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Antibacterial and Phytotoxic Profile of Selected Pakistani Medicinal Plants

[1]*Abdur Rauf,* [2]*Naveed Muhammad,* [3]*Ajmal Khan,*
[3,4]*Nizam Uddin,* [1]*Muhammad Atif and* [5]*Barkatullah*

[1]Institute of Chemical Sciences, University of Peshawar, Peshawar -25120, KPK, Pakistan
[2]Department of Pharmacy University of Peshawar, Peshawar, Pakistan
[3]H.E.J. Research Institute of Chemistry, International Center of Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan
[4]Center of Excellence in Marine Biology, University of Karachi, Karachi-75270, Pakistan
[5]Department of Botany University of Peshawar, Peshawar, Pakistan

Abstract: In the present research work three Pakistani medicinal plants were scrutinized for their antibacterial and phytotoxic potentials. The antibacterial and phytotoxic effects were determined for the crude methanolic extract and its subsequent solvent fractions. In case of antibacterial effect the chloroform and ethyl acetate fractions of all tested plants showed moderate antibacterial potential. All the tested plants were proved good phytotoxic at concentrations of 1000, 100 and 10 ppm. The phytotoxic effect of the tested plants was higher than antibacterial effect. In conclusion these plants can be used as good source of antibacterial and phytotoxic actions.

Key words: *Periploca aphylla* · *Ficus sarmentosa* · *Isodon rugosus* · Phytotoxic activity · Antibacterial activity

INTRODUCATION

Plants are natural source of producing wide number of bioactive chemical constituents in a most efficient way and with precise selectivity. Since the middle of the 19th century, different class of bioactive compounds have been isolated and characterized. Many of these are used as the active ingredients of the modern medicine, or as the *lead compounds* for new drugs discovery. Several plant derived medicines, are rich in phenolic compounds such as those used in protection against coronary heart diseases and carcinogenesis [1-2].

Periploca aphylla belongs to family Asclepiadaceae, is a large erect branched shrub, grown on different altitudes, all over South West Asia. *P. aphylla* is commonly used for the treatment of tumours and swellings, while the bark of the *P. aphylla* used as antipyretic [3]. *Ficus sarmentosa* is commonly found at N. W. Hills, Kashmir, Northern India, Bangla Desh, Burma and China. It is extremely variable and common wild species. The plant is found creeping on rocks and in crevices or climbing on other trees with the help of adventitious roots, up to c. 2300 m from sea level. It is an indigenous climber and its leaves are lopped for feeding all types of animals particularly by the marginal social segments occupying the steep terrain [4]. *Isodon rugosus* belong to family *Lamiaceae*. Is a deciduous shrub growing up to 1.5 m. It flowers from July to September and the seeds ripen from August to October. It has suitable for light (sandy), medium (loamy) and heavy (clay) soils. The suitable pH for this plant is acid, neutral and basic (alkaline) soils. It can propagate in semi-shade (light woodland) or no shade and prefers moist soil. One report mentioned that the plant is edible, but no data are reported to show which part of the plant is used [5]. In current study three medicinal plants were evaluated for their antibacterial and phytotoxic activities.

MATERIALS AND METHODS

Plant Material: *Periploca aphylla*, *Ficus sarmentosa* and *Isodon rugosus* were collected from Razagram, Toormang, Dir, Khyber Pakhtunkhwa Pakistan in month of December 2011. The plants were identified by Ghulam Jelani Department of Botany University of Peshawar Pakistan.

Corresponding Author: Abdur Rauf, Institute of Chemical Sciences, University of Peshawar, Peshawar, KPK, Pakistan. Tel: +92-346-9488944/313-9488944.

Extraction: Shade dried and crushed plant material of *Periploca aphylla*, *Ficus sarmentosa* and *Isodon rugosus* were subjected to hot extractions with methanol in soxhlet extractor for 48h.The solvent extract was concentrated under reduce pressure at 40°C using rotavapor and suspended in water and successively partitioned with n-hexane, chloroform, ethyl acetate and methanolic fractions.

Antimicrobial Assay: The antibacterial activity was done using modified agar well diffusion method as earlier discuss [6,7]. The Muller-hinton agar was used as medium. The cultures were incubated at 37°C for 24 to 72 hours. The broth culture (0.6 ml) of the test organism was placed in a sterile Petri-dish to which 20 ml of the sterile molten MHA was added. Holes were bored in to the medium using 0.2ml of the extract. Streptomycin was the standard antimicrobial agent at concentration of 2 mg /ml. Inoculation was done for 1 hour to make possible the diffusion of the antimicrobial agent into the medium. The diameters of the zone of inhibition of microbial growth were measured in the plate in millimeters.

Phytotoxic Activity: Crude extracts and various fractions of *Periploca aphylla*, *Ficus sarmentosa* and *Isodon rugosus* were tested against Lemna minor [8]. In this bioassay, three flasks were inoculated with a sufficient stock solution of (20 mg/ml) to achieve a final concentration of 500, 50 and 5µg/ml, respectively. To each flask, added a 20 ml medium 10 plants each one containing rosette of three fronds. Parquet was used as a standard growth inhibitor. The flasks were kept in growth cabinet for incubation up to seven days. After this growth, regulation in percentage was determined with reference to the negative control.

RESULTS AND DISCUSSION

When the crude methanolic extract and its subsequent solvent fractions *Periploca aphylla* were tested for their anti-bacterial potential against various gram positive and gram negative bacteria. The *E. coli* and *S. aureus* were completely resistant to the tested samples; while the growth of remaining tested bacteria were variably inhibited. The n-hexane fraction demonstrated antibacterial effect against *K. pneumonia*, *Straptodirimu, B. stearothermophihus and S. Typhimuriun* with zone of inhibition 10, 10, 12 and 12 mm respectively. The chloroform fraction exhibited somewhat better activity than n-hexane and inhibited the growth of *K. pneumonia*, *Straptodirimu, B. stearothermophihus and S. Typhimuriun* with zone of inhibition 12, 12, 14 and 12 mm respectively. The zone of inhibition resulted from the action of ethyl acetate against *K. pneumonia*, *Straptodirimu, B. stearothermophihus and S. Typhimuriun* was 10, 10, 12 and 10 mm respectively, while methanolic extract exhibited 12,10, 12 and 12 mm zone of inhibition against *K. pneumonia*, *Straptodirimu, B. stearothermophihus and S. Typhimuriun* respectively.

The antibacterial activity of the crude methanolic extract and its various solvent fractions of *Ficus sarmentosa* is presented in Table 2. All most all the tested bacterial were inhibited by tested extract/fraction. The n-hexane fraction showed 14, 10, 16, 12, 15 and 12 mm of zone of inhibition against *E. coli, S. aureus, K. pneumonia*, *Straptodirimu, B. stearothermophihus and S. Typhimuriun* respectively. When chloroform fraction was tested against these bacterial the zone of inhibition was as 12, 14, 16, 12, 14 and 14 mm respectively. Ethyl acetate fraction illustrated variable degree inhibition against *E. coli, S. aureus, K. pneumonia*, *Straptodirimu, B. stearothermophihus and S. Typhimuriun* with zone of inhibition 14, 12, 14, 13, 14 and 15 mm respectively. The crude methanolic extract was also active against all the tested bacteria and was responsible for 12, 14, 15, 12, 15 and 12 mm zone of inhibition against *E. coli, S. aureus, K. pneumonia*, *Straptodirimu, B. stearothermophihus and S. Typhimuriun respectively*.

The antibacterial profile of the crude extract of its various solvent fractions of *Isodon rugosus* is presented in Table 3. The growth E. coli was not inhibited by any of the tested samples and rest of bacteria showed sensitive to the tested samples. The n-hexane fraction showed 12, 16, 15, 10 and 10 mm zone of inhibition against *S. aureus, K. pneumonia*, *Straptodirimu, B. stearothermophihus and S. Typhimuriun respectively*. The antibacterial action of chloroform fraction in the form of zone of inhibition was 14, 12, 10, 10 and 10 mm against *S. aureus, K. pneumonia*, *Straptodirimu, B. stearothermophihus and S. Typhimuriun respectively*. The ethyl acetate fraction exhibited comparatively good activity against *S. aureus, K. pneumonia*, *Straptodirimu, B. stearothermophihus and S. Typhimuriun* with zone of inhibition 13, 14, 12, 12 and 14 mm respectively. The crude methanolic extract illustrated antibacterial action against *S. aureus, K. pneumonia*, *Straptodirimu, B. stearothermophihus and S. Typhimuriun* having zone of inhibition 14, 12, 12, 10 and 12 mm respectively.

The results demonstrated that all the tested plants have all most similar pattern of antibacterial profile. In the present modern era ample of synthetic antibacterial drugs

Table 1: Antibacterial activity of *Periploca aphylla*

Bacterial strain	Gram +/-	Streptomycin	*n*-hexane	Chloroform	Ethyl acetate	Methanol
E. coli	+	28	-	-	-	-
S. aureus	+	30	-	-	-	-
K. pneumonia	-	30	10	12	10	12
Straptodirimu	-	30	10	12	10	10
B. stearothermophihus	+	28	12	14	12	12
S. Typhimuriun	+	28	12	12	10	12

Key words: - = not active; Well size = 6mm

Table 2: Antibacterial activity of *Ficus sarmentosa*

Bacterial strain	Gram +/-	Streptomycin	*n*-hexane	Chloroform	Ethyl acetate	Methanol
E. coli	+	28	14	12	14	12
S. aureus	+	30	10	14	12	14
K. pneumonia	-	30	16	16	14	15
Straptodirimu	-	30	12	12	13	12
B. stearothermophihus	+	28	15	14	14	15
S. Typhimuriun	+	28	12	14	15	12

Key words: - = not active; Well size = 6mm

Table 3: Antibacterial activity of *Isodon rugosus*

Bacterial strain	Gram +/-	Streptomycin	*n*-hexane	Chloroform	Ethyl acetate	Methanol
E. coli	+	28	-	-	-	-
S. aureus	+	30	12	14	13	14
K. pneumonia	-	30	16	12	14	12
Straptodirimu	-	30	15	10	12	12
B. stearothermophihus	+	28	10	10	12	10
S. Typhimuriun	+	28	10	10	14	12

Key words: - = not active; Well size = 6mm

Table 4: Phytotoxic activity of *Periploca aphylla*

Fraction	Conc. (µg/ml) of sample	Fronds survived	Fronds died	% Growth Regulation
n-Hexane	1000	6	14	70
	100	8	12	60
	10	10	10	50
Chloroform	1000	4	16	80
	100	6	14	70
	10	8	12	60
Ethyl acetate	1000	5	15	75
	100	6	14	70
	10	8	12	60
Methanol	1000	4	16	80
	100	6	13	65
	10	8	12	60

Total no of fronds: 20. Conc... Of Standard drug 0.015 µg/mL

Table 5: Phytotoxic activity of *Periploca aphylla*

Fraction	Conc(µg/ml) of sample	Fronds survived	Fronds died	% Growth Regulation
n-hexane	1000	6	14	70
	100	8	12	60
	10	10	10	50
Chloroform	1000	4	16	80
	100	6	14	70
	10	8	12	60
Ethyl acetate	1000	4	16	80
	100	8	12	60
	10	10	10	50
Methanol	1000	4	16	80
	100	5	15	75
	10	10	10	50

Total no of fronds: 20. Conc... of Standard drug 0.015 µg/mL

Table 6: Phytotoxic activity of *Isodon rugosus*

Fraction	Conc(µg/ml) of sample	Fronds survived	Fronds died	% Growth Regulation
n-hexane	1000	15	5	50
	100	12	8	40
	10	10	10	25
Chloroform	1000	6	14	70
	100	8	12	60
	10	10	10	50
Ethyl acetate	1000	4	16	80
	100	6	14	70
	10	8	12	60
Methanol	1000	6	14	70
	100	10	10	50
	10	12	8	40

Total no of fronds: 20. Conc... of Standard drug 0.015 µg/mL

are available in market. The problems with available antimicrobial drugs is high price, none availability at right time, right place and right time, besides these the bacterial resistance is another changing problem. Keeping in view these problems associated with antibacterial drugs the search for new, effective, safe, easily available natural products is a big challenge for researcher. To cope with these problem our research group is working in exploring the antibacterial potential of Pakistani medicinal plant with the hope of finding effective, safe and cheap antibacterial medicine.

The *in vitro* phytotoxic effect of the crude methanolic extract and its solvent fractions of *Periploca aphylla* is presented in Table 4. The n-hexane fraction exhibited 70, 60 and 50% phytotoxic effect at 1000, 100 and 10 ppm respectively. The chloroform fraction showed a dose dependant phytotoxic effect as 80, 70 and 60%. Ethyl acetate caused a percent growth regulation as 75, 70 and 60% at the tested concentrations of 1000, 100 and 10 ppm respectively. The crude methanolic extract illustrated a phytotoxic effect at tested concentrations of 1000, 100 and 10 ppm as 80, 65 and 60% respectively.

The Phytotoxic activity of *Periploca aphylla* (crude extract and its various solvent fractions) is tabulated in Table 5. The n-hexane when tested at concentrations of 1000, 100 and 10 ppm, it protected the growth of *Lemnna* at dose dependent manner. The chloroform fraction exhibited the maximum phytotoxic effect and produced 80, 70 and 60% growth inhibition at concentration of 1000, 100 and 10 ppm respectively. The growth of *Lemnna* was significantly inhibited by ethy acetate and showed 80, 60and 50% effect. The crude methanolic extract was more phytotoxic at was demonstrated 80, 75 and 50% growth inhibition effect at concentration of 1000, 100 and 10 ppm respectively.

The phytotoxic profile of *Isodon rugosus* is presented in Table 6. A weak phytotoxic effect was observed with n-hexane fraction, while chloroform fraction demonstrated 70, 60 and 50 % phytotoxic effect at tested concentrations of 1000, 100, 10 ppm respectively. Ethyl acetate fraction was the most significant phytotoxic and shwoed 80, 70 and 60% phytotoxic effect at tested concentrations of 1000, 100 and 10 ppm respectively. The phytotoxic action of crude methanolic extract was higher than n-hexne and weaker than remaining tested samples.

The need of phytotoxic molecules out compounds connot be ignored in the present era as most of our agricultural products are affecting with weeds. These weeds are mostly controlled with synthetic compounds like DDT. Due to the side effect, high price and none availability of these drugs at right time, most of former are reluctant to use it. Moreover these synthetic herbicides have negative effects on human health. Due these drawbacks of weedicidal researchers are in struggle to investigate safe, effective and human health friendly phytotoxic medicine.

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