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Antifungal Activity of Some Saudi Plants Used in Traditional Medicine

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Abstract: Methanolic, chloroform and aqueous extracts of 11 medicinal plants used in folklore medicine in Saudi Arabia, were investigated for *in vitro* activity against four pathogenic fungi. The extracts at concentration of 0.5 mL plate^{-1} showed varying degrees of total inhibition of fungal growth. Extracts from *Salvadora persica* and *Vigna fragrans* showed the highest activity, followed by *Peganum harmala* and *Withania somnifera*, while *Polycarpaea corymbosa* demonstrated the least activity, when compared to 25 μg mL^{-1} Clotrimazole control antibiotic. The fungal strains tested differed significantly in their susceptibility to plant extracts, with complete inhibition in *Aspergillus fumigatus* and *Candida albicans*. The plants which exhibited a marked antifungal activity were shown to be rich in alkaloids, flavanoids, tannins and glycosides. These results support the traditional use of these plants in the treatment of some fungal infections.

Key words: Medicinal plants, folklore medicine, traditional medicine, antifungal activity, Saudi Arabia

INTRODUCTION

Higher plants have been used for centuries as remedies for human diseases. As a result of the increasing need for new and better drugs to heal diseases, research workers from different disciplines are jointly attempting to study rationally and scientifically the resources of medicinal plants (Azaizeh *et al*., 2003). Various antifungal agents have been explored, but the control of the fungal diseases has not yet been achieved (Omer and Elnima, 2003; Misra and Sahu, 1977; Goun *et al*., 2003). In the literature a number of compounds have been isolated from plants and their chemical structures were fully elucidated and many of them were tested for possible biological activities (Crombie *et al*., 1990; Mitscher *et al*., 1972). Many of the active compounds has found place in modern therapy and compounds vary from alkaloids, triterpenes, flavanoids, glycosides, to many minor classes of plant constituents (Vardamides *et al*., 2001; Edeoga and Ikem, 2002).

As yet, plants in Saudi Arabia have not been thoroughly investigated with regard to their biological activities (Mossa, 1986; Al-Taweel *et al*., 2004; Kadriya *et al*., 2004). Therefore in this study an attempt was done to investigate plants collected from different regions in the southern area for possible antifungal activities and these plants are commonly used in traditional medicine.

MATERIALS AND METHODS

Plant material: The study was carried out at the Department of Biology at Abha, during January to December 2003. Eleven plant species representing 10 botanical families were collected from the South area of Saudi Arabia (Table 1) and were washed with distilled water and dried in shade at room temperature. Plants were then authenticated by the Department of Botany, University of Kartoum, Sudan, a voucher specimens were deposited at Biology Department Herbarium.

Preparation of extracts: Ten gram of the coarsely powdered plant material were successively Soxhlet extracted with $CHCL_3$ and MeOH for 24 h. The extracts were evaporated under vacuum and the residues were separately dissolved or suspended in the same extracting solvent (10 mL) and kept in refrigerator till use. In addition, water extracts were prepared by adding distilled water to 10 g of coarsely powdered plant material in a conical flask and left to soak overnight. The residue was then filtered and the final volume was adjusted to 10 mL with distilled water and the solution used immediately.

Fungal strains: Four fungal species were obtained from the Department of Microbiology, University of King Khalid, Abha. These were *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger* and *Candida albicans*. Each organism was cultured on Sabourauds dextrose agar medium incubated at 25°C for 7 days, to obtain inoculums for testing.

Antifungal bioassay: Amount of 0.5 mL of each extract and 0.1 mL of each fungal suspension in sterile normal saline were mixed with 20 mL of pre-sterilized Sabouraud's dextrose agar medium which was maintained at 45°C, then

Table 1: Antifungal activity of some Saudi Arabian plants, *in vitro* tests

Plant (Botanical family)	Part (s) used	Inhibition of fungal growth: Ch A.fl	Ch A.fu	Ch A.n	Ch C.a	Me A.f	Me A.fu	Me A.n	Me C.a	H_2O A.fl	H_2O A.fu	H_2O A.n	H_2O C.a
Argemone mexicana L. (Papaveraceae)	L	+++	-	-	+++	-	++	++	-	-	++	++	-
Datura stramonium L. (Solanaceae)	L	++	+	+	++	++	++	++	+++	-	++	+	++
Fagonia cretica L. (Zygophyllaceae)	L	+	+	-	-	++	++	+++	+++	-	-	+	+
Grewia villosa Wildd. (Tiliaceae)	L	+++	+	+	+++	-	+	+	-	-	+	+	-
Nymphaea lotus L. (Nymphaeceae)	WP	+++	++	-	+++	-	++	-	-	-	+	+	-
Peganum harmala (Caryophyllaceae)	L	+	+	-	-	+++	+++	+++	+++	++	++	++	++
Polycarpaea corymbosa (L.) Lam (Caryophyllaceae)	WP	+++	-	-	-	-	++	++	-	-	++	++	-
Salvadora persica L. (Salvadoraceae)	St	+	+	+	++	++	+++	++	+++	++	+	++	++
Sterculia steigera Del. (Sterculiaceae)	Fr	+	-	-	+	-	+	-	-	-	+	+	-
Vigna fragrans Bak.f. (Papilionaceae)	R	+	+	-	+	+	+	+	+++	+	+	+	+++
Withania somnifera (L.) Dunal. (Solanaceae)	L	-	-	+	+	++	++	++	++	+	+	++	++
Clotrimazole 25 μg mL^{-1} (Reference drug)													

*Data are presented as follows, - = No inhibition of fungal growth, + = Slight inhibition, ++ = Moderate inhibition, +++ = Strong inhibition and the average of two separate experiments with five replicates in each treatment, A.fl = *Aspergillus flavus*, A.fu = *Aspergillus fumigatus*, A.n = *Aspergillus niger* C.a = *Candida albicans*, L = Leaves, St = Stem, WP = Whole Plant, Fr = Fruit, Ch = Chloroform, Me = Methonol, H_2O = Water

poured in sterile disposable petri dishes and left at 25°C for 7 days. In controls, sterile water, $CHCL_3$, MeOH and 25 μg mL^{-1} Clotrimazole reference antibiotic were used in place of the test extract. Each extract was tested against each organism using the method described by Groove and Randall (1955) and the total inhibition of fungal growth was measured and reported in Table 1.

Photochemical screening: Phytochemical screening was carried out using the methods adopted by Crombie *et al*. (1990) in a similar surveys.

RESULTS AND DISCUSSION

Thirty three plant extracts representing 11 plants belonging to 10 families were tested (Table 1). The results of the *in vitro* antifungal screening of plants used in Saudi Arabia folklore medicine are shown in Table 1. The plant extracts which possessed moderate growth inhibition or more against one or more fungal strains were considered to be active. Out of the 33 extracts examined 55% showed significant antifungal activity. 54% of extracts showed significant activity against *Candida albicans*, 64% against *Aspergillus niger*, 73% against *Aspergillus fumigatus* and 46% against *Aspergillus flavus*.

It is evident that extracts at concentration of 0.5 mL $plate^{-1}$ showed varying degrees of total inhibition in fungal growth. Extracts from *Salvadora persica* and *Vigna fragrans* showed the highest activity, followed by *Peganum harmala* and *Withania somnifera*, while *Polycarpaea corymbosa* demonstrated the least activity when compared to 25 μg mL^{-1} Clotrimazole control antibiotic. The fungal strains tested differed significantly in their susceptibility to plant extracts, with complete inhibition in *Aspergillus fumigatus* and *Candida albicans*. Out of the 11 plants tested, 9 plants showed a high activity against the four types of fungal species tested. With *Candida albicans*, *Aspergillus fumigatus* and *Aspergillus niger*, all methanolic and aqueous extracts exhibited fungicidal activity, except 4 extracts which showed no growth inhibitory action. This justifies the traditional use of the aqueous extracts.

Out of the most active 22 extracts, methanol extracts exhibited a well marked antifungal activity (82%), followed by aqueous extracts (64%) and chloroform (55%). This data is in agreement with previous reports elsewhere using different plants (Omer and Elnima, 2003; Okemo *et al*., 2003; Almagboul *et al*., 1988).

Some of the most active plants were phytochemically screened and the results are shown in Table 2. The plants which exhibited a marked antifungal activity were shown

Table 2: Phytochemical analysis of the most active antifungal plant samples

Plant species	Part used	Alkaloids	Flavonoids	Tannins	Saponins	Anthra-quinones	Cyanogenic glycosides	Sterols and/or triterpenes
Peganum harmala	L	+++	-	-	-	-	-	-
Withania somnifera	WP	+++	-	-	-	-	+++	-
Nymphaea lotus	WP	++	++	+	-	-	-	+
Vigna fragrans	R	-	-	-	+	-	±	++
Grewia vilosa	L	+++	+++	-	±	-	-	++

Constituents, + = Low, concentration, ++ = Medium concentration, +++ = High concentration, -= Not detectable, ± =Traces

to be rich in alkaloids, flafonoids, tannins and glycosides. Four of the active plants contain alkaloids, 2 contain flavanoids and alkaloids, 1 plant contain tannins. Sterols and saponins were detected in only 2 plants. None of the plants tested was found to contain anthraquinones.

It is not possible to make a direct correlation between the observed activity of the tested plant extracts *in vitro* and the actual effects when used *in vivo* for the diseases observed by the indigenous people and traditional healers. Therefore, it is important that the plant species which have demonstrated growth-inhibiting activity in this assay be further investigated to evaluate the significance of these extracts, clinical role and the medical system of indigenous people. Additional research is also necessary to isolate and characterize their active compounds for pharmacological testing.

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