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Research Article

Antibacterial, Antifungal, Insecticidal and Phytotoxicity Activities of *Indigofera geradiana*

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ABSTRACT
In the current study an exertion was done to perform biological investigation of *Indigofera geradiana* to scientifically authenticate its biological importance. The results obtained showed that all the fractions *n*-Hexane, EtOAc, Chloroform, Water and residue displayed no antibacterial activities against special bacterial strains. In case of antifungal activities the water fraction (F4) showed 10 % and residue fraction (F5) showed 35 % activity against *Callosbruchuanalis*. Subsequent the other hand in case of insecticidal activities the chloroform fraction (F3) showed 20% activity against *Rhyzopertha dominica* and residue fraction (F5) showed 20%*, Rhyzopertha dominica and Callosbruchuanalis* whereas in case of Phytotoxicity activity all the fractions showed significant phytotoxicity activities at (1000 μg/ml) and moderate activity at (100 μg/ml). Similarly, all the fractions showed low activities at (10 μg/ml).These results indicated that *M. ovalifolia* is very important from medicinal point of view.

Key words: *Indigofera geradiana*, antibacterial, antifungal, insecticidal and phytotoxicity, Activities, strains and aerial parts.

INTRODUCTION

Indigofera geradiana commonly known an (Indigo Himalayan) is a deciduous shrub belongs to genus Indigofera (family leguminosae) widely found in the tropical region of the globe. In Pakistan, it consists of about 24 species[3]. In the previous phytochemical investigation, various chemical constituents such as triterpenes, steroids, alkaloids, lignin, flavonoids, and acylphloroglucinols were isolated from various species of this genus[1]. Other compounds like saponins, quinines, tannins, garlic acid, caffeic acid, “rutin, myricetin, quercitrinmyricetin and galangin” were also reported[2]. In our phytochemical study of the various fractions of *Indigofera geradiana* were assayed for their Antibacterial, antifungal, insecticidal and phytotoxicity activities.

MATERIAL AND METHODS

Plant material: The medicinal plant *Indigofer*a *geradiana* were collected during the month of May, 2009 from Lower Dir in Northern areas of Pakistan. Taxonomic identification of the plant was done by Dr. Samin Jan, Associate Professor, Department of Botany, Islamia University, Peshawar, Pakistan. The voucher specimen (SJ-42) was deposited in the herbarium of Islamia University, Peshawar, Pakistan.
Extraction and isolation: Air shade dried powdered Aerial parts of *Indigofer*a *geradiana* 44 kg were subjected to extraction (x 3) with 5% aqueous methanol for one week. The combined extract was concentrated under reduced pressure by a vacuum rotary evaporator, to obtained brownish residue F1 (4.50 kg), which was fractionated by using chloroform and water to yield F2 (90 g) of chloroform and F3 (2.9 kg) of water fraction. The chloroform fraction was partitioned into *n*-hexane and methanol fractions afforded FX1A (12 g) and FX1B (73 g) respectively, using soxhlet extractor. Water fraction was also partitioned with ethyl acetate (EtOAc), as a result FX3A (1.7 kg) of ethyl acetate fraction was obtained, which was further fractionated using ether: petroleum ether (2:1) and water to get three fractions, FX3AC (700 g), FX3AB (240 g) and residue fraction FX3AA (570 g).
The fraction FX3AC was exposed to column chromatography on silica gel eluted with *n*-hexane- ethyl acetate; in increasing polarity to yielded sub fractions (A-G). The sub fractions (C-F) were combined based on TLC profile yielded 70 sub fractions. The sub fractions 42-65 were then mixed and chromatographed eluted with *n*-hexane-acetone in increasing polarity to obtain various fractions. Antibacterial activity: The microorganisms; *B. subtilis ATCC 6633, E. coli ATCC 25922, Straptodirimus, S. flexenari (clinical isolate), S. aureus ATCC 25923, P. aeruginosa ATCC 27853 and S. typhi ATCC19430* were used for evaluation of antibacterial activity. The organisms were stored in Muller hantin agar in the refrigerator at 4^0C prior to subculture. Antibacterial testing was carried out on the already developed agar well diffusion method to study the potency of the seeds extract of *Indigofera geradiana*. Broth media were prepared and the test

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Table 1: Antibacterial activity of the extract fractions of *M. o*valifolia stems bark

Microorganisms	Gram	F1	F2	F3	F4	F5
Escherichia coli	-	-	-	-	-	-
Bacillus subtilis	+	-	-	-	-	-
Shigella flexenari	-	-	-	-	-	-
Staphylococcus aureus	+	-	-	-	-	-
Seudomonas aeruginosa	-	-	-	-	-	-
Salmonella typhi	-	-	-	-	-	-
DMSO (-)	-	-	-	-	-	-
Imipenum 10 μg/Disc (+)	25	50	28	48	23	28

Well size 6 mm, F1= n-hexane, F2 = EtOAc, F3 = chloroform, F4 = Water, F5 = Residue.

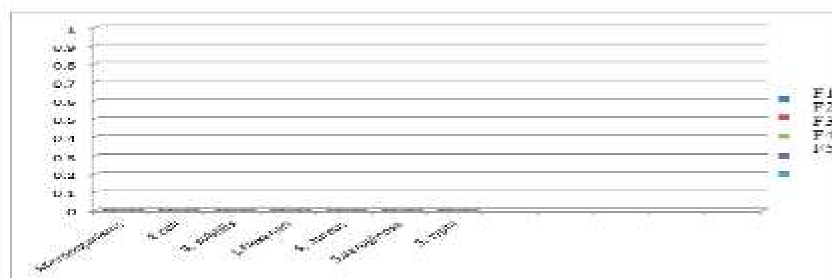

Fig. 1: Antibacterial activity of the extract fractions of M. ovalifolia stems bark.

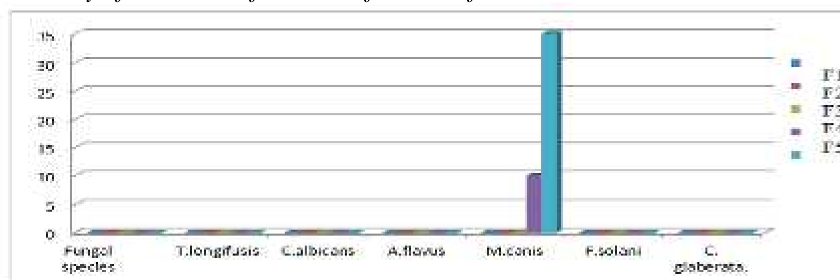

Fig. 2: Antifungal activity of the extract fractions of Indigofera geradiana.

Table 2: Antifungal activity of the extract fractions *M. ovalifolia* stem bark

Fungal species	Standard (μg/ml)	F1	F2	F3	F4	F5
Trichophyton longifusis	Miconazole 70.08	-	-	-	-	-
Candida albicans	Miconazole 110.8	-	-	-	-	-
Aspergilus flavus	Miconazole 20	-	-	-	-	-
Microsporum canis	Miconazole 98.4	-	-	-	10	35
Fusarium Solani	Miconazole 73.10	-	-	-	-	-
Candida glaberata.	Miconazole 110.8	-	-	-	-	-

F1= n-Hexane, F2 =EtOAc, F3 =chloroform, F4 =Water, F5 =Residue

Table 3: Insecticidal activity of the extract fractions of *M. ovalifolia* stems bark.

Insect	NOs	F1	F2	F3	F4	F5
Tribolium castaneum	100	-	-	-	-	-
Rhyzopertha dominica	100	-	-	20 %	-	20 %
Callosbruchuanalis	100	-	-	-	-	20 %

F1=n-Hexane, F2 =EtOAc, F3 = Chloroform, F4 = Aqueous, F5= Residue

organisms were transferred to the broth media from agar plate and were grown at 37^{0}C for 24 hours. After 24 hours 25 ml of MHA were poured into each petri plate and cooled in sterile condition. The fresh culture was prepared from day old culture, after solidification of MHA in plate, 0.6 ml of fresh culture of test organism were poured on to MHA. Wells of 6 mm diameter were digged in to the medium by using sterile borer and 22 mg of different fractions of the seeds of *Indigofera geradiana* were used against each organism. DMSO and standard antibiotic (Imipenum) were added into other wells. The plates were kept in sterilized inoculation chambers for 1 hour to facilitate diffusion of the antimicrobial agent into the medium. The plates were then incubated at 37 ^{0}C for 24 hours and the diameters of the zone of inhibition of microbial growth were measured in millimeters.[4]

Antifungal activity: The microorganisms; *T. longifusis, C. albicans ATCC 2091, A. flavus ATCC 32611, M. canis ATCC 11622, F. solani 11712* and *C. glaberata ATCC 2091* were used for antifungal assay. All these strains were

Table 4: Phytotoxic activity of the extract fractions of *M. ovalifolia* stem bark

Conc.of sample (μg/ml)	Stand. drug	F1	F2	F3	F4	F5
1000		100	100	100	35	100
100	0.015	70	75	75	20	75
10		05	20	0	10	0

F1=n-Hexane, F2 =EtOAc, F3 = Chloroform, F4 = Aqueous, F5= Residue

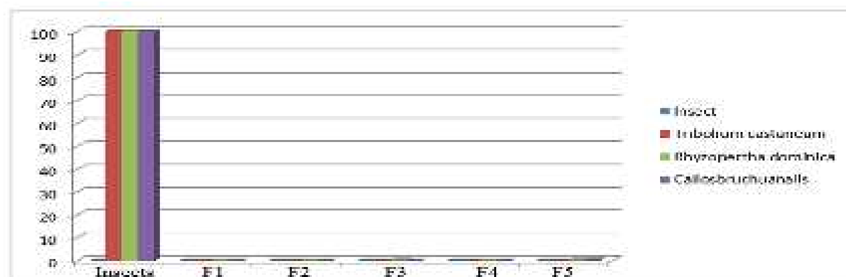

Fig. 3: Insecticidal activity of the extract fractions of M. ovalifolia stems bark.

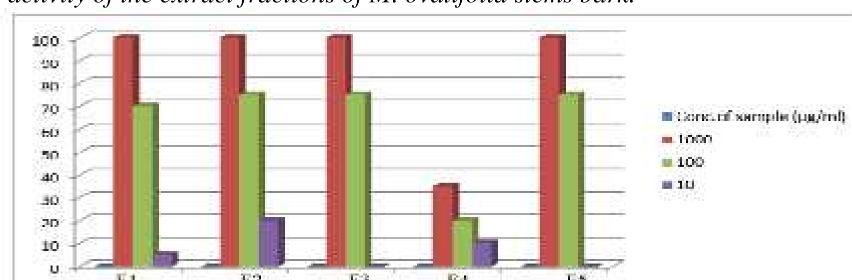

Fig. 4: Phytotoxic activity of the extract fractions M. ovalifolia stem bark

maintained on agar slant at 4^0C, the slant was allowed to activate at a temperature of 37 ^{0}C for duration of 3-4 days on nutrient agar (NA), for fungi, before any screening is made. The crude extract fractions were dissolved in DMSO (24 mg/ml) and sterile medium (5 ml) was placed in a test tube and inoculated with the sample solution (400μg/ml) which was then kept in a slanting position at room temperature for overnight. The tubes were inoculated by a piece of fungus (4 mm diameter) from seven day old culture. The samples were then incubated for 7 days at 28^0C and the fungal strain starts growth on the slant. The growth inhibition was observed and percentage growth inhibition was determined by calculating with reference to the positive control by applying the formula

$$\% \text{ Inhibition} = \frac{\text{100-linear growth and test (mm)}}{\text{linear growth in control (mm)}} \times 100$$

Miconazole was used as standard drug a positive control.[5-6]

Insecticidal activity: Different fractions were tested against various insects *viz, R. dominica, T. castaneum* and *C. bruchuanalis*. The sample for test was prepared by adding 20 mg of crude fractions with 2 ml acetone which was immediately kept in Petri dish covered with the filter papers. Later 24 hours, ten insects were retained in every plate which was incubated at 27 ^{0}C for 24 hours in 50% humid environment of growth chamber. The activity was examined as percent mortality, which was calculated with reference to the negative and positive controls. Permethrin was kept as a standard drug, while acetone and test insects were used as positive and negative controls.[7]

Phytotoxicity activity: Phytotoxic activity of various fractions of the seeds extract of *Indigofera geradiana* was tested against *Lamina minor*. The medium was prepared by mixing various constituents in 1000 ml distilled water and pH (5.5-6.5) was adjusted by addition of KOH solution. The medium prepare was autoclaved at 121^0C for duration of 15 minutes. The extract was dissolved in ethanol (20 mg/ml) which served as stock solution. Later on, nine sterilized flasks three for each concentration were inoculated with an amount of 1000 μl, 100 μl and 10 μl of the stock solution each of 1000,100 and 10μg/ml, respectively. The solvent was evaporated overnight in sterile condition. To every flask medium (20 ml) and *Lamina minor* containing a rosette of three fronds was mixed. All flasks were cotton plugged and allowed to grow in sterile condition for 7 days. The numbers of fronds per flask were counted and recorded on day seven and their growth regulation in percentage was determined by calculating with the formula given below.

$$(\%) \text{ Growth regulation} = 100 - \frac{\text{No. of fronds in the test samples}}{\text{No. of fronds in the control samples}} \times 100$$

The result was calculated with reference to the positive and negative control. In this study parquet was used as standard drug, while parquet and volatile solvents were used as positive and negative controls.[8]

RESULTS AND DISCUSSION

Antibacterial activity of the crude fractions: All fractions showed no antibacterial activity against selected bacterial strains (Table-1).

Antifungal activity: All the fractions of stem bark of *M. o*valifolia were also investigated for antifungal bioassay against the selected fungal strains; *T. longifusis, C. albicans, A. flavus, M. canis, F. solani, C. glaberata* (Table-2). The ethyl acetate (F2) and chloroform (F3) fractions showed 10 and 12% bioactivity against *M. canis.*

Insecticidal activity: All the fractions (Table- 3) evaluated for insecticidal activity against the selected insects; *T. castaneum, R. dominica* and *C. bruchuanalis.* The results are summarized in table-1 which clearly indicated that the chloroform fraction has 20 % activity against each insect while ethyl acetate has 20 % activity against *C. bruchuanalis.*

Phytotoxicity activity: The isolated fractions from the extract of stem bark of *M. o*valifolia were subjected for phytotoxicity. The results of the phytotoxicity bioassay of various fractions are shown in table-3 and figure-3. The *n*-hexane (F1), ethyl acetate (F2), Chloroform (F3), water (F4) and residue fraction (F5) showed significant phytotoxicity activity at 1000 μg/ml and low activity at 100 μg/ml concentration. However, the entire fractions showed no activity at10 μg/ml.

CONCLUSION

In the current study the leaves of medicinal plant *Millettia ovalifolia* was investigated for their bioassay to explore its phytochemical importance. The results obtained showed that this plant is very important from medicinal point of view, and it needs further phytochemical exploitation to isolate phytochemical constituents showing insecticidal and phytotoxic activities.

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