Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
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Asian Journal of Plant Sciences
Volume 1 Number 6: 708-709, 2002

Antifungal Properties of Some Indigenous Plants from Peshawar Valley

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Abstract: The research work was conducted to investigate the antifungal activity of hexane, chloroform, ethanol and water extracts of the leaves of *Melia azedarach* L., *Vitex negundo* and *Broussontia papyrifera* and fruit of *Datura anoxia* were evaluated against *Rhizopous niger, Fusarium chlamdosporum, Aspergillus niger, Stemphlium wallr* and *Hyloflora ramosa* using clotrimazole as a reference standard. The antifungal activity was observed for ethanolic and hexane extracts of *B. papyrifera*. The chloroform extracts of *M. azedarach* L. was active against *F. chlamdosporum* (6.6 mm) and the chloroform extract of *D. innoxia* was inactive against all of the fungi tested. The water extract of *D. innoxia* showed highest activities against fungi *S. wallr* (4.95 mm) and minimum activity was observed for *V. negundo* against *S. wallr* (3.30 mm). Hexane extracts of *B. papyrifera* showed the highest antifungal activity against *F. chlamdosporum* (8.36 mm) and minimum was observed for *V. negundo* against *R. niger* (2.85 mm). The antifungal activities for all of the plants extracts were weak and it is not possible to use these plant extracts for the control of fungal diseases.

Key words: *Melia azedarach* L., *Vitex negundo, Broussontia papyrifera, Datura anoxia,* extracts, antifungal activity

Introduction

Many plants are used as insecticides, molluscides and rodenticide (Evan, 1992; Poswal *et al.*, 1993; Anwar *et al.*, 1992; Daoud *et al.*, 1990). The plant fungal diseases are traditionally been controlled by chemical fungicides. The development of the resistance strains of pathogens against various chemical fungicides (Lin, 1981) and their toxic properties make limited the use of these chemicals. The use of plants or plant materials as fungicide is of great importance and need more attention (Bodde, 1982) and various plants products like gum, oil, resins etc. are used as fungicidal (Dwivedi *et al.*, 1990 and Daoud *et al.*, 1990). The biotic-control of plant diseases may have minimum adverse effect on physiological processes of plant and less environmental hazards (Isman, 1989). Biotic-fungicides, being a plant product are easily convertible into a common organic material and may creat fewer health problems compared to the synthetic alternatives.

The powdery mildews (*Erysipha polygoni* D.C.) of pea (*Pisum sativum* L.) was controlled by the use of sunflower (El-Sheriff *et al.*, 1980). The garlic extract, oil (Singh *et al.*, 1984) and juice (Harun and Labosky, 1985) showed the fungicidal properties against the *Fusarium* of watermelon. Bio-control of fungal diseases is not common and thorough investigations are required to find out the suitable plants that can be used to control the pathogenic fungi.

The objective of the research work was to study the antifungal activity of hexane, chloroform, ethanol and water extracts of the leaves of *Melia azedarach* L., *Vitex negundo* and *Broussontia papyrifera* and fruit of *Datura anoxia* plants.

Materials and Methods

Plant material: The study was carried out at the Department of Pharmacy, University of Peshawar during June to December 1994. Leaves of *Melia azedarach* L., *Vitex negundo* and *Broussontia papyrifera* and capsules of *Datura anoxia* were collected from Peshawar University Campus and were identified by the Department of Botany, University of Peshawar.

The plants material were collected during the month of June-July and were washed with distilled water and dried in shade. Dried plant materials (100 g each) were finely ground.

Extraction: The powdered plant material of each plant was macerated separately, for 72 h with ethanol (70%), chloroform, hexane and distilled water. Then extracts were separated by filtration and the residual plant material was further extracted with respective solvents using soxhlet extractor for 8 h to exhaust the material. The extracts were filtered and combined. The solvent was evaporated under reduced pressure and semi solid gummy material was obtained.

Microbial culture and growth conditions

Culture of fungi: *Rhizopous niger, F. chlamdosporum* and *Aspergillus niger* were cultured on bread, while *Stemphlium wallr* and *Hyloflora ramosa* were obtained from growth media used for plant tissue culturing. Spores of these fungi were re-cultured on potato dextrose agar (Difco, USA) plates at 28°C.

Antimicrobial assay: Sterile, filter paper discs of 6 mm diameter were impregnated with about 80 μg disc^{-1} of extract which have been dissolved in dimethyl sulphoxide (DMS) and placed in duplicates onto the potato dextrose agar plates, seeded with 0.2 ml of fungal suspension (ca. 10^8 cells ml^{-1}). The plates were then incubated at 28°C for 10-14 days (Ugarte *et al.*, 1987). The zone of inhibition around each disc was measured in mm. The results are presented as mean ± SD of zone of inhibition. Clotrimazole (20 μg disc^{-1}) was used as a reference standard for comparison.

Results and Discussions

Generally, all of the crude plants extracts showed the weak antifungal activities against the fungi under study. The hexane and chloroform extracts of *V. negundo* showed some weak inhibitory effects against all of the fungi tested (Table 1). The maximum inhibitory effect for hexane extract of *V. negundo* was observed against *A. niger* where the zone of inhibition was 6.24 ± 0.83 mm against the reference standard (16.60 ± 0.80 mm) and minimum activity was observed against *R. niger* (2.85 ± 0.78 mm). The chloroform extract of *V. negundo* showed the maximum activities against the *H. ramosa* where the zone of inhibition was 4.32 ± 0.56 mm against the reference drug 18.05 ±1.72 mm and the weakest activity was observed against *R. niger* (3.15 ± 0.30 mm). The ethanolic and water extracts showed weak activities (Table 1). The zone of inhibition of the ethanolic extracts of *V. negundo* was 5.76 ± 0.81 mm against the *A. niger* compared with the reference drug (19.00 ± 1.75 mm). The water extract showed the weak activities against *S. wallr* (3.30 ± 0.38 mm) and *H. ramosa* (4.32 ± 0.62 mm).

The hexane extract of *M. azedarach* L. showed weak antifungal properties against all of the organisms tested (Table 1), the maximum inhibitory effects were observed against *H. ramosa* (6.30 ± 0.84 mm), *A. niger* (5.28 ± 0.40 mm) and *F. chlamdosporum* (5.28 ± 0.74 mm). The chloroform extract showed antifungal property against only the *F. chlamdosporum* (6.60 ± 1.02 mm). The ethanolic extract was active against only *H. ramosa* and the zone of inhibition was 3.96 ± 0.53 mm compared with the reference standard where the zone of inhibition was 18.05 ± 1.72 mm (Table 1). The water extracts of *M. azedarach* was devoid of any antifungal activities (Table 1). The good antifungal activity of *M. azedarach* against *Alternaria,*

Table 1: Antifungal activity of different extracts of the plants under study

	Hexane extracts				Ref. drug	Chloroform extracts		
Fungi	*V. negundo*	*M. azedarach*	*D. innoxia*	*B. papyrifera*	*clotrimazole*	*V. negundo*	*M. azedarach*	*B. papyrifera*
F. chlamydosporium	5.28±0.65	5.28±074	5.24±0.31	8.36±1.03	22.02±1.64	4.40±0.37	6.6±1.02	-
B. *niger*	6.24±0.83	5.28±0.40	-	3.52±0.40	16.60±*0.83*	4.00±0.43	-	-
R. niger	2.85±0.78	4.75±0.63	-	5.89±0.51	19.00±*1.75*	3.15±0.30	-	-
S. wallr	5.25±1.02	4.35±0.51	-	3.45±0.53	15.83±*1.45*	3.50±0.41	-	3.45±0.32
H. ramosa	4.50±0.52	6.3±0.84	3.6±0.40	3.6±0.50	18.05±1.72	4.32±0.56	-	4.32±0.47

Continued with Table 1

	Ethanol extracts				Ref. drug	Water extracts		
Fungi	*V. negundo*	*M. azedarach*	*D. innoxia*	*B. papyrifera*	*clotrimazole*	*V. negundo*	*D. innoxia*	*B. papyrifera*
F. chlamydosporium	-	-	-	7.36±1.05	22.02±1.64	-	-	-
A. niger	5.76±0.81	-	4.53±0.76	4.96±0.53	16.60±0.83	-	4.00±0.61	-
R. niger	5.03±0.54	-	-	5.03±0.74	19.00±1.75	-	4.75±0.43	-
S. wallr	4..00±0.60	-	6.06±0.48	-	5.83±*1.45*	3.30±0.38	4.95±0.56	-
H. ramosa	6.04±0.53	3.96±0.75	-	5.04±0.45	18.05±1.72	4.32±0.62	4.50±0.47	3.6±0.13

Data are presented as the Mean ±SD of zone of inhibition of samples (80 μg disc^{-1}) and clotrimazole (20 μg disc^{-1}), the reference drug

Aspergillus and *Penicillium* spp. have been reported elsewhere (Daoud *et al.*, 1990) but in present studies all of these extracts showed weak antifungal activity against the tested fungi.

The aqueous extract of *D. innoxia* Mill was active against all fungi except *F. chlamdosporum*, the highest inhibitory effect was observed against *S. wallr* where the zone of inhibition was 4.95 ±0.56 mm compared with the reference drug 15.83 ± 1.45 mm. The ethanolic extract was active against only *A. niger* and *S. wallr* where the zone of inhibition was 4.53 ±0.76 and 6.06±0.48 mm, respectively (Table 1). The hexane extract was active only against *F. chlamdosporum* (5.24 ± 0.31 mm) and *H. ramosa* (3.60 ± 0.50 mm) and chloroform extract of *D. innoxia* Mill was inactive against all the fungi tested (Table 1).

The hexane extracts of *B. papyrifera* showed comparatively better activities against all fungi (Table 1). The good activity was observed against *F. chlamdosporum* where the zone of inhibition was 8.36 ± 1.36 mm and zone of inhibition for reference drug was 22.02 ± 1.64 mm. The chloroform extract of *B. papyrifera* was active only against *S. wallr* (3.45 ± 0.32 mm) and *H. ramosa* (4.32 ± 0.47 mm) (Table 1). The ethanolic extract was inactive only against *S. wallr* and showed weak antifungal activity against all of the fungi under study (Table 1), the highest activity was observed against *F. chlamdosporum* (7.36 ±1.05 mm).

The difference in the antifungal properties of these extracts is due to the fact that various solvents used as menstrum have different polarity and hence have different extraction power. The present studies showed that hexane, least polar, can be a better solvent used for extraction. The aqueous extract, being the more polar, showed the poor fungicidal activities against test fungi. The most obvious conclusion could be that more than one chemical might be extracted by single solvent and the effect of toxic moieties may be masked by other components of the plant. So constituent should be separately tested because there has been considerable attention given to the effect of constituents (Feeny, 1970; Rhoades and Cates, 1976; Bernays, 1978; Swain, 1979). The conditions for drying like temperature, humidity and light might destroyed the anti-fungal constituents (Khune *et al.*, 1985) where the suitable temperature is 40°C.

Weak antifungal properties were observed for the plants extracts against microorganisms understudy, further work is required to isolate the active constituents and test the antifungal properties of these compounds, this may help to find the compound responsible for antifungal activities. Comparatively better antifungal activity was observed in least polar solvent used as menstrum. Generally, aqueous extract showed poor activity against test organisms. The plants extracts under study showed weak antifungal properties against the test microorganisms and it is not possible to use these extracts on economical basis for bio-control of the fungi.

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