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Preliminary Comparative Phytochemical Screening and Biological Evaluation of *Desmodium elegans*

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Abstract: *Desmodium elegans* is medicinal plants commonly used for the treatment of jaundice, fever, paralysis, oedema, asthma, cold, constipation, cough, convulsion, cholestylithiasis and urolithiasis. The present investigation intended with various phytochemical screening and biological studies were carried out on the bark of *Desmodium elegans*. Preliminary phytochemical test of the crude extract and various fractions revealed the presence of, steroids, Saponins, tannins, reducing sugars, cardiac glycosides and caumarines. The antimicrobial activity and Phytotoxicity activities of crude extract and various fractions of *D. elegans* were investigated. Significance phytotoxic activity was observed at higher concentration.

Key words: *Desmodium elegans* · Phytochemical Screening · Phytotoxic Activity

INTRODUCTION

Plants is a rich source of natural products and natural product which is a platform for biological active principals of drugs and approximately 50% of drugs in clinical used today are derived from natural products [1]. Not only this, it is a starting material for molecular modeling through synthesis even if new one is not discovered. During 1981-2002, 61% of all the new drugs that were introduced were inspired by natural products. According to World Health Organization approximately 80% of world population especially in developed countries, traditional herbal medicines represent the primary source for their health care apart from 25% population of US [2]. *Desmodium elegans* is one of the members of genus Desmodium and family Leguminosae [3]. It is an erect woody deciduous Shrub growing 1.8 m to 2.5 m tall having stalked trifoliate leaves. Flowers are pale purple and arranged in terminal panicles from August-September. Fruit is sessile and propagate through seeds from September-December [3]. *D. elegans* has found wide applications in folk medicine and various parts of plant have been reported to be used for different purposes, for instance roots were used as carminative, diuretic and tonic and the powdered leaves were applying on cuts for healing wounds [3-5]. Previous studies have revealed that all members of genus *D. elegans* are rich sources of alkaloids. This plant has also been studied for the presence of alkaloids along with wax constituents obtained from the said plant [6- 7]. Due to the widely used of this plant as a folk medicine, we identified the bioactive secondary metabolite and their biological activities (antimicrobial, phytotoxic activities) were evaluated.

Collection of Plant Material: The aerial plant materiel was collected during the summer season from Gallyat area, KPK, Pakistan, in June 2010 and identified by Prof Dr. Farrukh Hussain, Dean, Department of Botany, University of Peshawar. Later on, a reference voucher (Bot. 726) was deposited in the herbarium of the Botany Department, University of Peshawar.

Extraction, Fractionation: The air dried powdered aerial parts (10 kg) of the plant were soaked with methanol for fifteen days at room temperature. Filtering with ordinary filter paper, repeated the process for three times. The combined methanolic filtrate was concentrated on rotary evaporator in vacuum at 40°C to afford a black gummy mass (330 g). The black gummy material was suspended in water and fractioned with solvent of gradually

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increasing polarity profile starting from n-hexane (3×2 L), dichloromethane (3×2 L) and ethyl acetate (3×2 L) respectively and subsequently concentrating these fractions on rotary in vacuum yielding n-hexane, dichloromethane, ethyl acetate respectively.

Antimicrobial Assay: Antimicrobial assay of the different fractions were performed using standard procedure [8] against particular Bacterial strains: Modified agar well diffusion method was implemented to test the antimicrobial potential of the fractions by well diffusion methods with the use of Muller-Hinton agar as medium. The culture was prepared in triplicate incubated at 37°C temperature for a period of 24 to 72 hours. 0.6ml of the broth culture of the test organism was put in sterile petri-dish and added 20 ml of the sterile molten MHA. Wells were bored into the medium using 0.2 ml of the fraction using Streptomycin (2mg/ml) as a standard of antimicrobial agent. Inoculation was done for 1 h to ensure the diffusion of the antimicrobial agent into the medium. The inoculated plates were incubated for 24 hours at 37°C. DMSO served as negative control. Diameters of the inhibition zone of microbial growth were measured in millimeter (mm).

Phytotoxicity Activity: *In vitro* phytotoxicity of crude extract/fractions was tested against Lemna minor. In this biological study, three flasks were inoculated with a necessary stock solution of (20 mg/ml) to get a final concentration of 500, 50 and 5µg/ml, respectively. Each flask was then added a 20 ml medium 10 plants each one containing rosette of three fronds. Parquet was used as a standard growth inhibitor. The whole 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.

Phytochemical Screening: The chemical tests were performed on the hexane, chloroform, ethyl acetate and methanolic extracts of *D. elegans* using standard procedure [9-10] to recognize the bioactive secondary metabolite.

Alkaloids: About 0.2 g of each of the fractions was warm with 2% H_2SO_4 for two minutes.The reaction were filtered and added a few drops of Dragendroff's reagent to each filtrate. Orange red precipitate indicates the presence of alkaloids moiety.

Tannins: A small quantity of each extract was mixed with Water and heated on water bath and filtered. A few drops of ferric chloride were added to each filtrate. A dark green solution indicates the presence of tannins.

Anthraquinone: About 0.5 g of each extract was boiled with 10 % HCL for few minutes on water bath. The reaction mixture was filter and allows to cool. Equal volume of $CHCl_3$ was added to each filtrate. Few drops of 10 % ammonia was added to each mixture and heated. Rose - pink color formation indicates the presence of anthraquinone.

Glycosides: Each extract was hydrolyzed with HCl and neutralized with NaOH solution. A Few drops of Fehling`s solution A and B were added to each mixture. Formation of red precipitate indicates the presence of glycosides.

Reducing Sugar: Each extract was shaken with distilled water and filtered. The filtrates were boiled with few drops of Feeling's Solution A and B for few minutes. An orange red precipitate indicates the presence of reducing sugars.

Saponins: About 0.2 g of each extract was shaken with 5 ml of distilled water and heated to boiling. Frothing (appearance of creamy miss of small bubbles) shows the presence of saponins.

Test for Flavonoids: About 0.2 g of each extract was dissolved in diluted NaOH and few drops of HCl were added. A yellow solution that turn colorless indicates the presence of flavonoids.

Phlobatanins: About 0.5 g of each extract was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl solution. Red precipitate shows the presence of phlobatanins.

Steroids: Exist 2 ml of acetic anhydride was added to the mixture of 0.5 g of each extract and H_2SO_4 (2ml). The color change from violet to blue or green in some samples indicates the presence of steroids.

Terpenoids: About 0.2 g of each extract was mixed with 2 ml of chloroform and concentrated H_2SO_4 (3ml) was carefully added to form a layer. The formation of a reddish brown coloration at the interface indicates positive results for presence of terpenoids.

Table 1: Phytochemical profiling of crude extract and various fractions of *D. elegans*

Chemical constituents	n-hexane	Dichloromethane	Ethyl acetate	Methanol	Crude Extract
Tannins	-	-	+	+	+
Alkaloids	-	+	+	+	+
Anthraquinones	-	-	-	-	-
Glycosides	-	-	-	-	+
Reducing sugar	-	-	-	+	+
Saponins	-	-	+	+	+
Flavonoids	-	-	-	-	-
Phlobatanins	-	-	-	-	+
Steroids	-	-	-	-	+
Terpenoids	+	+	+	+	+

Key words: +: present, - : absent

Table 2: Phytotoxic activity of crude extract/fractions of *D. elegans*.

Sample	Conc. of sample (µg/ml)	Fronds survived	Fronds died	% Growth Regulation
Hexane Fraction	05	20	0	0
	50	19	1	05
	500	05	15	75
Dichloromethane fraction	05	20	0	0
	50	20	0	0
	500	03	17	85
Ethyl acetate fraction	05	20	0	0
	50	19	01	05
	500	03	17	85
Methanol fraction	05	20	0	0
	50	18	02	10
	500	07	13	65
Crude residue	05	0	0	0
	50	0	0	0
	500	19	01	05

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

RESULTS

The preliminary phytochemical profiling of crude extract and fractions of the title plant are listed in Table 1. The results of Phytotoxicity activities of crude extractsalong with various fractions are shown in Table 2 while no antibacterial activity is observed in the present investigation.

In vitro pyhtotoxicity bioassay was conducted with the aim to evaluate the toxicity of crude and sub-fractions of crude extract of different parts of plants. Standard drug i,e, Paraquat was used in this experiment under control condition at a concentration of 0.015 µg/mL. The crude extract and sub fractions of crude extract exhibited phytotoxic activity at highest dose of 500 µg/ml. The crude extract and all of the fractions did'nt show significant inhibitory activities at tested concentration of 5 µg/ml and 50 µg/ml. These observations are displayed in Table 2.

DISCUSSION

The preliminary phytochemical screening of the bark of *D. elegans* revealed the presence of bioactive secondary metabolite such as saponins, glycosides, steroids, terpenoids, tannins and reducing sugar. This bioactive secondary metabolite showed the medicinal value of *D. elegans* which is used as anti-spasmodic, anti-ulcerative and arthopathies relaxant [4].

The extracts and fractions were furthers evaluated for their antibacterial potential against selected bacterial strain such as *Staphylococcus aureus*, Strap epidermis, *Escherichia coli* and Klebsiella pneumonia but no activity was observed. The Phytotoxicity activities were performed at different concentration. Different fractions showed activity at different level (Table 2). The low activities found in methanol crude residue while good activity was observed in the entire fractions. Chloroform and dichloromethane fractioned also showed significant

activity at higher concentration. The present finding showed that the bark of title plant can be taken in good quantity in order to reduce the risk of various types of diseases. The pharmacological activity of title plant was confirmed from the Phytotoxicity assay of crude extract various fractions.

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