2011) observed that methanolic extract showed moderate activity against *A. niger* and no activity against *A. flavus.* The aqueous extract moderate activity against *A. flavus* and against *A. niger* it showed zero activity. Both the extracts showed very high MIC values that was 800µg/ml against *A. flavus* and *A.niger.* In present study both fungi were showed resistance against methanolic extract of *S. chirata*.

The methanolic extracts of plants were subjected to screen for their antioxidant capacity using DPPH free radical scavenging assay. DPPH, as a stable free radical accepts protons from antioxidant substrate and decrease in absorbance. The decrease in absorbance is taken as a measure of the extent of radical scavenging. Therefore DPPH is widely used for the measurement of antioxidant activity of compounds as well as extracts. The study of Dutta *et al*., (2012) showed considerable DPPH radical scavenging activity of 50%methanolic extract of the *S. chirata* was 55.17 ±1.90% at the concentration of 15mg/ml and indicated dose dependent radical scavenging activities of the different extracts. The study of Dutta *et al*. (2012) also revealed that solvents with different polarity significantly alter the DPPH radical scavenging activity. According to Phoboo *et al*. (2010) aqueous and ethanolic extracts of *S. chirata* showed highest antioxidant activity as compare to its substitutes in DPPH radical scavenging method. Antioxidant potential plants contained high concentration of phenolic compounds and its relationship with Type-2 diabetes as explained by Sabu & Kuttan (2002). According to Kumar *et al*. (2011) free radicals have a role in hepatic injury and evaluated the antioxidant property of hepato-protective drugs and explored that *S. chirata* extract has 59.80 % DPPH radical scavenging activity at 200µg/ml and EC_{50} =315.83µg/ml. These values are agreed with the observation 70%

inhibition of free radicals in this experiment and justified their hepato-protective action. The investigation of methanolic extract of *S. chirata* at different concentrations from 50- 200µg/ml (Sharma *et al*., 2013) and found 51% to 90% antioxidant activity by DPPH scavenging assay.

Liver as a key organ of metabolism is continuously exposed to many challenges so in the result hepatic injury and viral infections. There is no synthetic drug, which claimed for the treatment of hepatic disorders in spite of advancement in modern medicine. Ranawat & Patel, (2013) conducted a study to evaluated hepato-protective effect of ethanolic extract of *Z. armatum* preparation and antioxidant effects on Paracetamol induced acute liver damage in rats. The findings supported its hepato-protective effect probably by its antioxidants capacity. Anti-oxidant activity of *Z. armatum* essential oil showed excellent results with IC_{50} value of 27.0±0.1µg/ml (Negi *et al.,* 2012).

Investigations have shown that the antioxidant activities of plants could be correlated with oxidative stress defense and different human diseases and aging process etc (Finkel & Holbrook, 2000). According to Hazra *et al.* (2010), in this respect flavonoids and other polyphenolic compounds have received the greatest attention. The methanolic extract of *T. bellerica* showed 55.6± 0.02% inhibition of DPPH free radicals and $IC_{50}$is 100µg/ml that not satisfied with the result of Hazra *et al*. (2010) that showed IC_{50} value of 70% methanol extract of *T. bellerica* was 1.45±0.02µg/ml.

CONCLUSIONS

The polarity of the solvent played an important part in potential antibacterial, antifungal as well as antioxidant activities. The search of antimicrobial and antioxidant agent of plant origin provides an alternative drug with no adverse effect. The present study provides the evidence of antimicrobial and antioxidant properties of these medicinal plants. Further work will be continued for the identification of the isolated pure compound and its biological effectiveness as an anti-bacterial or antioxidant agent.

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