

## Full Length Research Paper

# Antibacterial activity analysis of extracts of various plants against gram -positive and -negative bacteria

Anwar Khalid<sup>1</sup>, Amir Waseem<sup>1</sup>, Malik Saadullah<sup>2</sup>, Uzair-Ur -Rehman<sup>3</sup>, Sonia Khiljee<sup>4</sup>,  
Ayesha Sethi<sup>5</sup>, Muhammad Hassham Hassan Bin Asad<sup>6</sup>, Fatima Rasool<sup>7</sup>,  
Muhammad Khurram<sup>8</sup> Waqas and Ghulam Murtaza<sup>6\*</sup>

<sup>1</sup>Department of Chemistry, COMSATS Institute of Information Technology, Abbottabad 22060, Pakistan.

<sup>2</sup>Department of Pharmacy, University of Lahore, Lahore, Pakistan.

<sup>3</sup>Department of Biochemistry, Hazara University, Garden Campus, Mansehra, Pakistan.

<sup>4</sup>Department of Pharmacy, Faculty of Pharmacy and Alternative Medicines, the Islamia University of Bahawalpur, Bahawalpur, Pakistan.

<sup>5</sup>College of Pharmacy, GC University, Faisalabad, Pakistan.

<sup>6</sup>Department of Pharmaceutical Sciences, COMSATS Institute of Information Technology, Abbottabad 22060, Pakistan.

<sup>7</sup>College of Pharmacy, University of Punjab, Lahore, Pakistan.

<sup>8</sup>Department of Pharmacy, the University of Faisalabad, Faisalabad, Pakistan.

Accepted 26 October, 2016

The methanolic, hot water and cold water extracts of *Pistacia Integerrima* (gall), *Polygonum Bistorta* (root), *Swetia Charita* (stem) and *Zingiber officinale* (root) were screened against gram positive bacteria like *Bacillus subtilis* (ATCC 6633), *Enterococcus faecalis* (ATCC 14506) and *Staphylococcus aureus* (ATCC 6538), and gram negative bacteria like *Pseudomonas aeruginosa* (ATCC 27853) and *Salmonella typhi* (ATCC 14028) for their antibacterial efficiency. Discs agar diffusion technique was used. The maximum zone of inhibition of 19 mm of methanolic extract of *Zingiber officinale* was observed against *Staphylococcus aureus*, while the maximum zone of inhibition of 15 mm of cold water extract of *Zingiber officinale* against *Pseudomonas aeruginosa* and 15 mm of cold water extract of *Swetia Charita* against *Bacillus subtilis* were observed. Standard drugs with antibiotic action like ceftriaxone, ceftriaxone sodium, cefuroxime, ciprofloxacin, gentamycin, levofloxacin, metronidazole and tranexamic acid were also used against these bacteria and compared their results with that of the plants extracts.

**Key words:** *Pistacia integerrima*, *Polygonum bistorta*, *Swetia charita*, *Zingiber officinale*, antimicrobial activity, extract.

## INTRODUCTION

*Pistacia integerrima* (*Pistacia chinensis*) belong to the family Anacardiaceae with synonyms: *P. chinensis* Bge. ssp. *integerrima* (J. I. S.) Rech., locally called Kakar Singi, Kangar (Urdu, Hindko), Pistacio (English) (Samin et al., 2008). It is distributed in East Afghanistan, Pakistan,

Northwest and West Himalaya to Kumaon (Nasir and Ali, 1983). Its galls are tonic and expectorant; used in cough and asthma and they are also used for dyeing and tanning of cloth. Its LEAVES are used as fodder for cattle (Samin et al., 2008).

*Polygonum bistorta* (*Bistorta major*) belongs to the family Polygonaceae and is a perennial herb locally called Anjbaar (Urdu), Bistort (English) and distributed in Europe, Siberia, Iraq, Iran and Pakistan (Kurram) (Nasir and Ali, 1983). *Polygonum bistorta* has been used in

\*Corresponding author. E-mail: [gmdogor356@gmail.com](mailto:gmdogor356@gmail.com). Tel: +92-314-2082826. Fax: +92-992-383441.

**Table 1.** Test organisms.

Test organism	Gram stain	Diseases caused
<i>Bacillus subtilis</i>	Positive	Bronchial disease, Food poisoning.
<i>Enterococcus faecalis</i>	Positive	UTI, Bacterimia, Endocarditis, Meningitis.
<i>Pseudomonas aeruginosa</i>	Negative	Wound infection in burnt patients, UTI.
<i>Staphylococcus aureus</i>	Positive	Atopic dermatitis, Ritter' disease, Endocarditis.
<i>Salmonella typhae</i>	Negative	Typhoid, Paratyphoid, Food borne-illnesses.

Chinese folk medicine to treat dysentery with bloody stools, diarrhea in acute gastroenteritis, acute respiratory infection with cough, carbuncles, scrofula, aphthous ulcer, hematemesis, epistaxis, hemorrhoidal bleeding and venomous snake bite (State Pharmacopoeia Commission of China, 2005). The aqueous, different solvent extracts and isolated constituents of seven higher medicinal plants including *P. bistorta* (Polygonaceae) were screened *in vitro* for antibacterial activity by cup diffusion method against phytopathogenic *Xanthomonas pathovars* viz., *Xanthomonas axonopodis* pv. *malvacearum*, *Xanthomonas axonopodis* pv. *phaseoli* and *Xanthomonas campestris* pv. *vesicatoria* (Babu et al., 2007).

*Swertia chirata* belongs to the family Gentianaceae and is a biennial herb with local names like Chiretta (English), Chirayata (Hindi, Urdu), Chiretta- Senburi (Japanese) Tig-ta (Tibetan) . It is distributed in the temperate Himalayas from Kashmir to Bhutan and in the Khasia Hills of Meghlaya and Nepal (Chanda, 1976). It was investigated that *S. chirata* is a strong tonic and is an excellent remedy for a weak stomach, especially when this gives rise to nausea, indigestion and bloating; it has also been shown to protect the liver (Chevallier, 1996). According to previous studies (Bown, 1995; Grieve, 1984) this plant is similar in many respects to *Gentiana lutea*, a widely used restorative tonic of the digestive system. It also contains xanthones, which are reputedly effective against malaria and tuberculosis and also amarogentin, a glycoside that may protect the liver against carbon tetrachloride poisoning. The whole plant is an extremely bitter tonic digestive herb that lowers fevers and as a stimulant, the herb has a beneficial effect on the liver, promoting the flow of bile; it also cures constipation and is useful for treating dyspepsia. Biological activities of *S. chirata* are previously reported as the plant is anti-helminthic, antileishmaniac, anticholinergic, anticonvulsant, antiedemic, antiinflammatory, antimalarial, antipyretic, antitubercular, astringent, bitter cardio-stimulant, cholagogue, choleric, CNS depressant, emollient, hepatoprotective, hypnotic, hypoglycemic/antidiabetic, laxative, secretagogue, stomachic, tonic, undersedative and vermifuge (Joshi and Dhawan, 2005).

The ginger plant, *Zingiber officinale* (Zingiberaceae), has a biennial or perennial creeping rhizome, locally

called Adrak (Urdu) and Ginger (English). It is widely distributed throughout India, Sri Lanka, Malaysia, and Pakistan. In Pakistan, it is cultivated in the Hazara Region in Haripur and Tret, and also in the Sindh and Punjab Plains (Nasir and Ali, 1983). Ginger is an essential ingredient in many traditional Chinese medicines and has been used since the 4<sup>th</sup> Century BC. Africans and West Indians also use ginger medicinally and the Greeks and Romans use it as a spice (Melvin et al., 2009). The Chinese take ginger for a wide variety of medical problems such as stomachache, diarrhea, nausea, cholera, asthma, heart conditions, respiratory disorders, toothache and rheumatic complaints (Wagner and Hikino, 1965).

Among these four plants (Table 1), except *S. chirata*, the other three plants are included in the record of the Flora of Pakistan, No.146, 152 and 205 [National Herbarium (Stewart Collection) Pakistan Agricultural Research Council, Islamabad].

This study was performed to evaluate the antimicrobial activity of four plants; *Z. officinale* (Ginger), *P. chinensis* (*P. integerrima*), *B. major* (*P. bistorta*) and *S. chirata* (Cherrita) being conventionally used as medicines.

## MATERIALS AND METHODS

The current research work (BS9203-ML-2009-HU) was carried out in the Department of Biochemistry, Hazara University, Mansehra, Pakistan. Analytical grade methanol and n-hexane were purchased from Merck, Germany. Distilled water was prepared in the Microbiology Laboratory.

Centrifuge machine (5702, Eppendorf, Germany), rotary shaker (VRN-360, Gemmy Industrial Corporation, Pakistan), ready made blank antibiotic assay discs (Micropore, Pakistan) and water bath (Emmay, Pakistan) were used in this study.

Medicinal plants (Registration No. E073B) like *P. Integerrima*, *P. Bistorta*, *S. Charita* and *Z. officinale* were procured from the Botanical Research Centre, Hazara University and were authenticated by an expert botanist.

## Preparation of extracts

Medicinal plant parts, as specified in Table 2, were dried at room temperature under sterile conditions and then each plant material were separately converted to coarse powder using pestle and mortar, followed by its chopping to get fine powder. 2 g of each plant's ground material was soaked separately in 25 ml of each solvent that is methanol and cold distilled water (at room temperature)

**Table 2.** Plants part used.

Botanical name	Family	English name	Local name	Part used
<i>Pistacia Integerrima</i>	Anacardiaceae	Pistacio	Kakar Singi	Gall
<i>Polygonum Bistorta</i>	Polygonaceae	Bistort	Anjbaar	Root
<i>Swetia Charita</i>	Gentianaceae	Chirayata	Chiretta	Stem
<i>Zingiber officinale</i>	Zingiberaceae	Ginger	Adrak	Root

**Table 3.** Composition of Mueller Hinton agar.

Ingredient	Amount (g/L)
Beef extract powder	2.0
Acid digest of casein	17.5
Starch	1.5
Agar	17.0

temperature) and hot distilled water (temperature maintained up to 60°C in a water bath). After 24 h, the mixtures were shaken using the rotary shaker at 150 rpm for 24 h and filtered using Whatman's filter paper No. 1. The materials were again mixed with 25 ml of their respective solvents, and same process was repeated twice. The extracts of all plants after treating separately with 75 ml solvents were filtered, and the filtrate was transferred to test tubes followed by the removal of solvents to get completely dry extracts. The extracts were then stored in a refrigerator at 4°C until used. At the time of use, the extract of each medicinal plant were re-suspended in 2 ml of the respective solvent. The concentration of each final extract was 1 g of material/1 ml of solvent.

### Bacteria

Five species of bacteria (Table 2), three gram positive (*Bacillus subtilis* (ATCC 6633), *Enterococcus faecalis* (ATCC 14506) and *Staphylococcus aureus* (ATCC 6538)) and two gram negative (*Pseudomonas aeruginosa* (ATCC 27853) and *Salmonella typhi* (ATCC 14028)) were obtained from Ayub Medical College, Abbottabad, Pakistan and was used for testing.

### Preparation of media

The Culturing media used for the antibacterial assay was Mueller Hinton agar for the growth of respective bacteria. Mueller Hinton agar media (Table 3) was prepared in a conical flask by dissolving 38 g of powdered agar media in 1 L of distilled water. The flask was heated on an open flame to dissolve the medium completely and then the medium was sterilized in an autoclave at 121°C temperature for 15 min.

### Antibiotic discs

Ready-made blank sterilized discs of size 6 mm were used; each having maximum capacity of 30 l. The plant extracts were taken in separate bottles and the discs were placed in these extracts so that each disc could absorb adequate quantity. Prepared discs were stored at 4°C in the refrigerator till use. For negative control,

methanol paper discs prepared by dipping the disk into the methanol were used.

### Standard antibiotic discs

The standard antibiotic discs contained ceftriaxone, ceftriaxone sodium, cefuroxime, ciprofloxacin, gentamycine, levofloxacin, metronidazole and tranexamic acid in each disc.

### Bioassay

Antibacterial activity was determined by Standard Disc Diffusion Assay adapted from Taylor et al. (1995). For this purpose, 10 ml of sterilized media was poured into sterilized 15 ml Petri dishes. The 0.2 ml of  $10^{-4}$  dilution of 24 h old culture was used so as to ensure that the concentrations of these bacteria contained about  $1 \times 10^6$  CFU/ml. Bacterial cultures were seeded on agar media in Petri plates by the streaking method. Prepared antibiotic discs were applied on each plate and the plates were then incubated upside down at 37°C for 24 h. The inhibition zones were measured and recorded. The assessment of antibacterial activity was based on the measurement of the diameter of the inhibition zone formed around the disc.

### Determination of minimum inhibitory concentration (MIC)

MIC was determined by adopting plate dilution method of methanolic, hot water and cold water extracts of all medicinal plants. For this purpose, 10, 20 and 30 l extracts of each plant per disc were used against 0.2 ml of  $10^{-4}$  dilution of bacteria.

### Statistical analysis

For calculations, p-values were enumerated by SPSS Version 13.0 applying one-way-ANOVA with the statistical significance set at 5%.

## RESULTS

Different parts of a total of 5 selected folklore medicinal plant [(*P. integerrima* (Gall), *P. bistorta* (root), *S. chirata* (stem) and *Z. officinale* (root))] extracts have been tested for their antibacterial effect (Table 4) against five microbial species (*B. subtilis*, *E. faecalis*, *S. aureus*, *P. aeruginosa* and *S. typhi*) were recorded (Table 4). Results of standard antibiotic discs were also recorded against these microorganisms and comparison with the results of plant extracts.

**Table 4.** Antibacterial activity of methanolic, cold water and hot water extracts of *P. integerrima* (gall), *P. bistorta* (root), *S. chirata* (stem) and *Z. officinale* (root).

Bacteria	<i>B. subtilis</i> ATCC 6633			<i>E. faecalis</i> ATCC 14506			<i>S. aureus</i> ATCC 6538			<i>P. aeruginosa</i> ATCC 27853			<i>S. typhi</i> ATCC 14028			
	Extract conc. ( l)	30	20	10	30	20	10	30	20	10	30	20	10	30	20	10
<b>Plant extract</b>	<b>Zone of inhibition (mm)</b>															
<i>Pistacia integerrima</i>																
Methanolic	12	8	0	10	6	0	10.5	6	0	10	6.5	0	11	7	0	
Cold water	8	0	0	0	0	0	9	0	0	0	0	0	8	0	0	
Hot water	9	0	0	8	0	0	8	0	0	8	0	0	9	0	0	
<i>Polygonum bistorta</i>																
Methanolic	11	6.5	0	13	8	0	8	0	0	9	6	0	0	0	0	
Cold water	12	8	0	9	0	0	Nil	0	0	10	6	0	8	0	0	
Hot water	10	7	0	10	0	0	9	0	0	8	0	0	8	0	0	
<i>Swertia chirata</i>																
Methanolic	12	7.5	0	0	0	0	0	0	0	8	0	0	9	0	0	
Cold water	15	9	0	8	0	0	10	6	0	0	0	0	9	0	0	
Hot water	9	0	0	8	0	0	8	0	0	8	0	0	10	0	0	
<i>Zingiber officinale</i>																
Methanolic	17	11	6	8	0	0	19	12	7	16	9	6	10	6	0	
Cold water	9	0	0	8	0	0	10	7	0	15	10	0	8	0	0	
Hot water	9	6	0	0	0	0	8	0	0	9	0	0	8	0	0	

#### ***Pistacia integerrima* (gall)**

Methanolic extracts (30, 20 and 10 l dilution per disc) showed maximum zones of inhibition of 12, 8 and 0 mm respectively against *B. subtilis*, 11, 7 and 0 mm against *S. typhi*, 10.5, 6 and 0 mm against *S. aureus*, and 10, 6 and 0 mm against *E. faecalis* and 10, 6.5 and 0 mm against *P. aeruginosa*. The results are presented in Table 4.

Cold water extracts of 30 l of dilution showed non-significantly ( $p > 0.05$ ) highest zone of inhibition

of 9 mm against *S. aureus*, 8 mm against *B. subtilis* and *S. typhi*, and no activity was observed against *E. faecalis*, *P. aeruginosa*. As far as 20 and 10 l cold water extracts are concerned, they showed no activity against any of the five bacteria (Table 4).

Hot water extracts of 30 l dilution showed non-significantly ( $p > 0.05$ ) high zones of inhibition of 9 mm against *B. subtilis* and *S. typhi*, 8 mm against *E. faecalis*, *S. aureus* and *P. aeruginosa*. As for 20 and 10 l dilutions, they showed no results. The

results are also presented in Table 4.

#### ***Polygonum bistorta* (root)**

Methanolic extracts of 30, 20 and 10 l showed significantly ( $p < 0.05$ ) different zones of inhibition of 13, 8 and 0 mm, respectively against *E. faecalis*, 11, 6.5 and 0 mm against *B. subtilis*, 9, 6 and 0 mm against *P. aeruginosa* and 8., 0 and 0 mm against *S. aureus*, while no activity of any

dilution was detected against *S. typhi* (Table 4). Cold water extracts showed significantly ( $p < 0.05$ ) different zones of inhibition of 12, 8 and 0 mm against *B. subtilis*, 10, 6 and 0 mm against *P. aeruginosa*, 9, 0 and 0 mm against *E. faecalis*, 8, 0 and 0 mm against *S. typhi* and show no activity against *S. aureus*. The results are shown in Table 4.

Hot water extracts showed significantly ( $p < 0.05$ ) different zones of inhibition of 10, 7 and 0 mm against *B. subtilis* and 10, 0 and 0 mm against *Enterococcus faecalis*, 9, 0 and 0 mm against *S. aureus*, 8, 0 and 0 mm against *P. aeruginosa* and *S. typhi* (Table 4).

#### **Swertia chirata (stem)**

Methanolic extract having 30, 20 and 10 l dilution per disc extracts showed significantly ( $p < 0.05$ ) different zones of inhibition of 12, 7.5 and 0 mm against *B. subtilis*, 9, 0 and 0 mm against *S. typhi* and 8, 0 and 0 mm against *P. aeruginosa* and no activity against *E. faecalis* and *S. aureus* (Table 4).

Cold water extracts showed significantly ( $p < 0.05$ ) different zones of inhibition of 15, 9 and 0 mm against *B. subtilis*, 9, 0 and 0 mm against *S. typhi*, and 8, 0 and 0 mm against *E. faecalis* and 10, 6 and 0 mm against *S. aureus*, while no activity was observed against *P. aeruginosa* (Table 4).

30 l dilutions per disc of hot water extracts showed non-significantly ( $p > 0.05$ ) different zones of inhibition of 10 mm against *S. typhi*, 9 mm against *B. subtilis*, 8 mm against *E. faecalis*, *S. aureus*, and *P. aeruginosa*. No activity was observed for 20 and 10 l dilutions per disc against any of the bacteria. The detail is given in Table 4.

#### **Zingiber officinale (root)**

Methanolic extracts having 30, 20 and 10 l showed significantly ( $p < 0.05$ ) different zones of inhibition of 19, 12 and 7 mm against *S. aureus*, 16, 9 and 6 mm against *P. aeruginosa*, 17, 11 and 6 mm against *B. subtilis*, 10, 6 and 0 mm against *S. typhi* while values of 8, 0 and 0 mm were observed against *E. faecalis* (Table 4).

Cold water extracts showed significantly ( $p < 0.05$ ) different zones of inhibition of 15, 10 and 0 mm against *P. aeruginosa*, 10, 7 and 0 mm against *S. aureus*, 9, 0 and 0 mm against *B. subtilis*, 8, 0 and 0 mm against *E. faecalis* and *S. typhi* (Table 4).

Hot water extracts showed significantly ( $p < 0.05$ ) different zones of inhibition of 9, 6 and 0 mm against *B. subtilis* and 9, 0 and 0 mm against *P. aeruginosa*, 8, 0 and 0 mm against *S. aureus* and *S. typhi*, and no activity against *E. faecalis* (Table 4).

#### **Ceftriaxone**

The maximum zones of inhibition of 31 mm against *E.*

*faecalis* and *S. typhi*, 30 mm against *B. subtilis*, 26 mm against *S. aureus* and 25 mm against *P. aeruginosa* were recorded (Table 5).

#### **Ceftriaxone sodium**

The maximum zones of inhibition of 34 mm against *S. typhi*, 32 mm against *E. faecalis*, 29 mm against *P. aeruginosa* and 27 mm against *B. subtilis* and *S. aureus* were observed (Table 5).

#### **Cefuroxime**

The maximum zones of inhibition of 27 mm against *E. faecalis* and *P. aeruginosa*, 26 mm against *S. typhi*, 21 mm against *B. subtilis* and 10 mm against *S. aureus* were observed (Table 5).

#### **Ciprofloxacin**

The maximum zones of inhibition of 28 mm against *B. subtilis*, 27 mm against *E. faecalis*, 26 mm against *S. aureus* and *S. typhi* and 25 mm against *P. aeruginosa* were detected (Table 5).

#### **Gentamycine**

The maximum zones of inhibition of 30 mm against *P. aeruginosa*, 29 mm against *E. faecalis*, 22 mm against *B. subtilis* and *S. typhi* and 18 mm against *S. aureus* were observed (Table 5).

#### **Levofloxacin**

The maximum zones of inhibition of 29 mm against *P. aeruginosa*, 28 mm against *B. subtilis* and *E. faecalis*, 27 mm against *S. typhi* and 23 mm against *S. aureus* were indicated (Table 5).

#### **Metronidazole**

The maximum zones of inhibition of 18 mm against *S. typhi*, 17 mm against *B. subtilis*, 16 mm against *P. aeruginosa*, 13 mm against *E. faecalis* and 10 mm against *S. aureus* were recorded (Table 5).

#### **Tranexamic acid**

The maximum zone of inhibition of 21 mm against *E. faecalis*, 19 mm against *B. subtilis*, 17 mm against *S.*

**Table 5.** Antibacterial activity of standard antibiotics.

Antibiotic	Bacteria				
	<i>B. subtilis</i> ATCC 6633	<i>E. faecalis</i> ATCC 14506	<i>S. aureus</i> ATCC 6538	<i>P. aeruginosa</i> ATCC 27853	<i>S. typhi</i> ATCC 14028
	<b>Zone of inhibition (mm)</b>				
Ceftriaxone	30	31	26	25	31
Ceftriaxone sodium	27	32	27	29	34
Cefuroxime	21	27	10	27	26
Ciprofloxacin	28	27	26	25	26
Gentamycine	22	29	18	30	22
Levofloxacin	28	28	23	29	27
Metronidazole	17	13	10	16	18
Tranexamic acid	19	21	10	10	17
Methanol*	0	0	0	0	0

\*Methanol was used as control.

*typhi* and 10 mm against *S. aureus* and *P. aeruginosa* were recorded (Table 5).

#### Methanol (control)

Methanol, in a concentration of 30 l, was used as a control in this experiment and no inhibitory results were found against any bacterial strain (Table 5).

#### DISCUSSION

The antimicrobial effect of medicinal plants is well documented. The results of different studies provide evidence that some medicinal plants might indeed be potential sources of new antibacterial agents even against some antibiotic-resistant strains. Using the disk diffusion method in this study, it was observed that extracts of *P. Integerrima*, *P. bistorta*, *S. charita* and *Z. officinale* produce antibacterial activity against both gram negative (*P. aeruginosa* and *S. typhae*) and gram positive (*B. subtilis*, *E. faecalis* and *S. aureus*) pathogens. The profile of four medicinal plants used in this study is shown in Table 2. Although the result of each plant extract varied from that of the other against same pathogen and also against different pathogens, it was also observed that the antibacterial activity varied among methanolic, cold water and hot water extracts of the same plant against the same bacteria. Some bacteria show resistance against one type of extract, while other types of extract of the same plant show the best effect against the same bacteria. Eight standard antibiotics were also used and compared with the activity of the plant extracts.

The activity of each individual plant is comparatively discussed as follows:

Antibacterial activity of *P. integerrima* (Gall) was checked

against *B. subtilis*, *E. faecalis*, *S. aureus*, *P. aeruginosa* and *S. typhi*. Methanolic, cold water and hot water extracts were made for this purpose. Standard antibiotics used for the study were ceftriaxone, ceftriaxone sodium, cefuroxime, levofloxacin, tranexamic acid, gentamycine, metronidazole and ciprofloxacin.

The net results of *P. integerrima* (Gall) with five bacterial strains showed that this specific part of the plant has good antibacterial activity. The current study revealed that methanolic extracts of *P. integerrima* (Gall) can be used as an alternate of cefuroxime, metronidazole and tranexamic acid in the treatment of *S. aureus* infections and tranexamic acid in the treatment of *P. aeruginosa* infections, after conversion to aqueous medium.

The antibacterial activity of *P. bistorta* (root) was tested against *B. subtilis*, *E. faecalis*, *S. aureus*, *P. aeruginosa* and *S. typhi* and compared with standard antibiotics. The final result with five bacterial strains showed that the specific part of the plant has moderate antibacterial activity. Already tested is the antibacterial activity of different plants including *P. bistorta* and found recorded is the good antibacterial activity against *X. pathovars* and moderate activity against the remaining pathogens. In the current study, positive results were obtained for *P. bistorta* (root). Methanolic extracts of *P. bistorta* (root) can be used as an alternate for metronidazole in the treatment of *E. faecalis* infections, while cold water extracts can be used as an alternate for tranexamic acid in the treatment of *P. aeruginosa* infections.

Antibacterial activity of *S. chirata* (stem) was tested against *B. subtilis*, *E. faecalis*, *S. aureus*, *P. aeruginosa* and *S. typhi* with standard antibiotics. In the current study, positive results were obtained for *S. chirata* (stem). Cold water extracts can be used as an alternate to metronidazole and tranexamic acid in the treatment of *S. aureus* infections.

By using *Z. officinale* (root), antibacterial activity was tested against *B. subtilis*, *E. faecalis*, *S. aureus*, *P. aeruginosa* and *S. typhi* with standard antibiotics. The net results revealed that the specific part of the plant has good antibacterial activity and the results were similar to that previously reported Indu et al. (2006). Methanolic extracts of *Z. officinale* (root) can be used like tranexamic acid, gentamycine, cefuroxime and metronidazole in the treatment of *S. aureus* infections, metronidazole and tranexamic acid in the treatment of *P. aeruginosa*. It can be used as an alternate to metronidazole in the treatment of *B. subtilis* infections. Cold water extracts can be preferred upon tranexamic acid in the treatment of *P. aeruginosa* infections. It can also be used as an alternate to metronidazole and tranexamic acid in the treatment of *S. aureus* infections. Among the plants studied, *S. chirata* and *Z. officinale* possessed the highest antibacterial activity. Malu et al. (2009) have also studied the antibacterial activity of ginger extracts. The results elaborated that all the extracts except the water extract have antibacterial activity, while bacterial growth inhibition was also dose dependent.

Since methanol extract seems to possess the highest solubility/extractability for various plant metabolites, it is found to be more effective against bacteria. Thus, the stronger extraction capacity of methanol for these fruits became evident and yielded a greater number of active constituents having strong antibacterial activity. These findings were also previously supported (Melvin et al., 2009).

## Conclusion

The present results offers a scientific basis for the traditional use of cold and hot water and methanolic extracts of *P. integerrima*, *P. bistarta*, *S. chirata* and *Z. officinale*. However, further studies on these medicinal plants are necessary to determine their active constituent-activity relationship. The antibacterial activities could be enhanced if active components are purified and adequate dosage determined for proper administration. This may be the first preliminary report on the anti-microbial activity of these medicinal plants in Pakistan.

## REFERENCES

- Babu S, Satish S, Mohana DC, Raghavendra MP, Raveesha KA (2007). J. Agri. Technol., 33: 785.
- Bown D (1995). Encyclopaedia of Herbs and their Uses. Dorling Kindersley, London, UK.
- Chanda YR (1976). The wealth of India. Publication AND Information Directorate, New Delhi, India.
- Chevallier A (1996). The Encyclopedia of Medicinal Plants. Dorling Kindersley. London, UK.
- Grieve M (1984). A Modern Herbal. Penguin Publishers, London, UK.
- Indu MN, Hatha AAM, Abirosh C, Harsha U, Vivekanandan G (2006). Brazilian J. Microbiol., 37: 153.
- Joshi P, Dhawan V (2005). *Swertia chirayita*—an overview. Academic Press, New York, USA.
- Malu SP, Obochi GO, Tawo EN, Nyong BE (2009). Antibacterial activity and medicinal properties of Ginger (*Zingiber officinale*). Global J. Pure Appl. Sci., 15: 365-368.
- Melvin JM, Jayachitra J, Vijayapriya M (2009). J. Med. Plants Res., 3: 1134.
- Nasir E, Ali SI (1983). Flora of Pakistan National Herbarium (Stewart Collection). No: 152 (December 1983), No: 146 (1<sup>st</sup> October 1982), No: 205 (25<sup>th</sup> July 2001). Agric. Res. Council, Islamabad, Pakistan.
- Samin J, Khan MA, Din SU, Murad W, Hussain M, Ghani A (2008). Pak. J. Weed Sci. Res., 14: 169.
- Taylor RSL, Manandhar NP, Towers GHN (1995). Screening of selected medicinal plants of Nepal for antimicrobial activities. J. Ethnopharmacol., 46: 153-159.
- The State Pharmacopoeia Commission of P.R. of China. (2005). Pharmacopoeia of people's Republic of China, Beijing. Publishing House of Chemical Industry, I: 202.
- Wagner EH, Hikino H (1965). Economic and Medicinal Plants Research. Academic Press, New York, USA.