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BIOLOGICAL ACTIVITIES OF *POLYGONUM BARBATUM*

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Abstract: In continuation of our screening programme for biological activities of the higher plants, we have studied *Polygonum barbatum.* The aerial parts of this species were extracted successively with dichloromethane and methanol. Extracts were submitted to a battery of biological tests including cholinergic activity, spasmolytic activity, larvicidal activity, brine shrimp toxicity, antifungal and antibacterial activities. Dichloromethane extract showed brine shrimp toxicity and spasmolytic activity whereas the methanol extract was found to possess cholinergic activity.

Keywords: Biological activity, Polygonaceae, *Polygonum barbatum*, screening, spasmolytic.

INTRODUCTION

The genus *Polygonum* (Polygonaceae) comprises of about 150 species. The presence of diverse secondary metabolites like flavonoids [Isobe *et al.* 1980, Isobe *et al.* 1981], anthraquinones [Jayasuriya *et al.* 1992, Kang and Woo 1982], phenylpropanoids [Brown *et al.* 1998], proanthocyanidins [Islambekov *et al.* 1969] have been reported from species of the genus *Polygonum*. Moreover, biological activities of *Polygonum* species such as antihypertensive effect of *P. perfoliatum* [Lian 1983], myocardial protective action of *P. multiflorum* [Yim *at al.* 1998], antiallergic effect of *P. tinctorium* [Kim 2000] and antiviral activity of *P. punctutam* [Kott *et al.* 1999] have been investigated but there has been no report on the biological activity of *Polygonum barbatum*, a species very commonly found in Pakistan. We undertook the biological study of this species. In folk medicine, the plant has carminative, astringent and cooling effects.

MATERIALS AND METHODS

PLANT MATERIAL

The aerial parts of *Polygonum barbatum* were collected by Muhammad Younas Syad on December 1999 from the surroundings of Bahauddin Zakarriya University, Multan. The plant material was authenticated by Dr. Altaf Ahmad Dasti, Associate Professor, Department of Botany, B. Z. University, Multan.

EXTRACTION

The air-dried material was ground and extracted successively with dichloromethane and methanol. The extraction was carried out at room temperature and under constant agitation for 24 hrs.

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THIN LAYER CHROMATOGRAPHY (TLC)

TLC was carried out on precoated silica gel 60 F_{254} aluminium sheets (Merck). The TLC plates were developed in chromatograhic tanks made of glass, saturated with an appropriate solvent system. The principle solvent systems employed were *n*-hexane-ethylacetate (1:1) and chloroform-methanol-water (65:35:05). The compounds on the TLC plates were revealed in UV light at 254 and 366 nm and subsequently either exposed to the iodine vapors or sprayed with the Godin reagent.

CHEMICALS

The following reference materials were obtained from the sources specified: acetylcholine perchorate, atropine sulphate, potassium chloride (Sigma Chemicals Co, St Louis, MO, USA) and thiopental sodium (Abbott Laboratories, Pakistan). All chemicals used were of the highest purity grade available. All drugs were dissolved in distilled water and dilutions were made fresh in normal saline (0.9% sodium chloride) on the day of experiment.

ANIMALS

Animals (Rabits of either sex) used in this study were housed at the Animal House of the Agha Khan University, maintained at 23-25 ºC and were given a standard diet and tap water ad libitum.

GLASSWARE CLEANING

Procedure used for cleaning the glassware was outlined below:
Debris, if any was taken out from desired glassware. Glassware was soaked in conc. H_2SO_4 for 8-9 hrs, rinsed with running tap water and washed with household detergent followed by rinsing with tap water. Cleaned glassware was air dried and kept in closed cabinet.

STERILIZATION

Glassware

Cleaned test tubes and flasks were plugged with cotton wool stoppers and wrapped in craft paper. Cotton plugs were also inserted in the month end of pipettes. Petri dishes and graduated pipettes were placed in their respective cases.

Inoculating Wires and Loops

Inoculating wires and loops and points of forceps were sterilized on an ordinary Bunsen burner. They were made red on heat (Flame) and cooled before use.

Mouth of Culture Tubes and Cotton Wool Stoppers etc.

During the culture technique mouths of culture tubes, cotton wool stoppers, open sides of petri dishes were sterilized by flaming i.e. by

passing the article through the Bunsen flame without allowing it to become red hot at the time of media pouring, cavity formation (well formation) and adding of extracts.

Environment
To ensure complete aseptic working conditions, the inoculation chamber, table-top and hands were sterilized with rectified spirit before starting work.

Test Media for Bacteria
Nutrient agar medium (Merck, supplied by G.M. Chemical Stores Multan) was used for the stock culture and petri plate screening work.

Preparation of Medium
Dissolved 20 gm of nutrient agar media in distilled water by gentle heating. Allowed the pH to 7.0 ± 0.2 and sterilized by heating in an autoclave at 121 ºC for 15 minutes.

Preparation of Inoculum
Antimicrobial activity of the extracts of *P. barbatum* was determined by using *Sarcina leutea* (donated by PCSIR Laboretories Lahore) and *Escherichia coli* (Provided by Pathology Department, Nishtar Hospital Multan) as the test organisms. For the present experimental work test organisms were maintained by biweekly transfer on agar slant (Nutrient agar medium). A fresh slant was inoculated with the test organisms by using platinum wire loops and incubated in an incubator (Memmert 854 schwabach) for 24 hrs at 37 ºC, 5 ml of sterilized normal saline was poured in the slant and a resulting suspension was used to inoculate a measuring flask (250 ml) having 100 ml of solidified nutrient agar medium and incubated for 24 hrs at 37 ºC. The growth was harvested by washing with 50 ml of sterilized normal saline transferred to sterilized glass flasks and stored under refrigeration.

Addition of Inoculum to the Media
After autoclaving, the media was allowed to cool to 50 ºC and previously prepared microbial suspension (inoculum) was added in 1000 ml of media. The flask was shaken gently to distribute the suspension evenly in the entire media. Quantities (20 ml approximately) of inoculated media were poured in the previously sterilized and cooled petri dishes (100 mm diameter). After solidification of media, the petri dishes were put in the refrigerator at 4 ºC for at least 30 minutes before use on the same day.

Preparation of Standard Solution
The standard (or reference) solution of ampicillin trihydrate micronized (donated by Hamaz Pharmaceuticals Pvt. Limited, Multan) was prepared

by dissolving in water. Similarly 15 mg ml^{-1} solution of methanol extract of *P. barbatum* was prepared in methanol and 15 mg ml^{-1} solution of dichloromethane extract of *P. barbatum* was prepared in dichloromethane.

ANTIMICROBIAL ACTIVITY

Well Diffusion Method

The antimicrobial activity of the standard and extract solutions was determined by an agar well diffusion (cylinder plate method) on the already prepared plates of the inoculated media. Three wells with diameter 8 mm were cut per plate with a borer, and sealed with a drop of inoculated sterile media. All the solutions (0.1ml of each) were poured in the wells by dried sterilized 1ml pipette. The petri dishes were incubated at 37 ºC for 24 hrs.

Disk Diffusion Method

Cylindrical disks (Whattman No. 1 filter paper disks having a diameter of 10 mm were made and sterilized) of the same concentrations (as is present in 0.1ml of solution) were prepared for both standard and extract solutions. Three disks per petri dish were applied (one of the each extract solutions and one of the standard solution).

CHOLINERGIC AND SPASMOLYTIC ACTIVITIES

The cholinergic and spasmolytic activities of the dichloromethane and methanol extracts of the aerial parts of *P. barbatum* were studied by using rabbit jejunum of a local breed of either sex (housed at the Animal House of the Agha Khan University, Karachi). Animals had free access to water but food was withdrawn 24 hr prior to experiment. Rabbits were killed and gut segments 2 cm long were suspended in Tyrode's solution bubbled with a mixture of 95% oxygen and 5% carbon dioxide and maintained at 37°C. the composition of the Tyrode's solution was (mM): KCl 2.68, NaCl 136.9, $MgCl_2$ 1.05, $NaHCO_3$ 11.90, NaH_2PO_4 0.42,$CaCl_2$ 1.8 and glucose 5.55. Intestinal responses were recorded isotonically using Bio Science transducers and an oscillograph. Under these experimental conditions rabbit's ileum behaved as a quiescent preparation and is considered more useful for detecting spasmogenic or cholinergic activity. Each tissue was allowed to equilibrate for at least 30 min before the addition of any drug. Acetylcholine was used as the positive control as the spasmogenic agent [Gilani *et al*. 2000].

ANTIFUNGAL ASSAYS

The antifungal test against *Cladosporium cucumerinum* was carried out on a TLC plate. After developing with suitable solvent systems, the TLC plates were well dried with an air dryer in order to remove the solvents completely. The developed and dried TLC plates were sprayed with a

conidial suspension of *C. cucumerinum* in a nutrition medium and incubated in a moist atmosphere for 2-3 days. The inhibition of fungal growth was assessed by visual examination [Homans and Fuchs 1970].

LARVICIDAL ACTIVITY

Eggs of *Aedes aegypti* were incubated in tap water for 24 hrs at 25 ^{o}C. Twenty instar II or III larvae were placed in 10 ml of tap water, to which the crude plant extract or pure compound dissolved in DMSO was added. Final concentration of DMSO was 1%. The survival rate was evaluated after incubation at 25 °C for 24 hrs by visual examination [Zarroug *et al.* 1988].

BRINE SHRIMP TOXICITY TEST

Eggs of brine shrimp were incubated in a petri dish containing artificial seawater (35 gm litre^{-1}) for 32 hrs. Hatched larvae were transferred into a second petri dish and incubated another 24 hrs to allow for molting to 2nd instar. This process allows obtaining a homogenous population of 2nd instar larvae. Extracts or pure compounds were solubilized in DMSO with a maximum concentration of 50 μg μl^{-1} (for final test solution of 500 ppm). Six geometrical dilutions were prepared in DMSO to obtain identical solvent concentration in all samples. The larvae were counted into groups of 10 and placed in 1 ml of artificial seawater, to which had been added the sample to be tested in DMSO. The final concentration of DMSO was 1%. Survival was evaluated after incubation at 25 °C for 24 hrs by observing under a dissection microscope [Meyer *et al*. 1982].

RESULTS AND DISCUSSION

The aerial parts of *Polygonum barbatum* were extracted successively with dichloromethane and methanol. These extracts were subjected to a battery of biological tests including cholinergic activity, spasmolytic activity, larvicidal activity, brine shrimp toxicity, antifungal activity and antibacterial activities. The results are summarized in Tables 1 and 2.

Table 1: Results of the screening of different extracts of *P. barbatum* for their cholinergic, spasmolytic and antibacterial activities.

Extract	Cholinergic activity	Spasmolytic activity	Antibacterial activity*
Dichloromethane extract	-	+	-
Methanol extract	+	-	-

* *Sarcina leutea* and *Escherichia coli* were used as test organisms.

Table 2: Results of the screening of different extracts of *P. barbatum* for their brine shrimp toxicity, antifungal and larvicidal activities.

Extract	Antifungal activity*	Larvicidal activity**	Brine shrimp toxicity***
Dichloromethane extract	-	-	+
Methanol extract	-	-	-

* Activity was determined in a bioautographic assay on TLC plate against *Cladosporium cucumerinum* and *Candida albicans* at 100 μg of the sample.
** Activity was determined at 500 ppm after 24 hours against *Aedes aegypti*
*** Activity was determined at 500 ppm after 24 hours.

The dichloromethane extract has a dose dependant (0.1 - 0.5 mg ml^{-1}) spasmolytic effect on rabbit jejunum that is devoid of any relaxant effect on the potassium chloride induced contraction up to 1 mg ml^{-1}. Whereas its methanol extract has shown dose dependent (0.01 - 0.3 mg ml^{-1}) cholinergic effect that is atropine sensitive. The spasmogenicity is without a co-relaxant effect. The dichloromethane extract was also found to possess marked brine shrimp toxicity. Antibacterial, antifungal and larvicidal activities were found to be absent in both dichloromethane and methanol extracts of *P. barbatum*.

Cholinergic activity of plant constituents like pilocarpine obtained from *Pilocarpus jaborandi* (Rutaceae) and physosigmine from *Physostigma venenosum* (Legumenosae) are clinically used in the treatment of glaucoma where they serve to increase the irrigation of the eye and relieve pressure. The spasmolytic constituents such as hyoscine, commonly found in plants belonging to Solanaceae family (*Atropa belladonna*, *Hyoscyamus, Datura* and *Duboisia* species), are used to relieve the colic pains. In the present study, it is interesting to note that both cholinergic and spasmolytic activities have been shown by the same species (*P. barbatum*).

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