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BIOLOGICAL AND PHARMACOLOGICAL EFFECTS OF *MYRICARIA ELEGANS* ROYLE.

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ABSTRACT: The methanolic (80%) crude extract of *Myricaria elegans* Royle. was investigated for various *invitro* biological and pharmacological activities including antifungal, antibacterial, insecticidal, phytotoxic, and brine-shrimp cytotoxic activities. The crude extract was found to be highly effective inhibitor against *Trichophyton longifusus*, (70%), *Candida albicans* (60%) and *Microsporum canis* (60%). The extract also displayed remarkable phytotoxic activity at different concentrations against the *Lemna acquinoctialis* Welv. This extract, however, did not show any antibacterial, insecticidal activities and Brine shrimp cytotoxicity during this study.

KEY WORDS: *Myricaria elegans*, Pharmacological & biological activities, Phytotoxicity, Antifungal Activities

INTRODUCTION

Myricaria elegans royle. (Tamariscineae) is a bush with striate slender stem (Hooker, J.D., 1982). The plant is found in Western Himalaya, Western Tibet and from Garwhal to Ladak at altitude of 6000 15000 feet (Kirtikilf and Basu, 1918). The leaves of the plant are applied to the bruises and the twigs are browsed by sheep and goats in Ladak (Watt, 1972).

The overall literature shows that very little attention has been given to study this plant scientifically; therefore, the present study was designed to evaluate the therapeutic value *M. elegans* in the modern phytomedicines. In the current study we investigated the various *in-vitro* biological and pharmacological acti vities including antifungal, antibacterial, insecticidal, phytotoxic, and brine-shrimp cytotoxic activities of the crude methanolic extract of *M. elegans*

MATERIAL AND METHOD

Plant material

Myricaria elegans Royle. was collected in July 2002 from the hills of Sawat, NWFP, Pakistan. The plant was identified by plant taxonomist, Prof. Jehandar Shah (Vice Chancellor, University of Malakand, Chakdara, NWFP, Pakistan).

Extraction

The plant (stem) was chopped into small pieces and shade-dried. The dried plant material was powdered and exhaustively extracted with methanol (80 %) by percolation at room temperature. The combine extract was filtered off and dried at low temperature in rotary evaporator. The pharmacological activities were performed by using different concentrations of the crude extract as per requirements of the individual assay method.

In- Vitro Antifungal Bioassay

The in vitro antifungal bioassay of the crude methanolic extract was performed by agar tube dilution method (Atta-ur-Rehman *et al.,* 1995). The crude extract was evaluated against clinical specimens of *Trichophyton longifusus, Candida albicans, Aspergilus Flavus, Microsporum canis, Fusarium solani* and *Candida glaberata.* A control experiment with test substance (medium supplemented with appropriate amount of DMSO) was carried out for verification of the fungal growth. The extracts (24 mg) dissolved in sterile DMSO (1.0 ml), served as stock solution. Sabouraud dextrose agar (SDA) (4ml), was dispensed into screw cap tubes which were autoclaved at 121°C for 15 minutes and then cooled to 50°C. The non-solidified SDA media was poisoned with stock solution *(66.6JlI),* giving the final concentration of 400 *Jlg* of the extract/ml of SDA. Each tube was inoculated with a piece (4mm diameter) of inoculum removed from a seven days old culture of fungi. For nonmycelial growth, an agar surface streak was employed.

Inhibition of fungal growth was observed after 7days of incubation at 28:tl °C.

In Vitro Phytotoxic Bioassay

The phytotoxic bioassay was performed by following the modified protocol (Mc Laughlin et al. 1991), using *Lemna acquinoctialis* Welv. The medium was prepared by mixing various constituents in 100 ml

Table. Susceptibilities of Different Fungi to Crude Extract of *Myricaria elegans* Royle

Name of Fungi	Linear Growth (mm)		Inhibition (%)	Standard Drugs
	Sample	Control		
Trichophyton longifusus	30±1.8	98±2.1	70.00	Miconazole
Candida albicans	40±3.1	101±3.7	60.00	Miconazole
Aspergilus flavus	100±2.6	98±2.6	0.00	Amphotericine-B
Microsporum canis	40±3.6	99±4.1	60.00	Miconazole
Fusarium solani	90±1.6	100±4.1	10.00	Miconazole
Candida glaberata	80±5.6	99±4.3	20.00	Miconazole

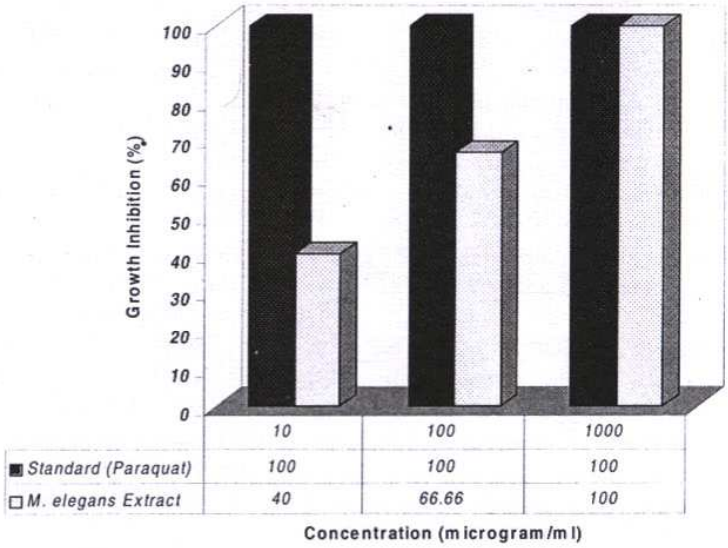

Fig. Phytotoxic Activity of Crude Extract ofMyricaria elegans Royle.

distilled water, and the pH was adjusted (5.5 - 6.5) by adding KOH solution. The medium was then autoclaved at 121°C for 15 minutes. The extracts (15.0mg) dissolved in ethanol (1.5 ml) serving as stock solution. Nine sterilized flasks, three for each concentration, were inoculated with 2000 µl, 200 µl and 20 µl of the stock solution to give the final concentration of 1000, 100, and 10 ppm respectively. The solvent was allowed to evaporate overnight under sterile conditions. To each flask, medium (20 ml) and plants (10), each containing a rosette of three fronds, of *Lemna acquinoctialis* Welv., were added. One other flask supplemented with solvent and reference growth inhibitor (Paraquat), served as negative control. All flasks were plugged with cotton and kept in the growth cabinet for seven days. The number of fronds per flask were counted and recorded on day seven. Results were analyzed as growth regulation in % age and calculated with reference to negative control.

Antibacterial Activity

The extract was screened against various human pathogens including *Corynebacterium diphtheriae, Escherichia coli, Klebsiella pneumoniae, Proteus morganni, Pseudomonas aeruginosa, Salmonella typhi, Shigella boydii, Staphylococcus aureus*, and *Streptococcus pyogenes* by agar well diffusion method (Atta-ur-Rahman, 1991). Nutrient agar plates were

swabbed with a 2 - 8 h broth culture of respective bacteria. Wells (6 mm diameter) were dugged in the media in each of these plates using a sterile metallic borer with centers at least 24 mm apart. Sample (3 mg/ml of DMSO) were then added in their respective wells using sterilized dropping pipettes. Other wells supplemented with DMSO and reference antibacterial drug (lmipenum, 10 Ilg/disc) serving as negative and positive controls, respectively. The plates were immediately incubated at 37 DC for 14 -19 h. Activity was determined by measuring the diameter of zones showing complete inhibition (mm). Growth inhibition was calculated with reference to positive control.

Brine-shrimp Cytotoxicity

Artemia salina (brine-shrimp eggs) were used to determine the cytotoxic activity of the various samples (Meyer, 1982). Three different concentrations (10, 100 and 1000 Ilg/ml) of test sample was prepared by dissolving in DMSO as the solvent. Seawater was prepared by dissolving commercially available sea salt (3.8 g) into tap water (1 1). Brine shrimps hatched in seawater media at 27DC for 48 h. Ten shrimps, seawater (5 ml) and different amounts of test sample were put in a vial. Two other vials were supplemented with solvent and reference cytotoxic drug serving as negative and positive controls respectively. Etoposide (LDso = 7.465 Ilg/ml) was used as the standard reference cytotoxic drug. All vials were incubated at 25 - 27DC for 24 h and the survived brine shrimps were counted. The data was analyzed with Finney computer program to determine LDso values with 95% confidence interval.

Insecticidal bioassay

Tribolium castaneum, Sitophylus oxyzae, Rhyzopartha dominica, and *Trogoderma granarum* were used to determine the insecticidal activity of the samples (Naqvi and Parveen, 1991). The insecticidal activity of plant extract was determined by direct contact application using filter paper. 1 ml of the samples was applied by micropipette to filter papers (90 mm diameter), which gave 1571.33 Ilg/cm2 concentration. After drying under a fume hood for 2 min, each filter paper was placed in the petri dish and then 10 adults of each of *Tribolium castaneum, Sitophylus oxyzae, Rhyzopartha dominica,* and *Trogoderma granarum* were placed in each petri dish which was covered with a lid. A check batch was treated with solvent for determination of solvent effect. A control batch was kept for the determination of environmental effects. Another batch was supplemented with reference insecticide (permethrin, 235.71Ilg/cm2). All these were kept without food throughout 24 h exposure period. Mortality count was done 24 h after treatment.

Statistical Analysis

Data are presented as (J :t SD and compared using student *t-test.* All the statistical calculations were carried out using MinitabTM, a statistical package.

RESULTS AND DISCUSSION

To the best of our knowledge and from the literature search it was revealed that no earlier scientific study has been undertaken to evaluate the *M. elegans* for therapeutic potential in phytomedicines or its value for the isolation of bioactive chemical constituents. Therefore, the current study was designed and undertaken to screen *M. elegans* to confirm and provide scientific basis for its use in traditional system of medicine (Watt, 1972) and also to explore some new biological and pharmacological activities of this plant. Moreover, the present study will provide a base to the researchers to carry out studies for the isolation of biologically active compounds.

Antifungal activity of the crude extract was performed against *Trichophyton longifususi, Candida albican, Aspergilus flavus, Microsporum canis, Fusarium solani, Candida glaberata.* Growth in the medium containing the crude extract was determined by measuring the linear growth (mm), and growth inhibition (%) was calculated with reference to the negative control. The results of antifungal activities (Table) indicated that the crude extract of *Myricaria elegans* Royle. posses an overall significant activity. The crude extract showed a good fungal inhibition against *Trichophyton longifusus* (70%), *Candida albicans* (60%) and *Micosporum canis* (60%). The same extract displayed only very week potential for inhibition of the growth of *Candida glaberata* (20%) and *Fusarium solani* (10%) while it exhibited no antifungal activity against the fungal species *Aspergilus flavus.* The overall antifungal activities results showed that the crude extract of *M. elegans* has the potential to be an antifungal agent against *Trichophyton longifsus, Candida albicans* and *Microsporum canis.* Further investigations must be performed to examine its antifungal properties at a

higher concentration. However, even with the current concentration and results it can be recommended for detailed antifungal activities studies including the isolation of bioactive antifungal constituent(s). The discovery of a potent and safe herbal remedy will be a great achievement in fungal infection therapies. It is vital for systemic fungal infections that usually occur in immuno-compromised patients, as toxicities induced by commercial antifungal drugs, are often observed in these patients due to the high dosage and prolonged therapy (Somchit *et al.*, 2003).

The Phytotoxic activity of the crude extract was tested against the *Lemna acquinoctialis* Welv. Results of this activity of the crude extract of *Myricaria elegans.* on *Lemna acquinoctialias* Walv. were interpreted by analyzing the growth regulation in %age, calculated with reference to the negative control. Paraquat was used as standard growth inhibitor. The results (Figure) showed that the crude extract posses remarkable phytotoxic activity against *Lemna aequinoctialias* Welv. and caused complete (100%) growth inhibition of the plant at the highest tested concentration (1000).lg/ml) while a good (66.6 %) inhibitory activity at lower concentration (100).lg/ml). Even at the lowest tested concentration (10).lg/ml) it displayed a moderate phytotoxic activity (40%). The results of this bioassay reflects that the plant extract can be used as an effective phytotoxic agent.

The crude extract of *Myricaria elegans* was also tested for antibacterial and insecticidal activities. and Brine Shrimp cytotoxicity studies but was found to be devoid of any activity in these bioassays.

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