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STUDIES ON THE ANTIFUNGAL PROPERTIES OF INDIGENOUS PLANTS FROM KARACHI REGION

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Screening was carried out to study the antifungal properties of extracts of indigenous plants from the Karachi region. These studies are based on 50 crude extracts of higher plants belonging to 28 families, viz, *Apocynaceae, Asclepiadaceae, Cucurbitaceae, Compositae, Caricaceae, Crassulaceae, Euphorbiaceae, Lythraceae, Labiatae, Gentianaceae, Meliaceae, Moraceae, Menispermaceae, Myrtaceae, Nyctaginaceae, Oleaceae, Orchidaceae, Palmae, Papilionoidae, Polygonaceae, Solanaceae, Rhamnaceae, Ramunculaceae, Umbelliferae, Valerianaceae, Verbinaceae, Vitaceae, Zygophyllaceae.* The results showed that 18 % of the plants possessed some level of antifungal activity.

Key words: Antifungal, Plants, Karachi.

INTRODUCTION

For more than 40 years a number of investigators [1-6] have conducted surveys on antimicrobial substances, mainly from higher plants. These studies include mostly preliminary data on crude extracts. However, recent studies have also been done on the chemicals responsible for antimicrobial activities [7-13]. It is generally considered that variations in the results by the previous workers may be partly due to the presence of certain substances in crude extracts which exert antagonistic effect during testing of antifungal components. Also substances could be present in the extract that stimulate the growth of microorganisms, and hence interfere with the effect of inhibitory substances [14].

It is well known that fungal infections are more prevalent in hot and humid areas. This includes plant diseases as well as those of animals and human beings. As such, it was considered worthwhile to take up a programme for screening antifungal substances from indigenous plants, which in final analysis could possibly be used for the control of such diseases. These studies are amply justified because there exists considerable scope for new fungicides from natural resources.

MATERIALS AND METHODS

Plant material used. All plants wild or cultivated were collected fresh from different places in the district of Karachi. Various parts of the plant used during these studies include-roots, stems, leaves, flowers, fruits and seeds.

Preparation of plant extracts. Parts of the fresh plant were chopped into small pieces. About 100 g of plant material was soaked into 300 ml of 90 % either ethanol, or benzene, or petroleum ether, or water for 2-3 days at room temperature. In each case, the solvents used have been mentioned in the table. The crude extracts were obtained from soaked plant material by the percolation method, first after 48 hr and then twice after 24 hr. The pooled extracts were concentrated under high vacuum.

Preparation of samples for testing. Plant extracts were not equally soluble in water, and therefore, a solution containing 100 ml of 50 % ethanol and 5g of an emulsifier Triton$^{\circ}$ (Rohm and Haas, Philadelphia, Pa 19105) was used to emulsify all and extracts. The above plant extracts were weighed to give 5 % concentration (i.e. 0.05 g of plant extract dissolved in 1 ml solution of ethanol and triton mixture). Aliquots of this concentration were used to test antifungal activity.

Test organisms. Antifungal activity was tested against two common pathogenic fungi of fruits namely, *Aspergillus niger* and *Alternaria* spp. Both were isolated from infected citrus fruits. Cultures of these fungi were maintained on Sabouraud's Dextrose Agar at 4°.

Antifungal test. The diffusion plate method was used to test plant extracts (15). O.1 ml of the fungal spore suspension (grown for 3 days in 10 ml of Sabouraud's Dextrose Broth) was thoroughly mixed with 25 ml of melted Sabouraud Dextrose Agar and poured into sterilized petri-plates. When the agar was set, 5 holes of 6 mm diameter bore were made on each of the seeded plate. The

four outer holes were filled with 0.2 ml of the testing sample and the central hole with 0.2 ml of 5% standard commercial fungicide "Tecto" Merck Sharp & Dohme International, USA) for the purpose of comparing the antifungal activity of the extracts. Simultaneously another set was made with 0.2 ml of the base solution (used for dissolving plant extracts) as control in the central well of the petri-plate. All these experiments were performed in duplicate. The petri-plates were incubated at 28° for 3-5 days.

The zones of inhibition produced by plant extracts were compared with the zone produced by the standard fungicide. To show approximately an equal zone of inhibition as compared to "Tecto", sign (++) was used. Smaller zones are indicated by (+), whereas the (–) sign was used when no zone of inhibition was observed.

All culture plates were examined twice weekly for a period of 3 weeks and the results tabulated.

RESULTS AND DISCUSSION

Comprehensive data on antifungal activities shown by different plant extracts are represented by tables. Plant extracts which possessed inhibition zone 15-20 mm dia against test organisms were considered to be active and represented by (++). Out of the 50 plant extracts tested, only 9 (18 %) showed antifungal activity against *Aspergillus niger* but none of them showed any activity against *Alternaria* spp. It is possible that *Aspergillus niger* was more sensitive to the chemical constituents of plant extracts as compared to *Alternaria* spp. This seems more true since *Aspergillus niger* is a fast growing fungus as compared to *Alternaria.* Those plants which showed antifungal property were *Cichorium intybus* L., *Ricinus communis* L., *Ocimum basilicum* L., *Capsicum annum* L., *Psophocarpus tetragonobolus* (L.) DC., *Fagonia critica* L., *Trachyspermum ammi* (L.) Sprague and *Vitis venifera* L.

Table 1.

S. No.	Plant			Part used	Extracting solvent	Antifungal activity	
	Family	Botanical name	Common name			*Aspergillus niger*	*Alternaria spp.*
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
1.	Apocynaceae	*Rhazya stricta* Dcne.	Sewar	Leaves	Alcohol	(–)	(–)
2.	–do–	–do–	"	Roots	"	(–)	(–)
3.	–do–	–do–	"	Stem	"	(–)	(–)
4.	–do–	–do–	"	Seeds	"	(–)	(–)
5.	–do–	*Ervatamia divaricata* (L.) R. Br.	Chandni	Leaves	"	(–)	(–)
6.	–do–	*Nerium indicum* Mill	Kaner	Flowers	"	(–)	(–)
7.	–do–	*Vinca rosea* L.	Sada bahar	Shoots	"	(–)	(–)
8.	Asclepiadaceae	*Calotropis procera* (Willd.) R. Br.	Aak	Leaves	"	(–)	(–)
9.	Compositae	*Artemisia absinthium* L.	Vilayati afsantin	Leaves	"	(–)	(–)
10.	–do–	*Cichorium intybus* L.	Kasni	Flowers	"	(+)	(–)
11.	–do–	*Blumea balsamifera* DC.	Kakronda	Fruits	"	(–)	(–)
12.	–do–	*Tagetes erecta* L.	Marigold	Shoots	"	(–)	(–)
13.	–do–	*Chrysanthemum cinerariae-folium* (Trev.) Vis.	Pyrethrum	Leaves	"	(–)	(–)

(continued. . . . .)

(Table 1, continued)

14.	Cucurbitaceae	*Momordica charantia* L.	Bitter gourd	Leaves	"	(–)	(–)
15.	Caricaceae	*Carica papaya* L.	Papaya	Leaves	"	(–)	(–)
16.	Crassulaceae	*Bryophyllum pinnata* (Lam.) Oken	Zakhm-e-hayat	Leaves	"	(–)	(–)
17.	Euphorbiaceae	*Ricinus communis* L.	Castor bean	Leaves	"	(+)	(–)
18.	–do–	*Phyllanthus emblica* L.	Emblic imyrobalam	Leaves	"	(–)	(–)
19.	–do–	*Euphorbia antiquorum* L.	Thor	Stem	Water	(–)	(–)
20.	Punicaceae	*Punica granatum* L.	Pomegranate	Inner skin of fruits	Alcohol	(–)	(–)
21.	Labiatae	*Ocimum basilicum* L	Tulsi (Niazbo)	Leaves	"	(+)	(–)
22.	Gentianaceae	*Swertia chirata* (Wall) C.B. Cl	Chirata	Leaves	"	(–)	(–)
23.	Meliaceae	*Melia azadirach* L.	Persian lilac	Leaves	"	(–)	(–)
24.	–do–	*Azadirachta indica* L. A. Juss.	Neem	Leaves	"	(–)	(–)
25.	Moraceae	*Morus alba* L.	Mulberry	Leaves	"	(–)	(–)
26.	Menispermaceae	*Tinospora cordifolia* (DC) Miers.	Gilo	Leaves	"	(–)	(–)
27.	Myrtaceae	*Syzygium cumini* (L.) Skeel Syn. Eugenia Jambolana	Jamun	Seeds	"	(–)	(–)
28.	–do–	*Psidium guajava* L.	Guava	Leaves	"	(–)	(–)
29.	–do–	*Eucalyptus globulus* Labill	Eucalyptus	Leaves	"	(–)	(–)
30.	Nyctaginaceae	*Bougainvillae glabra* choisy	Bougainvillae	Leaves	"	(–)	(–)
31.	Oleaceae	*Nyctanthes arbortristis* L.	Harsinghar	Leaves	"	(–)	(–)
32.	–do–	*Jasminum sambac* Ait.	Jasmin	Leaves	"	(–)	(–)
33.	Orchidacea	*Orchis latifolia* L.	Salap	Rhizomes	"	(–)	(–)
34.	Palmae	*Cocos nucifera* L.	Coconut	Leaves	"	(–)	(–)
35.	Leguminosae	*Glycyrrhiza glabra* L.	Licorice	Roots	Water	(–)	(–)
36.	–do–	*Psophocarpus tetraqonolobus* (L.) DC.	Winged bean	Seeds	Benzene	(+)	(–)
37.	–do–	*Pongamia glabra* Vent.	Karanja	Seeds	Alcohol	(–)	(–)
38.	Polygonaceae	*Rheum emodi* Wall. ex Meissn.	Rewand chini	Leaves	"	(–)	(–)
39.	Solanaceae	*Nicotiana tabacum* L.	Tobacco	Leaves	"	(–)	(–)
40.	–do–	*Capsicum annum* L.	Capsicum	Fruits	Pet. ether	(+)	(–)
41.	–do–	*Datura metal* L.	Datura	Leaves	Alcohol	(–)	(–)

(continued. . . . .)

(Table 1, continued)

42.	Rhamnaceae	*Zizyphus jujuba* Lam. non Mill	Ber	Leaves	"	(–)	(–)
43.	Ranunculaceae	*Nigella sativa* L.	Kalonji	Seeds	Petroleum ether	(–)	(–)
44.	–do–	–do–	"	Seed	Alcohol	(–)	(–)
45.	Valerianaceae	*Valeriana wallichii* DC.	Mushkbala	Roots	"	(–)	(–)
46.	Verbenaceae	*Lantana camara* L.	Ghaneri	Shoots	"	(–)	(–)
47.	Vitaceae	*Vitis vinifera* L.	Grape	Leaves	"	(+)	(–)
48.	Umbelliferae	*Trachyspermum ammi* (L.) Sprague	Ajwain	Seeds	Petroleum	(++)	(–)
49.	–do–	–do–	"	Leaves	Alcohol	(+)	(–)
50.	Zygophyllaceae	*Fagonia cretica* L.	Sachi booti	Whole plant	"	(+)	(–)

Gupta and Benerjee [16] have not found antifungal activity against *A. niger* in *Ricinus communis* and *Ocimum basilicum* of West Bengal origin. This may be contributed to variation in the phytochemical composition of same plant species growing in different climatic conditions of the subcontinent.

The antifungal screening results suggest that the antimicrobial activity is not restricted to any morphological parts of the plant. In *C. annum* the extracts from fruits gave positive results, whereas, *P. tetragonobolus* seeds indicated some activity. In a single case of *F. critica,* the whole plant showed effectiveness, and was therefore, considered important for further studies.

On the basis of the present studies it seems that there is considerable scope in exploiting the indegenous plant resources for antifungal substances. This is more true because many of these plants are not found in the advanced countries and consequently they have not been properly exploited for such activities. These studies would increase the probability of spotting some plants with active antifungal properties from indigenous resources.

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