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Asian Pacific Journal of Tropical Biomedicine

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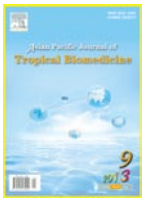

Document heading doi:10.1016/S2221-1691(13)60143-4 © 2013 by the Asian Pacific Journal of Tropical Biomedicine. All rights reserved.

In vitro biological screening of the stem of *Desmodium elegans*

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PEER REVIEW

Peer reviewer

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Comments

Generally the presentation of the paper is well, methodology is appropriate and the results are presented properly.
Details on Page 714

ABSTRACT

Objective: To explore the medicinal importance of the stem of *Desmodium elegans*, methanolic extract, and its different solvent fractions were evaluated for brine shrimp lethality, insecticidal and phytotoxicity, antifungal, and antibacterial activities.

Methods: The methanolic extract and its solvent fractions were tested for cytotoxic, phytotoxic, insecticidal, antifungal, and antibacterial effects using our previous published protocols.

Results: The methanolic, DCM, ethyl acetate and n-butanol fractions exhibited insecticidal effect against *Callosobruchus analis* and *Rhyzopertha dominic*. The methanolic extract, n-hexane, DCM ethyl acetate and n-butanol showed 75, 85, 85, 65 and 5% phytotoxicity at the tested concentration of 500 μg/mL respectively. The solvent fractions (DCM and ethyl acetate) were effective against *F. solani* (10% and 20% inhibition respectively). All the tested samples were devoid of cytotoxic and antibacterial effects.

Conclusions: It was concluded that this plant can be practiced for control of weeds and insects.

KEYWORDS

Leguminosae, *Desmodium elegans*, Brine shrimp cytotoxicity, Phytotoxic, Insecticidal and antimicrobial activities

1. Introduction

Plants are sound source of natural product in a most efficient way and with precise selectivity[1,2]. From the beginning of human civilization men were using different medicinal plants as traditional medicines for their health care. These plants have the ability to produce various valuable classes of compounds with interesting bioactivities[1-4]. Plants have been used as a source of medicine in different custom and form the basis for sophisticated traditional systems and have come up a sole potential candidate to fulfill the basic needs of mankind. In recent decades interest in exploiting biological activities of flora and fauna is getting high attention owing to cost effectiveness, easy availability and without harmful effects[5,6]. With growing interest in medicinal plants consumption have triggered global scientists in the field of natural products to study these plants for various

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Foundation Project: Supported by HEC, Pakistan with grant number 77-98/hec-bvi.

Article history:
Received 28 Jun 2013
Received in revised form 30 Jun, 2nd revised form 10 Jul, 3rd revised form 20 Jul 2013
Accepted 22 Aug 2013
Available online 28 Sep 2013

biological studies and to isolate bioactive constituents[7-13]. *Desmodium* is a genus of large flowering plant family Leguminosae, also called as Fabaceae or pea family with 650 genera and 18 000 species, mostly consisting of herbs, undershurbs or shrubs. The genus *Desmodium* is represented by 300-350 species distributed in tropical and temperate areas of the world except Europe and New Zealand[14]. Traditionally *Desmodium* plants have been used by native people to treat several diseases like jaundice, fever, paralysis, oedema, asthma, cold[15,16], constipation, cough, convulsion[17].

2. Materiel and methods

2.1. 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. Then a reference voucher (Bot. 726) was deposited in the herbarium of the Botany Department, University of Peshawar.

2.2. Extraction and fractionation

The air dried powdered aerial parts (10 kg) of the plant were soaked with methanol for 15 d at room temperature. Filter with ordinary filter paper, and repeat the process for three times. The combined methanolic filtrate was concentrated on rotary evaporator in *vacuo* 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 increasing polarity profile starting from n-hexane (3×2 L), dichloromethane (3×2 L), ethyl acetate (3×2 L) and n-butnol (3×2 L) respectively and subsequently concentrating these fractions on rotary in vacuo yielding n-hexane, dichloromethane, ethyl acetate and n-butnol respectively.

2.3. Brine shrimp lethality assay

This bioassay scheme was carried out according to literature protocol described by standard procedures[18,19]. The eggs of brine shrimp (*Artemia salina* leach) were hatched in sea water. Different concentration of test sample *viz.* 10, 100 and 1000 μg/mL respectively were prepared and then the shrimps were added to vial containing 5 mL sea water. After lapse of 24 h, counting of active nauplii was done and data was further processed with a Finney computer program to estimate LD_{50} values with 95 % confidence interval according to literature[20].

2.3.1. Insecticidal activity

Tribolium castaneum, *Sitophilus oryzea*, *Rhyzopertha dominica*, *Trogoderma granarium* and *Callosobruchus analis* were used as tested insects in this study with Permethrin, which is a standard insecticide according to standard procedure method[21]. Methanolic extract and fractions were dissolved in a suitable volatile solvent. Rearing of insects was maintained in laboratory on sterile breeding media under controlled conditions in plastic containers. Insects of the same age and size were used during the experiment. Filter paper strip measuring the size of petri plate was attached to respective plates. Test sample was introduced onto the strip of filter paper and allowed to stand for 24 h until volatilization is achieved.

After completing volatilization of the solvent, ten healthy insects of uniform age and size of each species were put in each plate covered with a lid. After three days of storage at laboratory conditions in a temperature humidity control growth cabinet (25 °C and 50% humidity), counting was done to determine the number of survival in each plate. Results were obtained in terms of percent mortality by the following formula.

$$\text{Percent Mortality} = \frac{100-\text{No. of insects survived in test}\times 100}{\text{No. of insects survived in control}}$$

2.3.2. Phytotoxic activity

Crude extract and its sub fractions were screened for Phytotoxicity Assay so called *Lemna minor* bioassay against *Lemna minor* L[22]. Stock solution (20 mg/mL) were prepared for this bioassay and then incubated in sterilized flask to have concentration of 500, 50 and 5 mg/mL respectively. To each flask, 20 mL medium along with 10 plants containing a rosette of three fronds were introduced and incubated in growth chamber at 28±1 °C for 7 d after which results were recorded in terms of growth regulation in percentage with reference to the negative control.

2.3.3. Antibacterial and antifungal assays

These assays were carried out on six bacterial and five fungal cultures. The reference bacterial strains used in this test were *Shigella flexeneri* (clinical isolate), *Escherchia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus*. Similarly tested organisms for fungal assay were *Candida albicans*, *Aspergillus flavus*, *Fusarium solani*, *Microspoum canis*, and *Candida glaberata*. These cultures were kept and maintained at 48 °C on agar slant. The tested organism were put on nutrient agar

or Sabouraud glucose agar (SGA) and activated at 37 °C for 24 h for the bacteria and fungi organisms respectively, prior to use for test[23, 24].

3. Results

3.1. Brine shrimp lethality assay

The crude methanolic extract of its subsequent solvent fractions were tested for their preliminary cytotoxic effect. None of the tested samples demonstrated any promising cytotoxic effect in brine shrimp bioassay clearly from Table 1.

Table 1
Cytotoxic profile of *Desmodium elegans*.

	Dose (μg/mL)	No. of shrimps	No. of survivors	No. of larvae died	LD_{50} (μg/mL)	Standard drug	LD_{50} (μg/mL)
Methanolic extract	10	30	28	2	2290.3940	Etoposide	7.4625
	100	30	23	7			
	1000	30	18	12			
n-Hexane fraction	10	30	25	5	3742.744	Etoposide	7.4625
	100	30	20	10			
	1000	30	18	12			
Dichloromethane fraction	10	30	27	3	10199.99	Etoposide	7.4625
	100	30	24	6			
	1000	30	20	10			
Ethyl acetate fraction	10	30	25	5	11572.466	Etoposide	7.4625
	100	30	22	8			
	1000	30	19	11			
Butanol fraction	10	30	28	2	1148.0440	Etoposide	7.4625
	100	30	22	8			
	1000	30	16	14			

3.2. Insecticidal activity

The methanolic extract and its various solvent fractions were tested against three insects, *viz*, *Tribolium castaneum*, *Rhyzopertha dominica* (*R. dominic*) and *Callosobruchus analis* (*C. analis*) as shown in Table 2. All the tested samples were devoid of insecticidal effect against *Tribolium castaneum*. The subfraction, DCM showed 40% mortality against *C. analis* and 20% effect against *R. dominic* along with methanolic extract exhibited 20% effect against the same insect. Regarding the remaining tested samples n-butanol demonstrated 20% activity against *R. dominic* and *C. analis* each and EtoAC showed 20% against *R. dominica*.

3.3. Phytotoxic activity

The methanolic extract and its solvent fraction showed good phytotoxic effect clearly from Figure 1. The phytotoxic effect was only observed at higher dose (500 μg/mL) in all cases. The methanolic extract, n-hexane, DCM ethyl acetate and n-butanol showed 75, 85, 85, 65 and 5% at the tested concentration of 500 μg/mL respectively.

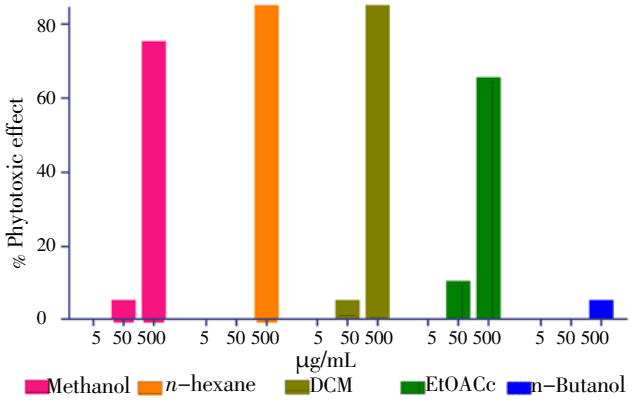

Figure 1. Phytotoxic activity of *Desmodium elegans*.

3.4. Antifungal activity

Regarding the fungicidal assay the methanolic extract and ethyl acetate fraction demonstrated 20% and 10% activity against *M. canis* respectively (Table 3). The solvent fractions (DCM and ethyl acetate) were effective against *F. solani* (10% and 20% inhibition respectively). The rest of tested samples were free of fungicidal activities.

Table 3
Antifungal activity of *Desmodium elegans*.

Fungal Strain	% Inhibition					Standard drug
	AK7	AK8	AK9	Ak10	AK11	
C. albicans	–	–	–	–	–	Miconazole
A. flavus	–	–	–	–	–	Amphotericin-B
M. canis	20	–	–	10	–	Miconazole
F. solani	–	–	10	20	–	Miconazole
C. glabarata	–	–	–	–	–	Miconazole

Key* AK7: Methanolic extract, AK8: n-hexane, AK9: DCM, AK10: EtOAc, AK11: Butanol

Table 2
Insecticidal profile of *Desmodium elegans*.

Sample	Insecticidal assay of ethanolic extract and fractions								
	T. castaneum			*R. dominica*			*C. analis*		
	No. of survivors	No. of died	% Mortality	No. of survivors	No. of died	% Mortality	No. of survivors	No. of died	% Mortality
AK7	10	0	0	10	0	0	8	2	20
AK8	10	0	0	10	0	0	10	0	0
AK9	10	0	0	8	2	20	6	4	40
AK10	10	0	0	8	2	20	10	0	0
AK11	10	0	0	10	0	0	8	2	20

Concentration of test sample=1019.10 μg/cm and Concentration of Standard Drug=235.9 μg/cm^2

AK7: Methanolic extract, AK8: n-hexane, AK9: DCM, AK10: EtOAc, AK11: Butanol

4. Discussion

Over the centuries, phytopharmaceuticals has been utilized by different communities of the world[25]. Local communities of different regions of Pakistan expecially rural areas have century's knowledge about medicinal uses of the plants growing in their areas. These medicinal plants are used to care for a large number of diseases[26]. In Pakistan, this trend is well established in the name of Hikmat/Tibb. Approximately, 600-1 000 medicinal plants of the country have been used in the management of different pathological conditions by more than 40 000 registered and a large number of unregistered Hakims or Tabibs[27]. The control of weeds in the crops is a big challenge to researchers in the present era. Our tested plant sproved very significant phytotoxic. The use of methanolic extract and its various solvent fractions will be helpful in controlling the weeds in agriculture. Pakistan is an agricultural country and the need of weeds control for Pakistan is more helpful as compared to other parts of the world. Beside the phytotoxic effect the plant was also proved week insecticidal. The control of weeds in the field crops and insect in the stored grain is mostly performed by synthetic chemicals which are mostly un environmental-friendly and toxic for human in high concentration or for prolong use. To minimize such toxic effect of insecticidal and weedicidal agent the search of natural agent having related potential is the demand of agriculture. With the hope of finding new, effective, easily available and safe agrochemical we screened the methanolic extract and various solvent fractions for phytotoxic and insecticidal effect.

In conclusion, the stem of *Desmodium elegans* has no significant phytotoxic effect and weak insecticidal effect. The plant is also weak source of antifungal agent. The crude methanolic extract and all solvent fractions were devoid of antibacterial and cytotoxic effect.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The corresponding author is thankful to the planning development division, University of Peshawar for their partial financial support to conclude this assignment. This study was supported by HEC, Pakistan with grant number 77-98/hec-bvi.

Comments

Background

Desmodium is a genus of large flowering plant family Leguminosae also called Fabaceae or pea family with 650 genera and 18 000 species, mostly consisting of herbs, undershurbs or shrubs. The genus *Desmodium* is represented by 300-350 species distributed in tropical and temperate areas of the world except Europe and New Zealand. Traditionally Desmodium plants have been used by native people to treat several diseases like jaundice, fever, paralysis, oedema, asthma, cold, constipation, cough, convulsion.

Research frontiers

In this research paper the methanolic extract and its various solvent fractions of the stem of Desmodium elegans were evaluated for brine shrimp lethality, insecticidal and phytotoxicity, antifungal, and antibacterial activities

Related reports

The GCMS of fixed oils isolated from stem of *Desmodium* elegans as well the phytochemical screening of various solvent fractions has been published by authors. The present study first time reporting these pharmacological activities. The methodology used for this study such as cytotoxicity (brine shrimp), insecticidal, phytotoxic and antimicrobial are well established procedures and even the authors have published lot of papers on this area of pharmacological activities.

Innovations and breakthroughs

Data indicates that this medicinal plant is a potential source for microbiologist as well for agrochemist. The present study provides scientific evidence for using this plant in the killing of insects, weeds and even microbes.

Applications

The paper has reported valuable uses of this plant. In controlling the microbial resistance and weeds the plant may play a vital role.

Peer review

Generally the presentation of the paper is good,

methodology is appropriate and the results are presented properly. Based on the results the authors reported a new application of this valuable medicinal plant. The results of cytotoxicity indicates that this plant can be tested for its anticancer potential. The phytotoxic activity of the plant encourages the agrochemist to use the solvent fractions in the management of weeds and the antimicrobial. Results also revealed that these tested fractions of the plant should be subjected for isolation of secondary metabolites and then should be tested for their antimicrobial activities.

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