ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF NINE SPECIES OF FAMILY RANUNCULACEAE FROM DIR KOHISTAN VALLEY, KPK, PAKISTAN

ALI HAZRAT[1,2*], JEHANDAR SHAH[2], MOHAMMAD NISAR[1] AND KHAN SHER[2]

[1]Dept. of Botany, University of Malakand, Chakdara, Dir Lower, Khyber Pakhtunkhwa-Pakistan
[2]Dept. of Botany, Shaheed Benazir Bhutto University, Sheringal, Dir Upper, Khyber Pakhtunkhwa-Pakistan
[*]Corresponding author e-mail: ali_hazrat8@yahoo.com

Abstract

The aim of this study was to evaluate the crude methanol extract and subsequent fractions of nine species of Ranunculaceae. They were screened for antibacterial and antifungal activities. Against tested pathogens, crude extract and subsequent fractions demonstrated moderate to excellent antibacterial activities. Highest antibacterial activity was displayed by the Methanol fraction from three species showing significant activity, while antifungal activity was found in two species.

Introduction

Family Ranunculaceae consist of about 50 genera and 2000 species in the world while in Pakistan it is represented by 22 genera and 150 species, distributed in the north temperate and arctic regions (Riedl, 1990).

A number of local Hakims claim that these plants species have been 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 cold.

In literature, a variety of pharmacological activities have been reported on various plants species. In Pakistan studies on antimicrobial activity of plants is still at pioneer stage. For this purpose crude aqueous or alcohol extracts are used. Ahmad *et al.*, (2009) studied the antimicrobial activities of some species of Boraginaceae of hilly area of Malakand University. The primary health care products were studied by Shandesh *et al.*, (2009). Masood *et al.*, (2001) isolated compounds from the whole plant of *Anemone obtusiloba*, obtusibinin and obtusilobin, two new saponins, from the ethanolic extract of *A. obtusiloba* of Family Ranunculaceae. According to Raza *et al*., (2001) crushed dried roots of *Delphinium denudatum* and the extract was used as a folk remedy for the treatment of epilepsy. Gilani *et al*., (2001) worked on the traditional medicine of *Nigella sativa* seeds for the healing of a variety of diseases including asthma, diarrhea, spasmolytic and bronchodilator activities. 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 and Aly, (2005) reported that *Nigella sativa* and *Syzygium aromaticum* oil are used for the treatment of inflammatory diseases and have antioxidant properties. The extensive study was conducted by Taous *et al*., (2005) who obtained methanolic extract from the whole plants of *Paeonia emodi* tested in vitro biological activities including antifungal, antibacterial and insecticidal. Shaheen *et al*., (2005) collected the aerial parts of *Aconitum* and it tested for anti-inflammatory, antioxidant activity. Similarly the leaves of *Aloe vera* antimicrobial activities were tested by Agarry *et al*., (2005). The medicinal plants pectoral guide of Pakistan was written and published by Shinwari *et al*., (2006) and they highlighted 466 medicinal plants in this book. The antiparasitic activity of *Nigella sativa* was reported by Ayaz *et al*., (2007). Based on the reported literature on the biological activities of Ranaunculaceous nine plant species have been tested in this paper for antimicrobial and antifungal activities for the first time from Dir Kohistan area KPK, Pakistan.

Materials and Methods

Nine species of Ranunculaceae were collected from Dir, Kohistan, KPK, Pakistan in June to August 2010. They were identified with the help of available literature (Nasir & Ali, 1995). The voucher specimens (R01-R09) were deposited in the herbarium of the University of Malakand and Shaheed Benazir Bhutto University Sheringal.

Extraction: The plants were dried in shade and ground 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 R01 to R09.

Fungal and bacterial strains: Tests were performed on six fungal and six bacterial reference strains. Bacterial strains were *Escherchia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633, *Shigella flexeneri* (clinical isolate), *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853 and *Salmonella typhi* ATCC 19430. Fungal strains includes *Trichophyton longifusus* (clinical isolate), *Candida albicans* ATCC 2091, *Aspergillus flavus* ATCC 32611, *Microspoum canis* ATCC 11622, *Fusarium solani* 11712 and *Candida glaberata* ATCC 90030. They were maintained on agar slant at $48C^0$. The strains were activated at $37.8C^0$ for 24 h or nutrient agar (NA) or Sabouraud Glucose Agar (SGA) respectively for bacteria and fungi, prior to any screening.

Table1. Plants used for Antibacterial and Antifungal activities.

Plant Name	Code #	Part used	Weight in g	Chemical used
Ranunculus hirtellus Royle	R01	Whole plant	200g	Methanol
Ranunculus muricatus L.	R02	Whole plant	200g	Methanol
Ranunculus sceleratus L.	R08	Whole plant	200g	Methanol
Ranunculus arvensis L.	R04	Whole plant	200g	Methanol
Ceratocephala falcata (L.) Pers.	R05	Whole plant	200g	Methanol
Delpinium uncinatum Hk. f. &T.	R06	Whole plant	200g	Methanol
Nigella Sativa L.	R07	Whole plant	200g	Methanol
Adonis aestivalis L.	R03	Whole plant	200g	Methanol
Aconitum heterophyllum Wall. Ex Royle	R09	Whole plant	200g	Methanol

Hole diffusion method: Agar Well Diffusion Method was used to screen extracts for their antibacterial activity (Ahmad *et al.,* 2009).

Results and Discussion

1. **Antibacterial activity:** The antibacterial activities of the extracts obtained from the plants under study by the diffusion method are shown in Table 2. Agar Well Diffusion Method was used to screen crude extracts for their antibacterial activity. *S. Aureus, E. coli, P. aeruginosa, S. typhi, B. subtilus* and *S. flexenari* were used as test organisms. 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. All these cultures are kept at 4°C prior to testing they were sub-cultured in liquid nutrient broth and incubated at 30°C for 18-24 hrs and then used for the screening. The results indicated (Table 2), that the crude extract of (R1, R2, R3, R4, R6 and R8) showed no activity and (R5, R7 and R9) showed significant activity (with 16-30 mm inhibition zone).

Table 2. Antibacterial activities of fractions (R1-R09) against E. ***coli, B. subtilis, S. flexeneri, S. aureus, P. aeruginosa*** **and S.** ***typhi.***

S.C	CPE	ZI S	CC	Z ISD
R1	100	0.0±0.0	100	35.7± 2.2
R2	100	0.0±0.0	100	35.7± 2.2
R3	100	0.0±0.0	100	39.4± 1.2
R4	100	0.0±0.0	100	39.4± 1.2
R5	100	15.11±0.2 to 17.11+0.2	100	30.3± 0.2
R6	100	0.0±0.0	100	31.2± 1.3
R7	100	15.00±0.3 to 17.0±4.3	100	30.3± 0.2
R8	100	0.0±0.0	100	30.3± 0.1
R9	100	15.01±0.4 to 18.6±5.2	100	30.3± 0.1

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

2. Antifungal Activity: Fungi cause great losses in agriculture as well as in the forest and food industry. Different chemicals, so called fungicides, have been used to prevent and kill fungi in various environments. The drawback of using fungicides is their potential have negative effects on the environment and living organisms, for instance toxicity to humans, birds and animals, accumulation in soil and water and build-up resistance in pathogen populations. Nowadays, the public demand has grown for more environmental friendly methods to prevent fungal spoilage of food and feed (Ahmad *et al.*, 2009). The goal of our study was to find the antifungal potential of Ranunculaceae that could be used for biological control of fungi in different areas.

Various plant extracts listed in the (Table 1) were tested against six strains of fungi their antifungal activities (Table 3). From the results it can be seen that R07 showed low antifungal activity while R08 showed good antifungal activity and rest of the species showed no activity.

Table 3. Antifungal activity of fractions (R01-R09) against *C. albicans*[1], *T. longifusus*[2], *A. flavus*[3], *M. Canis*[4], *F. solani*[5] and *C. glabrata*[6].

Sample Code.	% inhibition of the sample	Std.Drug MIC µg/mL (Miconazole)	Std.Drug MIC µg/mL (Miconazole)	Std.Drug MIC µg/mL (Miconazole)	Std.Drug MIC µg/mL (Miconazole)	Std.Drug MIC µg/mL (Miconazole)
		1 & 2	**3**	**4**	**5**	**6**
R1	0.0±0.0	110.8	20	98.4	73.25	110.0
R2	0.0±0.0	110.8	20	98.4	73.25	110.0
R3	0.0±0.0	110.8	20	98.4	73.25	110.0
R4	0.0±0.0	110.8	20	98.4	73.25	110.0
R5	0.0±0.0	110.8	20	98.4	73.25	110.0
R6	0.0±0.0	110.8	20	98.4	73.25	110.0
R7	32±0.1 to 35±0.1	110.8	20	98.4	73.25	110.0
R8	60± 0.1± to 67±0.1	110.8	20	98.4	73.25	110.0
R9	0.0±0.0	110.8	20	98.4	73.25	110.0

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