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Full Length Research Paper

Agrochemical potential of crude methanolic extract of plant and its hexane, ethyl acetate and butanol fraction

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Vitex agnus-castus **is a folkloric medicinal plant widely used in traditional medicines against various diseases. The agrochemical potential of crude methanolic extract of plant and its hexane, chloroform, ethyl acetate and butanol fractions were assessed through measuring their antibacterial, phytotoxic, insecticidal and cytotoxic activities (*in vitro*). All the fractions exhibited excellent herbicidal activity against *Lemna aequinoctialis welv*. in which chloroform fraction completely inhibited the plant growth at 500 µg/ml concentration. These fractions were also found significant active against various bacteria. Methanolic extract and its fractions did not show any insecticidal activity against *Tribolium castaneum* and *Callusobruchus analis*. Preliminary cytotoxicity tests were done with the five fractions using the larvae of the brine shrimp, *Artemia salina*. These fractions were however found to be relatively non-toxic.**

Key words: *Vitex agnus-castus*, Verbenaceae, antibacterial, cytotoxicity, insecticidal, phytotoxicity.

INTRODUCTION

The world human population is growing rapidly and is estimated to be 10 billion by the year 2050. To cope with the nutritional requirements of this population, more food and fibber would be needed. However, a stiff obstacle in the enhancement of food production from the available resources is the infestation of crops by pests, weeds and diseases, which even increases with increasing intensity of production. The overall global loss of crop production due to these factors is estimated to be 42%, which calls for urgent need for preventive measures.

Currently, the synthetic chemical pesticides are being used for prevention of crop production. However, some serious flaws are associated with the use of these synthetic pesticides including pest resistance and negative impact on natural enemies in addition to environment and health related concerns (Tewary et al., 2005; Pavela, 2004; Hernandez et al., 1999). These problems have resulted in the renewed interest in the development and use of botanical pesticides, which could be an appropriate and non-hazardous alternative to the currently used synthetic agrochemicals as the natural products (Addor, 1995). Numerous plants produce secondary metabolites, which provide them with protection against predation or infection and thus a source of their existence (Ciccia et al., 2000; Khokra et al., 2008). The plants with aforesaid properties, their extracts or purified active constituents can act as insecticides, herbicides or bactericides and thus could be the candidates for application for agricultural purpose. *Vitex agnus-castus* Linn. (Verbenaceae) is locally known as “Hub-el-faked” and “Sumbhalu-ke-bij”. It is a well known plant which is abundantly found in Pakistan (Chadha, 1976). It is traditionally used as an emmenagogue, sedative, anaphrodisiac and galactagogue (Bruneton, 1993). Ethanolic extract of the *V. agnus-castus* is used as a homeopathic drug (agnus castus) for the treatment of impotence and central nervous system disorders (Schwabe, 1987). Flowers of the plant are effective in diarrhoea and liver affections. Powder of its green parts is used as internal antihemorrhagic agent (Usmanghani et al., 1997). To find some new natural sources of pesticides from botanical origin, we screened the crude extract of *V. agnus- castus*

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and its hexane, chloroform, ethyl acetate and butanol fractions and through measuring their antibacterial, phytotoxic, insecticidal and cytotoxic potential.

MATERIALS AND METHODS

Plant material

The aerial parts of *V. agnus-castus* Linn. (40 Kg) were collected in September 1997 from Khost (Quetta, Pakistan) and were dried in air. The plant was identified by Dr. Rasool Bakhsh Tareen, Department of Botany, Baluchistan University, Quetta, Pakistan. A herbarium specimen (VS # 1445) of this plant was deposited at the department of Botany, University of Baluchistan, Quetta, Pakistan.

Extraction and fractionation

Air-dried plant of *V. agnus-castus* (17 kg) was extracted with methanol (50 L) at room temperature (30°C) for 15 days. After evaporation of the solvent, a crude extract (800 g) was obtained, which was dissolved in distilled H_2O (3 L) and defatted with petroleum ether (9 L). The defatted aqueous extract was further fractionated using various solvents mixture (chloroform, ethyl acetate and butanol, each 9 L) to obtain $CHCl_3$, EtOAc and butanol fractions, respectively.

Lemna Welv. phytotoxic bioassay

This test was performed according to the modified protocol of McLaughlin et al. (1991). The test compounds were incorporated with sterilized E-medium at different concentrations, that is 5, 50, and 500 µg/ml in methanol. Sterilized conical flasks were inoculated with compounds of the desired concentration prepared from a stock solution and allowed to evaporate overnight. Each flask was inoculated with sterilized E-medium (20 ml) and 10 plants of *Lemna aequinocitalis* Welv. each containing a Roselle of three fronds. Other flasks were supplemented with methanol serving as a negative control and reference inhibitor, that is parquat serving as a positive control. Treatments were replicated three times and the flasks incubated at 30°C in a Fisons Fi-Totran 600H growth cabinet for seven days, 9000 lux light intensity, 56±10 rh (relative humidity), and 12 h day length. Growth of *L. aequinocitalis* in the compound-containing flask was determined by counting the number of fronds per dose and growth inhibition in percentage calculated with reference to the negative control with the help of the following formula.

$$\text{Growth regulation (\%)} = \frac{\text{100 – Number of fronds in test flasks}}{\text{Number of fronds in negative control}} \times 100$$

Antibacterial activity

All fractions were screened against *Bacillus cereus, Staphylococcus aureus, Corynebacterium diptheriae, Escherichia coli, Klebsiella pneumoniae, Proteus mirabills, Pseudomonas aeroginos* and *Salmonella typhi* strains. For antibacterial screening, 3 mg of sample was taken and dissolved in 3 ml of DMSO (1 mg/ml). Molten nutrient agar (45 ml) was poured on the sterile Petri plates and allowed to solidify. Bacterial lawn was made on these nutrient agar plates by dispensing 7 ml sterile soft agar containing 100 ml of cultures of the test organisms. Wells were drugged with a 5 mm sterile metallic borer at appropriate distance, then 100 µl of sample was poured into each well, and the plates were incubated at 37°C for 24 h. The results in terms of zones of inhibition were recorded. The drug, amoxicillin was used as a positive control, while DMSO was used as a negative control.

Insecticidal bioassay

The insecticidal activity of plant extracts was determined by direct contact application using filter paper (Ahn et al., 1995). The extracts (200 mg) dissolved in methanol (3 ml) were applied to filter papers (90 mm diameter). After drying, each filter paper was placed in Petri dish and 10 adults each of *Tribolium castaneum* and *Callosobrachus analis* were placed in each Petri dish and 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 [pyrethroids (Coopex), 1571.33 µg/cm^2]. All these were kept without food for 24 h after which mortality count was done. All treatments were replicated three times and reported as the average. For calculation of percentage mortality the following formula was used:

$$\text{Mortality (\%)} = \frac{\text{100 – Number of survival in samples}}{\text{Number of survival in control}} \times 100$$

Cytotoxicity test

The extracts were tested using initial concentrations of 10, 100 and 1000 µg/ml in vials containing 5 ml of brine and 30 shrimps in each of three replicates, using the method of McLaughlin (1988). Survivors were counted after 24 h. The data were processed using a Finney program on a simple computer and LD_{50} values were obtained.

RESULTS AND DISCUSSION

Phytotoxic activity

The herbicidal potential of the methanolic extract of *V. agnus-castus* and its fractions was evaluated against *L. aequinoctialis* Welv. (Table 1). The extract obtained from *V. agnus-castus* and its fractions have excellent phytotoxicity at the highest tested concentration (500 µg/ml).

At this concentration, $CHCl_3$ fraction showed inhibited the plant growth by 100%. Hexane and EtOAc fractions and crude methanolic extract also showed significant phytotoxic activity and caused 98.41, 84.12 and 82.53% inhibition of *L. aequinoctialis* Welv., respectively at same concentration. However, at this concentration a good inhibitory activity was observed for the butanol fraction (69.84%). At 50 µg/ml, a moderate phytotoxic activity was observed for butanol, $CHCl_3$ and methanol fraction and caused 50.90, 58.18 and 58.18% inhibition of *L. aequinoctialis* Welv., respectively, whereas a weak activity observed for hexane and EtOAc fractions and caused 20 and 40%, respectively. At 5 µg/ml, a moderate phytotoxic activity was observed for butanol and methanol fraction and caused 65 and 45% inhibition of

Table 1. Result of *Lemna* Welv. phytotoxic bioassay (growth of inhibition in percnatage).

S/N	Fractions	Concentrations		
		500 µg/ml	50 µg/ml	5 µg/ml
1	Methanolic (M)	82.53	58.18	45.00
2	Hexane (H)	98.41	20.00	21.66
3	Chloroform (C)	100.00	58.18	36.66
4	Ethyl acetate (E)	84.12	40.00	33.33
5	Butanolic (B)	69.84	50.90	65.00

Table 2. Zone of inhibition of fractions against selected gram-positive bacteria in mm.

S/N	Name of bacteria	Amoxicillin	Fractions				
			Methanol	Hexane	Chloroform	Ethyl acetate	Butanol
1	*Bacillus cereus*	11.0	-	7.5	7.0	-	6.5
2	*Corynebacterium diptheriae*	20.0	-	-	-	-	6.0
3	*Escherichia coli*	19.0	-	-	-	-	-
4	*Klebsiella pneumoniae*	10.0	-	-	8.0	11.0	10.0
5	*Proteus mirabills*	26.0	-	-	-	-	-
6	*Pseudomonas aeroginosa*	20.0	-	10.0	6.5	10.0	10.0
7	*Salmonella typhi*	22.5	12.5	11.0	10.0	12.5	10.0
8	*Staphylococcus aureus*	9.0	-	-	-	-	-

L. aequinoctialis Welv., respectively whereas a weak activity observed for hexane, $CHCl_3$ and EtOAc fraction and caused 21.66, 36.66 and 33.33%, respectively. The results obtained in the current study indicated that the methanolic extract of *V. agnus-castus* and its fractions have tremendous phytotoxic activity against *L. aequinoctialis* Welv. (Table 1). Therefore the tested plant might be useful as natural herbicide and could be a rich source of bioactive agrochemicals.

Antibacterial activity

Crude methanolic extract exhibited significant antibacterial activity against *Salmonella typhi* with inhibition zones of 12.5 mm, while hexane fraction exhibited activity against *Bacillus cereus, Pseudomonas aeroginosa* and *Salmonella typhi*. Chloroform fraction also exhibited significant activity against *B. cereus, Klebsiella pneumoniae, P. aeroginosa* and *S. typhi*, while ethyl acetate showed activity against *K. pneumoniae, P. aeroginosa* and *S. typhi*. Butanolic fraction showed activity against *B. cereus, K. pneumoniae, P. aeroginosa* and *S. typhi* (Table 2). The results obtained indicated that the methanolic extract of *V. agnus-castus* and its fractions have significant antibacterial activity against *B. cereus, K. pneumoniae, P. aeroginosa* and *S. typhi* (Table 2).

Insecticidal activity

The insecticidal activity of the crude methanolic extract of *V. agnus-castus* and its fractions was tested against pests: *T. castaneum* (common grain pest) and *C. analis*. Crude methanolic extract and its fractions: hexane, chloroform, ethyl acetate and butanol did not exhibit any inhibitory activity against the tested insects species inhibitory the growth of *T. castaneum* and *C. analis*, by 0% mortality.

Cytotoxicity

In a study on the phytomedicinal investigations of this plant, none of these fractions showed considerable brine shrimp cytotoxicity. This declares the safe nature of this plant extract and thus provides non-toxic and environmental friendly alternate sources of pesticides to the synthetic ones.

Conclusions

The present study has identified several fractions of methanolic extract of *V. agnus-castus* which could be candidate for the commercial botanical pesticide formulation regarding their phytotoxic and antibacterial

activities. Moreover, the isolation, purification and investigation of the active principles responsible for the phytotoxic and antibacterial activities will be another landmark in the development of a verifiable application of these materials for the control of the pest vector.

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