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Full Length Research Paper

Evaluation of *Viburnum grandiflorum* for its *in-vitro* pharmacological screening

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In the present study the crude ethanolic and its succeeding solvent fractions of the aerial and roots parts of *Viburnum grandiflorum* were investigated for their *in-vitro* pharmacological (antifungal, phytotoxic and insecticidal) profile. In case of antifungal activity the *n*-hexane fraction was active against *Microsporum canis* having 35 percent zone of inhibition and ethyl acetate fraction were active against *Fusarium solani* with 10% zone of inhibition. The ethyl acetate fraction of aerial part was the most significant antifungal against *F. solani* with 30% zone of inhibition while the crude extract was 10% active against *M. cani.* The *n*-hexane, chloroform fractions and ethanolic extract of the root were significant phytotoxic having 70, 75 and 80% inhibition respectively. In case of aerial part the most significant phytotoxic effect was revealed by ethyl acetate with 85% inhibition followed by ethanolic extract with 80% growth of inhibition. The crude ethanolic extract of the aerial part showed 20, 40 and 40% mortality against *Tribolium castaneum, Callosbruchus analis* and *Rhyzopertha dominica* respectively. The *n*-hexane, chloroform and ethyl acetate fractions showed 20% mortality against *C. analis* and *R. dominica.* All the tested samples were significant insecticidal against *R. dominica*. The *n*-hexane fraction of root was failed to produce any insecticidal effect, while the chloroform fraction was effective against *C. analis* and *R. dominica* with 20% mortality. The crude ethanolic extract and its ethyl acetate fraction showed 40% mortality *C. analis* and *R. dominica*.

Key word: *Viburnum grandiflorum*, antifungal, phytotoxic and insecticidal.

INTRODUCTION

Viburnum, a genus of the family Adoxaceae (formerly Capripoliaceae), consists of more than 230 species, mostly distributed in the temperate or subtropical zones from South America to South East Asia and the majority of them are endemic (Lobstein et al., 1999). Six species of genus *viburnum (viburnum cotonifolium, viburnum tinus, viburnum cylindricum*, *viburnum opulus, viburnum mullaha, viburnum grandiflorum*) distributed in Pakistan. The genus *viburnum* is well known in folk medicine for their spasmolytic, sedative and anti-asthmatic properties (Jarboe et al., 1966). *Viburnum prunifolium* specifically used for menstrual cramps, as anti-abortive agent and for prevention of postpartum bleeding (Calle et al., 1999). *Viburnum* species are used in treatment of different diseases, such as diarrhea, rheumatoid arthritis and tumefaction (Yunnanica, 1991). Anti-diabetic, anti-oxidant and anti-bacterial.

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METERIALS AND METHODS

V. grandiflorum stem and roots were collected from Tandya-cancer, cytotoxic, urine relaxant and antinociceptive activity, uterine excitability, molluscicidal, and diuretic has been reported from *Viburnum* (Parveen et al., 1998). The triterenoids, iridoid glycosids, flavon glucoside neovibsanin, triterpene saponin, furcatin, norisoprenoids, phenolic compounds, new vibsane diterpenes and

Table 1. Anti fungal activity of *V. grandiflorum* root extract.

Name of fungus	*n*-hexane	Chloroform	Ethyl acetate	Ethanolic	Micnazole
Trichphyton longifusus	-	-	-	-	-
Candida albicans	-	-	-	-	110.8
Aspergillus flavus	-	-	-	-	-
Microsporum canis	35	-	-	-	98.4
Fusarium solani	-	-	10	-	73.25
Candida glabrata	-	-	-	-	110.8

lupane triterpenes isolated from *viburnum* genus (Shen et al., 2004; Fukuyama et al., 2004; Fukuyama et al., 2002). In continuation of our research work on Pakistani medicinal plants we tested the crude ethanolic extract as well as subsequent solvent fractions for its various biological screenings.

Ni district Hazara, Khyber Pakhtunkhwa, Pakistan in the month of July, 2009 plant was identified by an eminent taxonomist of Department of Botany Hazara University and a specimen voucher was deposited in the university Herbarium.

Extraction preparation

The plant materials were subjected for ethanolic extract and its various solvent fractions following recommended protocols (Muhammad and Saeed, 2011; Barkatullah and Muhammad, 2011; Saeed et al., 2010; Muhammad et al., 2012). Shade dried plant material 8.5 kg root and14.5 kg aerial part (stem) was grinded to powder mechanically with heavy duty local grinder. Soaked with ethanol for three weeks and subjected to extraction until exhaustion of plant materials. The extracts were then concentrated under reduced pressure using rotary evaporator at low temperature (40°C). Then the crude ethanolic extract was suspended in water and successively partitioned with *n*-hexane, chloroform and ethyl acetate. The crude extract and subsequent solvent were screened for its various in-vitro pharmacological potentials.

Antifungal activity

Stock solution of each extract was prepared by dissolving at a concentration of 24 mg/ml in DMSO and stored in a refrigerator till further used. Sabouraud dextrose agar (SDA) media for fungal growth was prepared by mixing Sabouraud 40% glucose agar and agar in distilled water with acidic pH (pH 5.5 to 5.6) and then autoclaved at 121°C for 15 min (Atta-ur-Rahman 1991). Media was then cooled to 45 to 50°C and 20 ml of molten SDA medium was aseptically transferred into each sterilized Petri dish (4 cm diameter). All dishes were then inoculated with 4 mm diameter piece of inoculums detached from a seven days old culture of fungus. Once the agar was hardened, 8 mm wells were bored using a sterile cork borer, then from each stock solution, extracts were transferred to a separate well having a final concentration of 400 µg/ml and the plates were incubated for 24 h at 29°C. Two wells in each petri dish were supplemented with DMSO and reference antifungal drug Miconazole (0.2 mg/ml) dissolved in DMSO (sigma) serve as negative and positive control.

Phytotoxic activity

The phytotoxic activity of crude and all subsequent solvent fractions of *V. grandiflorum* stem and roots were evaluated using *Lamna minor* plant (Muhammad and Saeed, 2011; Atta-ur-Rahman, 1991). 15 mg of respective extract was dissolved in 1.5 ml of respective solvent and from this solution transfer 5, 50 and 500 µl to the flask (3 flasks for each concentration). This concentration was equivalent to 10, 100 and 1000 µg/ml respectively. The solvent was allowed to evaporate overnight under sterilized condition in laminar flow. 20 ml of E. medium was added to each flask. Other flasks (3 for each) were supplemented with E. medium and standard drug (paraquat, 0.015 µg/ml) served as negative and positive control. To each flask ten plants with 2 to 3 fronds were transferred and kept all the flasks under about 12 h day light conditions. Plants were observed daily and on the seventh day the numbers of fronds were counted. The percentage growth inhibition was recorded with reference to the negative control using the following formula:

$$\text{Inhibition \%} = 100 - \frac{\text{Number of fronds in test sample}}{\text{Number of fronds in negative control}} \times 100$$

Insecticidal activity

In vitro insecticidal assay was carried out for the crude extract and its various solvent fractions against *T. castaneum, R. dominica* and *C. analis* following method available in literature (Saeed M et al. 2010). The test sample was prepared (200 mg of crude extract was dissolved in 3 ml of methanol and served as stock solution). The sample (1019.10 µg/cm^2) was loaded over the filter paper of appropriate size (9 cm or 90 mm) on Petri plate using micropipette. The plate was left overnight (24 h) to evaporate the solvent. Next morning, 10 healthy and active insects of each species of same size and age were added to each plate including control (methanol) and standard drug (Permethrin, 239.50 µg/cm^2). Thereafter the plates were incubated in growth chamber at 27°C for 24 h with 50% relative humidity. The percent mortality was calculated using the following formula;

$$\text{\% Inhibition} = 100 - \frac{\text{Number of insect alive in test}}{\text{Number of insect alive control}} \times 100$$

RESULTS AND DISCUSSION

The crude ethanolic extract and succeeding solvent fractions of the roots and aerial part of *V. grandiflorum* were tested against various strains of fungi that is, *Trichphyton longifusus, Candida albicans, Aspergillus flavus, Microsporum canis, Fusarium solani* and *Candida glabrata* as shown Table 1. The crude extract and chloroform fraction of the root was not active against any of the tested fungi, while *n*-hexane fraction was active against *M. canis* having 35 percent zone of inhibition and

Table 2. Anti fungal activity of *V. grandiflorum* aerial part (stem) extract.

Name of fungus	*n*-hexane	Chloroform	Ethyl acetate	Ethanolic	Micnazole
Trichphyton longifusus	-	-	-	-	-
Candida albicans	-	-	-	-	110.8
Aspergillus flavus	-	-	-	-	-
Microsporum canis	-	-	-	30	98.4
Fusarium solani	-	-	10	-	73.25
Candida glabrata	-	-	-	-	110.8

Table 3. Phytotoxic activity of *V. grandiflorum* root extract.

Extract	No of fronds	Percentage growth inhibition		
		10 ppm	100 ppm	1000 ppm
Control	20	-	-	-
n-hexane	20	10	20	80
Chloroform	20	10	25	75
Ethyl acetate	20	-	5	30
Ethanolic	20	-	15	70

Table 4. Phytotoxic activity of *V. grandiflorum* aerial part (stem) extract.

Extract	No of fronds	Percentage growth inhibition		
		10 ppm	100 ppm	1000 ppm
Control	20	-	-	-
n-hexane	20	-	-	75
Chloroform	20	-	35	75
Ethyl acetate	20	10	25	85
Ethanolic	20	10	15	80

ethyl acetate fraction were active against *F. solani* with zone of inhibition 10 %. The antifungal activity of the aerial parts against the mentioned fungi is presented in Table 2. It is very interesting that the ethyl acetate fraction has the most significant antifungal effect against *F. solani* with 30 % zone of inhibition while the crude extract was 10% active against *M. cani.* The *n*-hexane and chloroform fractions of the aerial parts were non active against any of the test fungus.

M. canis is an organism that can cause tinea capitis in humans, and simple ringworm in pets. (Ginter-Hanselmayer et al., 2004). Despite its name, its major reservoir in companion animals is the domestic cat and dog (Wu et al., 2009). *Fusarium* species are common hyaline soil saprophytes and plant pathogens, which have been frequently reported as etiologic agents of opportunistic infections in humans. These infections have usually been limited to superficial mycoses, but recently the number of infections of deep tissues and disseminated infections have greatly increased, especially in patients with underlying immunosuppressive conditions (Godoy et al., 2004). The crude ethanolic extract and ethyl acetate fractions of both parts can be used in the management of mycosis caused by *M. canis* used in the management of mycosis caused by *M. canis* and *Fusarium* species.

The crude ethanolic along with subsequent solvent fractions of root and aerial part of *V. grandiflorum* were tested for their phytotoxic activity. The *n*-hexane, chloroform and ethanolic fraction of the roots were significant phytotoxic having 70, 75 and 80% inhibition respectively as shown in Table 3. The *n*-hexane and chloroform fractions were the most significant as it shown the phytotoxicity as lower dose also. In case of aerial part the most significant phytotoxic effect was shown by ethyl acetate with 85% inhibition followed by ethanolic extract with 80% growth inhibition as shown in Table 4. The *n*-hexane and chloroform fractions were significant at higher doses. The phytotoxic effect of the root was weaker than aerial part as clear from Table 3 and 4. The crude ethanolic extract of root was better insecticidal against all the three tested insects as shown in Table 5. *C. analis* and *R. dominica* were sensitive for all the applied samples while *Tribolium castaneum* was resistant

Table 5. Insecticidal activity of various fractions of aerial part (stem) extract of *V. grandiflorum.*

Extract	Mortality percent		
	Tribolium castaneum	***Rhyzopertha dominica***	***Callosbruchus analis***
Control	-	-	-
n-hexane	-	20	40
Chloroform	-	20	20
Ethyl acetate	-	20	40
Ethanolic	20	40	40

Table 6. Insecticidal activity of various fractions of root extract of *V. grandiflorum*.

Extract	Mortality percent		
	Tribolium castaneum	***Rhyzopertha dominica***	***Callosbruchus analis***
Control	-	-	-
n-hexane	-	-	-
Chloroform	-	40	20
Ethyl acetate	20	40	40
Ethanolic	20	40	40

to the tested samples except ethanolic extract. The crude ethanolic extract of the aerial part was the most significant insecticidal against all tested samples as presented in Table 6. The crude ethanolic extract of the aerial part showed 20, 40 and 40% mortality against *T. castaneum, C. analis* and *R. dominica* respectively. The *n*-hexane, chloroform and ethyl acetate fractions showed 20% mortality against *C. analis* and *R. dominica.* All the tested samples were significant insecticidal against *R. dominica*. The *n*-hexane fraction of root was failed to produce any insecticidal effect, while the chloroform fraction was effective against *Callosbruchus analis* and *R. dominica* with 20% mortality. The crude ethanolic extract and its ethyl acetate fraction showed 40% mortality *C. analis* and *R. dominica*. There are three more similar insects with *T. castaneum*, all are known as red beetles attack stored grain products such as flour, cereals, meal, crackers, beans, spices, pasta, cake mix, dried pet food, dried flowers, chocolate, nuts, seeds, and even dried museum specimens (Via, 1999). These beetles have chewing mouthparts, but do not bite or sting. The red flour beetle may elicit an allergic response. These beetles are two of the most important pests of stored products in the home and grocery stores. The use of ethanolic extract as insecticidal for the protection of flour, meals, crackers etc is strongly recommended. As the crude extract is phytotoxic and insecticidal therefore it is very interesting that if the extract is used as weedicidal it can acts as insecticidal as the same time. The crude ethanolic is very interesting on agriculture point of view as having double action at the same time.

It is concluded that the underground and aerial parts of the *V. grandiflorum* can be safely used as antifungal especially against *M. canis* and *Fusarium* species. Beside the antifungal action the plant is a potential source of insects and weeds control.

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