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Biological Activity of *Dipterygium glaucum*

[1]Saeed Ahmed, [1]Mohammad Ashraf, [2]Abdul Jabbar, [3]Khalid Hussain Janbaz,
[1]M. Shafiq Khan, [1]Nisar-ur-Rahman and [2]Munawar Hussain
[1]Department of Pharmacy, [2]Department of Chemistry,
The Islamia University of Bahawalpur, Bahawalpur, Pakistan
[3]Faculty of Pharmacy, B.Z. University, Multan, Pakistan

Abstract: The present research demonstrates the biological activity of *Dipterygium glaucum* since extensive literature survey has shown no documented biological activity of this plant. Ethanol (80%) extract (A) of the plant was subfractioned by hexane (B), ethyl acetate (C) and butanol (D). These fractions (1 mg mL^{-1}) of *D. glaucum* showed 45 to 100% phytotoxicity as determined by the inhibition of *Lemna minor* plant growth but showed no cytotoxicity by brine shrimp lethality assay. DPPH radical scavenging activity of fraction 'A' was 87% whilst other fractions had antioxidant activity below 35%. When fraction 'A' was tested for antispasmodic activity, spontaneous contractions were recorded at 0.1-3.0 mg mL^{-1} concentration in isolated rabbit jejunum preparations. It also inhibited K^+ induced contractions to 60% at 1-3 mg mL^{-1} level, suggesting a calcium channel blockade activity. The n-hexane (B), ethyl acetate (C) and n-butanol (D) fractions exhibited no or little antibacterial, antifungal, antileishmanial and insecticidal activities as compared with their respective controls. This is the first report on the biological activity of *D. glaucum*.

Key words: Biological activity, capparidaceae, *Dipterygium glaucum*

INTRODUCTION

Dipterygium glaucum belongs to Capparidaceae family and is a monotypic genus with only one species *D. glaucum* that is found in Egypt, Arabia, Sudan and Pakistan. *D. glaucum* is branched undershrub, upto 60 cm tall and woody at the base (Jafri, 1973). Extensive literature survey has shown no reported biological activity of this plant. The present work therefore describes the biological activity of the various fractions of the crude 80% ethanolic extract of the whole plant.

MATERIALS AND METHODS

D. glaucum was collected from Cholistan desert and identified by Dr. M. Arshad (Cholistan Institute of Desert Studies, where a specimen is deposited). Plant was dried in shade and soaked in 80% ethanol for 2-3 weeks. After solvent evaporation, residue (A) was suspended in water and extracted with n-hexane (B), ethyl acetate (C) and n-butanol (D) and its various dilutions were made for the determination of the biological activity as described (Atta-ur-Rehman *et al*., 2001). Antispasmodic activity was determined as described earlier (Gilani *et al*., 1994). Briefly, abdomen of rabbits of local breed was cut open and jejunum was taken out. Segments of 2 cm length were suspended in Tyrode's solution aerated with a mixture of 95% oxygen and 5% carbon dioxide at 37 °C. Tissues were allowed to equilibrate for 30 min before the addition of test fractions. The spontaneous rhythmic movements were recorded isotonically using BioScience transducers and an oscillograph.

RESULTS AND DISCUSSION

D. glaucum ethanol (80%) extract was studied for its antispasmodic activity in rabbit jejunum (Table 1). It exhibited inhibition of spontaneous contractions of jejunum at 0.1-3.0 mg mL^{-1}. To test whether this effect is mediated through the blockade of Ca^{2+} influx, a high dose of K^+ (50 mM) was used to depolarize the tissue. Addition of test sample caused 60% inhibition of the K^+ pre-contracted jejunum as compared to the control verapamil (Table 1). It is therefore said that spasmolytic (antispasmodic) activity may be due to any active constituent found in the extract that should be isolated and identified.

Antioxidant activity of extract was found maximum (87%) at 200 μg mL^{-1} as compared with the stndared (92%) as determined by DPPH radical scavenging method whilst other fractions exhibited low levels of antioxidant activity (Table 2). As far as the antileishmanial activity is

Corresponding Author: Saeed Ahmed, Department of Pharmacy, The Islamia University of Bahawalpur, Bahawalpur, Pakistan

Table 1: Antispasmotic activity of the crude ethanolic extract

Spontaneous contraction	K^+-induced contraction
Contraction decreases at 0.1-3.0 mg mL^{-1}	Slow decrease in contraction at 1-3 mg mL^{-1}. About 60% relaxation at 3 mg mL^{-1}

Table 2: Antioxidant and antileishmanial activity of various fractions

Fractions	Antioxidant activity (%) (200 μg mL^{-1})	Antileishmanial activity IC_{50} values (μg mL^{-1})
A	87	>100
B	35	>100
C	19	>100
D	12	>100
Propyl gallae (standard)	92%	-
Amphotericin B (0.19 μg mL^{-1})	-	100% inhibition

Antioxidant activity was determined by DPPH radical scavenging method using propyl gallate as standard at 200 μg/mL levels. Antileishmanial activity was determined by 96-well serial dilution protocol and data expressed in terms of IC_{50} values (μg mL^{-1}). Amphotericin B at 0.19 μg mL^{-1} gave 100% mortality of parasites (n = 3)

Table 3: Phytotoxicity and cytotoxic activity of various fractions

Fractions	(1 mg mL^{-1})	(0.1 mg mL^{-1})	(0.01 mg mL^{-1})
80% Ethanol (A)			
Phytotoxicity	45%	30%	25%
Cytotxicity	0	0	0
n-Hexane (B)			
Phytotoxicity	100%	50%	25%
Cytotxicity	33%	0	0
Ethyl acetate (C)			
Phytotoxicity	100%	55%	40%
Cytotxicity	7%	0	0
n-Butanol (D)			
Phytotoxicity	70%	45%	38%
Cytotxicity	0	0	0

Phytotoxicity was determined by the inhibition of growth of *Lemna minor* plant using paraquat as standard drug (0.9025 μg mL^{-1}). Cytotoxicity was measured by brine shrimp method and number of larvae survived after addition of various concentrations of test sample was determined. Permethrin (236 μg cm^{-3}) was used as standard. Results are expressed in terms of percent (n = 3)

Table 4: Insecticidal activity was measured by contact toxicity method against insects

Insects	80% Ethanol (A)	n-Hexane (B)	Ethyl acetate (C)	n-Butanol (D)
Tribolium castaneum	0	0	0	0
Sitophilus oryzae	0	0	0	0
Rhyzopertha dominica	0	20%	0	0
Callosbruchus analis	0	0	0	0

Test sample (1572.7 μg cm^{-2}) was applied to filter paper in petri dish and placed at room temperature. The number of insects survived at 1 day were counted. Permethrin (236 μg cm^{-2}) was used as standard that gave 100% mortality

Table 5: Antibacterial activity of various fractions

Bacteria	A	B	C	D	Control
Escherichia coli	-	-	-	-	++++
Bacillus subtilis	+	+	-	+	++++
Shigella flexenari	-	-	-	-	++++
Staphylococcus aureus	-	-	-	-	++++
Psudomonas aeruginosa	+	+	+	+	++++
Salmonella typhi	-	-	-	-	++++

Test samples, 3 mg mL^{-1} in DMSO, were placed in wells of 6 mm diameter against standard drug, 10 μg $disc^{-1}$. Zone of inhibition of standard drugs was 27-33 mm and '+' indicates zone of inhibition in 10-14 mm (n = 3). Symbols '++++' indicate 27-33 mm zone of inhibition, '+' is 10-14 mm zone of inhibition and '–' sign means no activity

Table 6: *In vitro* antifungal activity of various fractions by agar tube dilution protocol

Fungus	A	B	C	D
Trichophyton longifusus	0	0	0	0
Candida albicans	0	0	26%	25%
Aspergillus flavus	0	0	0	0
Microsporum canis	0	0	0	0
Fusarium solani	0	0	0	0
Candida glaberata	0	0	0	0

Standard drug miconazole showed 70-98% inhibition of fungal growth. Test samples (0.4 mg mL^{-1} in DMSO) were added to growing fungi at 27°C for 7-days and %inhibition of linear fungal growth measured in mm (n = 3)

concerned, no such activity was found in *in vitro* assays as compared with the control (Table 2).

All fractions exhibited phytotoxic activity against *Lemna* plant in dose-dependent manner (Table 3) where in n-hexane (B) and ethyl acetate (C) fractions showed 100% phytotoxicity at 1 mg mL^{-1} followed by 70% mortality of plant by n-butanol fraction (D) as compared with the control. It is assumed that some phytotoxic constituent(s) are present in the fractions responsible for the activity. However, these fractions demonstrated little (fraction B) or no (fraction C or D) insecticidal or cytotoxicity (Table 3 and 4). Similar profiles have been seen when these fractions showed no antibacterial or antifungal activity (Table 5 and 6).

In summary, the 80% ethanol extract of this plant demonstrated antioxidant and antispasmodic activities with low phytotoxicity levels whilst n-hexane, ethyl acetate and butanol fractions showed considerable high levels of phytotoxicity. These fractions were devoid of any antileishmanial, insecticidal, cytoxicity, antibacterial or antifungal activities as determined by the given experimental methods. Further work is ongoing on the isolation and identification of chemical constituents of this plant.

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