Pak. J. Bot., 45(4): 1369-1374, 2013.

ANTIBACTERIAL ACTIVITIES OF SIXTEEN SPECIES OF MEDICINAL PLANTS REPORTED FROM DIR KOHISTAN VALLEY KPK, PAKISTAN

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Abstract

The methanol extract of 16 species of medicinal plants were screened for their antimicrobial activity, using Agar well diffusion method. They were tested against 6 species of tested pathogens *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Bacillus subtilis* and *Bacillus cereus*, crude extract and subsequent fractions demonstrated moderate to excellent antibacterial activities. Highest antibacterial activity was displayed by the methanol fraction showed good and significant activity in 10 species of medicinal plants.

Introduction

There are three traditional system of medicine, namely Ayurveda, Siddha and Unani present in India and Pakistan (Hazrat *et al*., 2011). Ethnomedicine is a type of research in an area that deals with medicines derived from plants, animals or minerals and used for the treatment of different ailments based on indigenous pharmacopia, tradition and of herbal magic (Hazrat *et al*., 2007).A number of local Hakims claim that these plants species are used for the treatment of various ailment like fever, gout, rheumatism, sour throat, cough, pain in body tonic, antperiodic, vomiting, appetizer, astringent, anathematic, diarrhea, gastric pain, stomach ache and cure cold.

In literature, a variety of pharmacological activities have been reported on various plants species. In Pakistan the antimicrobial activity is still at pioneer stage therefore, there is a constant need to find new and useful therapeutic agents and also achievable antimicrobial activities, for this purpose mostly by using crude aqueous or alcohol extracts. In this connection Ahmad *et al*., (2009) studied the antimicrobial activities of some species of Boraginaceae of hilly area of Malakand university. The primary health care products that are socially accepted and scientifically valuable, for all human being are studied by (Shandesh *et al*., 2009). Additional work was conducted by Masood *et al*., (2001) on the phytochemical isolation of compound from the whole plant of *Anemone obtusiloba* .The compound obtusilobinin and obtusilobin, two new saponins, were isolated from the ethanolic extract of *A. obtusiloba* of Ranunculaceae. Raza *et al*., (2001) crushed out the dried roots of *Delphinium denudatum* the extract were used as a folk remedy for the treatment of epilepsy. The other workers also used the same worked on the traditional medicine of *Nigella sativa* seeds for the healing of a variety of diseases including asthma, diarrhea, spasmolytic and bronchodilator activities were also found Gilani *et al*., (2001). Similarly the work of Abdul & Khan, (2005) also emphasized upon the sensitivity of the crude extracts of *Clerodendrum inerme* against some of the human pathogenic bacteria. Five plant extracts (Petrol, Benzene, Methanol, Ethly acetate and Aqueous) under six different concentrations (500µg/ml, 1mg/ml, 2mg/ml, 5mg/ml, 10mg/ml and 15mg/ml) were tested by disk diffusion method. Abdel & Aly, (2005) reported that *Nigella sativa* and *Syzygium aromaticum* oil were used for the treatment of inflammatory diseases and had antioxidant properties. The extensive study was conducted by Taous *et al*., (2005) are used the methanolic extract derived from the whole plants of *Paeonia emodi* for various in vitro including antifungal, antibacterial and insecticidal activities. Same strategic work was conducted by, Shaheen *et al*., (2005) they collected the aerial parts of *Aconitum* leaves and tested for anti-inflammatory, antioxidant activity. Similarly the leaves of *Aloe vera* were tested for antimicrobial activities (Agarry *et al*., 2005). The medicinal plants pictorial guide of Pakistan was written and published by Shinwari *et al*., (2006) they highlighted 466 medicinal plants in his book. The antiparasitic activity of *Nigella sativa* was reported by (Ayaz *et al*., 2007). Based on the reported literature on the biological activities of medicinal plants, 16 plant species were tested for antimicrobial activities for the first time from Dir Kohistan area KPK, Pakistan.

Materials and Methods

Sixteen species of high valued medicinal plants were collected from Kohistan, Dir Upper, KPK, in June to August 2009. They plants were identified with the help of available literature and standard procedure (Nasir & Ali, 1972-1994; Ali & Qaiser, 1995-2007). The voucher specimens (R10-R25) were deposited in the herbarium of the University of Malakand and Shaheed Benazir Bhutto University for future research activities.

Extraction: The plants were dried in a shade and grind into small pieces under sterile conditions, 200g portion of each plant was percolated with commercial grade methanol (3x1L) at room temperature. The extracts obtained were concentrated in vacuum at 40 °C or using rotary evaporator to yield crude methanol extracts. All the fractions were transferred to glass vials with a screw cap and labeled as R10 to R25.

Bacterial strains: Tests were performed on six bacterial reference strains provided by HEJ (Hussain Ebrahim Jamali Institute of Chemistry) Karachi. Bacterial strains were *Escherchia coli, Bacillus subtilis*, *Shigella flexeneri* (clinical isolate), *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi*. They were maintained on agar slant at 48^0C. The strains were activated at 37.8^0C for 24 h or nutrient agar (NA) or Sabouraud Glucose Agar (SGA) for bacteria, prior to any screening.

Results

A total of 16 medicinal plant species extract were used for antimicrobial activity collected from the unexplored study area given in Table 1.

The test sample that contained antibacterial agent inhibited the growth of bacterial stains producing a zone of inhibition i.e., observing a clear zone where the growth of bacteria had not occurred. The antibacterial activities for the extracts against six pathogenic strains obtained from the plants under study by the diffusion method are shown in Tables 2-7.

Table 1. Plants used for antimicrobial activities.

S.No.	Plant Name	Code #	Part used	Weight in gm	Chemical used
1.	*Pistacia chinensis*	R10	Bark	200g	Methanol
2.	*Monotheca buxifolia*	R11	Fruit	200g	Methanol
3.	*Diospyros kaki*	R12	Bark	200g	Methanol
4.	*Rumex hastatus* L.	R13	Leaves	200g	Methanol
5.	*Helianthus annus* L.	R14	Leaves	200g	Methanol
6.	*Mentha longifolia* L.	R15	Leaves	200g	Methanol
7.	*Solanum nigrum* L.	R16	Fruit	200g	Methanol
8.	*Dodonaea viscosa* L.	R17	Leaves	200g	Methanol
9.	*Cannabis sativa* L.	R18	Leaves	200g	Methanol
10.	*Thymus vulgaris L.*	R19	W.plant	200g	Methanol
11.	*Rosmarinus officinalis* L.	R20	Leaves	200g	Methanol
12.	*Salvia officinalis* L.	R21	Leaves	200g	Methanol
13.	*Ocimum basilicum* L.	R22	Leaves	200g	Methanol
14.	*Achillea millefolium* L.	R23	Flower	200g	Methanol
15.	*Punica granatum* L.	R24	Pericarp	200g	Methanol
16.	*Polygonum plebjum* R. Br.	R25	W.plant	200g	Methanol

Note: R= Sample code W. Plant= Whole plant g = gram

Table 2. Antibacterial activities of fractions (R01-R25) against *Escherichia coli*.

S.C	CPE	ZI S	CC	Z ISD
R10	100	26.3±0.5	100	36.1 ± 0.2
R11	100	30.1±0.5	100	36.1 ± 0.2
R12	100	27.8±0.5	100	36.1 ± 0.2
R13	100	25.4±0.3	100	39.8 ± 0.3
R14	100	36.6±0.1	100	39.8 ± 0.3
R15	100	26.1±0.5	100	39.8 ± 0.3
R16	100	20.0±0.0	100	25 ± 0.3
R17	100	18.6±1.1	100	25 ± 0.3
R18	100	23.3±0.5	100	25 ± 0.3
R19	100	0.0±0.0	100	36.1 ± 0.2
R20	100	0.0±0.0	100	36.1 ± 0.2
R21	100	0.0±0.0	100	36.1 ± 0.2
R22	100	0.0±0.0	100	36.1 ± 0.2
R23	100	0.0±0.0	100	36.1 ± 0.2
R24	100	0.0±0.0	100	36.1 ± 0.2
R25	100	20.3±0.2	100	36.1 ± 0.2

Note:
1. S.C= Sample code
2. CPE (mg/ml) = Concentration of plant extract (mg/ml)
3. ZIS = Zone of inhibition of sample (mm ± SE)
4. CC (mg/ml) = Concentration of Ciprofloxacin (mg/ml)
5. ZISD = Zone of Inhibition of Std. Drug (ciprofloxacin) (mm)
6. SE = Standard error

Table 3. Antibacterial activities of fractions (R10-R25) against *Bacillus subtilis*.

S.C	CPE	ZI S	CC	Z ISD
R10	100	22.7 ± 0.4	100	25.6 ± 0.3
R11	100	27.0 ± 0.2	100	25.6 ± 0.3
R12	100	26.1 ± 0.1	100	25.6 ± 0.3
R13	100	26.0 ± 0.6	100	31.1 ± 0.5
R14	100	21.0 ± 0.2	100	31.1 ± 0.5
R15	100	23.4 ± 0.2	100	31.1 ± 0.5
R16	100	0.0 ± 0.0	100	0.0 ± 0.0
R17	100	10.00 ± 0.0	100	0.0 ± 0.0
R18	100	13.66 ± 0.2	100	0.0 ± 0.0
R19	100	0.0 ± 0.0	100	36.1 ± 0.2
R20	100	26.66 ± 0.2	100	36.1 ± 0.2
R21	100	0.0 ± 0.0	100	36.1 ± 0.2
R22	100	0.0 ± 0.0	100	36.1 ± 0.2
R23	100	0.0 ± 0.0	100	36.1 ± 0.2
R24	100	25.00 ± 0.4	100	36.1 ± 0.2
R25	100	23.11 ± 0.2	100	36.1 ± 0.2

Note:
1. S.C= Sample code
2. CPE (mg/ml) = Concentration of plant extract (mg/ml)
3. ZIS = Zone of inhibition of sample (mm ± SE)
4. CC (mg/ml) = Concentration of Ciprofloxacin (mg/ml)
5. ZISD = Zone of Inhibition of Std. Drug (ciprofloxacin) (mm)
6. SE = Standard error

Table 4. Antibacterial activity of fractions (R10-R25) against *Bacillus cereus*.

S.C	CPE	ZI S	CC	Z ISD
R10	100	29.1 ± 0.3	100	32.7 ± 0.3
R11	100	20.0 ± 0.5	100	32.7 ± 0.3
R12	100	3001 ± 0.2	100	32.7 ± 0.3
R13	100	20.3 ± 0.4	100	26.1 ± 0.4
R14	100	24.6 ± 0.4	100	26.1 ± 0.4
R15	100	25.0 ± 0.4	100	26.1 ± 0.4
R16	100	16.33 ± 1.1	100	0.0 ± 0.0
R17	100	15.66 ± 0.3	100	0.0 ± 0.0
R18	100	11.33 ± 1.5	100	0.0 ± 0.0
R19	100	0.0 ± 0.0	100	36.1 ± 0.2
R20	100	0.0 ± 0.0	100	36.1 ± 0.2
R21	100	0.0 ± 0.0	100	36.1 ± 0.2
R22	100	0.0 ± 0.0	100	36.1 ± 0.2
R23	100	0.0 ± 0.0	100	36.1 ± 0.2
R24	100	26.00 ± 0.4	100	36.1 ± 0.2
R25	100	20.00 ± 0.0	100	36.1 ± 0.2

Note:
1. S.C= Sample code
2. CPE (mg/ml) = Concentration of plant extract (mg/ml)
3. ZIS = Zone of inhibition of sample (mm ±SE)
4. CC (mg/ml) = Concentration of ciprofloxacin (mg/ml)
5. ZISD = Zone of Inhibition of Std. Drug (ciprofloxacin) (mm)
6. SE = Standard error

Table 5. Antibacterial activity of fractions (R10-R25) against *Staphylococcus aureus*.

S.C	CPE	ZI S	CC	Z ISD
R10	100	20.0 ± 0.3	100	20.1 ± 0.4
R11	100	25.3 ± 0.1	100	20.1 ± 0.4
R12	100	31.3 ± 0.1	100	20.1 ± 0.4
R13	100	21.3 ± 0.4	100	26.1 ± 0.4
R14	100	20.6 ± 0.4	100	26.1 ± 0.4
R15	100	23.0 ± 0.4	100	26.1 ± 0.4
R16	100	17.33 ± 1.1	100	0.0 ± 0.0
R17	100	16.66 ± 0.3	100	0.0 ± 0.0
R18	100	13.33 ± 1.5	100	0.0 ± 0.0
R19	100	0.0 ± 0.0	100	36.1 ± 0.2
R20	100	0.0 ± 0.0	100	36.1 ± 0.2
R21	100	0.0 ± 0.0	100	36.1 ± 0.2
R22	100	0.0 ± 0.0	100	36.1 ± 0.2
R23	100	0.0 ± 0.0	100	36.1 ± 0.2
R24	100	22.00 ± 0.4	100	36.1 ± 0.2
R25	100	26.00 ± 0.0	100	36.1 ± 0.2

Note:
1. S.C= Sample code
2. CPE (mg/ml) = Concentration of plant extract (mg/ml)
3. ZIS = Zone of inhibition of sample (mm ± SE)
4. CC (mg/ml) = Concentration of ciprofloxacin (mg/ml)
5. ZISD = Zone of Inhibition of Std. Drug (ciprofloxacin) (mm)
6. SE = Standard error

Table 6. Antibacterial activity of fractions (R10-R25) against *Pseudomonas aeruginosa*.

S.C	CPE	ZI S	CC	Z ISD
R10	100	16.1 ± 0.2	100	23.7 ± 0.1
R11	100	24.3 ± 0.1	100	23.7 ± 0.14
R12	100	19.1 ± 0.3	100	23.7 ± 0.1
R13	100	24.3 ± 0.5	100	32.4 ± 0.1
R14	100	18.3 ± 0.4	100	32.4 ± 0.1
R15	100	17.3 ± 0.1	100	32.4 ± 0.1
R16	100	20.0 ± 0.0	100	0.0 ± 0.0
R17	100	17.66 ± 0.8	100	0.0 ± 0.0
R18	100	14.33 ± 0.6	100	0.0 ± 0.0
R19	100	22.0 ± 0.0	100	36.1 ± 0.2
R20	100	0.0 ± 0.0	100	36.1 ± 0.2
R21	100	0.0 ± 0.0	100	36.1 ± 0.2
R22	100	24.0 ± 0.0	100	36.1 ± 0.2
R23	100	0.0 ± 0.0	100	36.1 ± 0.2
R24	100	23.00 ± 0.4	100	36.1 ± 0.2
R25	100	14.00 ± 0.0	100	36.1 ± 0.2

Note:
1. S.C= Sample code
2. CPE (Mg/ml) = Concentration of plant extract (Mg/ml)
3. ZIS = Zone of inhibition of sample (mm ±SE)
4. CC (Mg/ml) = Concentration of Ciprofloxacin (mg/ml)
5. ZISD = Zone of Inhibition of Std. Drug (ciprofloxacin) (mm)
6. SE = Standard error

Table 7. Antibacterial activity of fractions (R10-R25) against *Salmonella typhi*.

S.C	CPE	ZI S	CC	Z ISD
R10	100	14.9 ± 0.5	100	24.3 ± 0.3
R11	100	22.0 ± 0.2	100	24.3 ± 0.3
R12	100	17.4 ± 0.4	100	24.3 ± 0.3
R13	100	22.5 ± 0.2	100	26.5 ± 0.2
R14	100	20.3 ± 0.3	100	26.5 ± 0.2
R15	100	22.0 ± 0.5	100	26.5 ± 0.2
R16	100	16.66 ± 1.1	100	0.0 ± 0.0
R17	100	14.33 ± 0.4	100	0.0 ± 0.0
R18	100	24.0 ± 0.3	100	0.0 ± 0.0
R19	100	0.0 ± 0.0	100	36.1 ± 0.2
R20	100	0.0 ± 0.0	100	36.1 ± 0.2
R21	100	0.0 ± 0.0	100	36.1 ± 0.2
R22	100	0.0 ± 0.0	100	36.1 ± 0.2
R23	100	0.0 ± 0.0	100	36.1 ± 0.2
R24	100	0.0.00 ± 0.4	100	36.1 ± 0.2
R25	100	15.00 ± 0.0	100	36.1 ± 0.2

Note:
1. S.C= Sample code
2. CPE (mg/ml) = Concentration of plant extract (mg/ml)
3. ZIS = Zone of inhibition of sample (mm ± SE)
4. CC (mg/ml) = Concentration of ciprofloxacin (mg/ml)
5. ZISD = Zone of Inhibition of Std. Drug (ciprofloxacin) (mm)
6. SE = Standard error

Discussion

Plant parts used along with their acquisition code number are given in Table 1. In present study Agar Well Diffusion method was used as reported by Ahmad *et al.*, (2009) to evaluate the antibacterial potential of selected medicinal plants against bacterial strains *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Bacillus subtilis* and *Bacillus cereus*. The current study showed no correlation between antibiotic resistance and susceptibility of test strains with plant extracts (Ahmad & Beg, 2001). Out of all the tested medicinal plants 62.5% plants *Pistacia chinensis, Monotheca buxifolia, Diospyros kaki, Rumex hastatus, Helianthus annus, Mentha longifolia, Solanum nigrum, Dodonaea viscosa, Cannabis sativa, Polygonum plebjum* were found to be potential against all the tested pathogenic strains with significant activity ranging 16-39 mm inhibition zone. While 37% plants *Thymus vulgaris, Rosmarinus officinalis, Salvia officinalis, Ocimum basilicum, Achillea millefolium, Punica granatum* were found to have no antibacterial activity.

Recommendations: The medicinal plants showed significant activities may be selected for further phytochemical screening to pin out the qualitative and quantitative active constituents responsible for antibacterial activities.

Acknowledgement

This work was carried out under the indigenous Ph.D. programme sponsored by the Higher Education Commission, Pakistan which is gratefully acknowledged.

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(Received for publication 10 January 2012)