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ANTIMICROBIAL PROPERTIES OF HIGHER PLANTS OF KARACHI REGION

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Abstract. The antibacterial properties of ethanolic extracts of various parts of 27 wild and cultivated higher plants of Karachi region have been studied *in vitro*. Ethanol extracts of six plants showed, through radial diffusion assay technique, activity against 16-22 test organisms which included 13 pathogenic bacteria and 10 dermatophytic fungi. The antifungal activity has been compared with that of a known pharmaceutical product. Use of the extracts of the plants which showed promising results, in dermatophytic conditions, are discussed.

From time immemorial drugs from plant source served as mainstay for the treatment of most of the human diseases and are still in practice in many parts of the world. The present studies are in continuation of the previous series (Attia)[1-2]. This paper deals with the *in vitro* antibacterial and antifungal testing, by radial diffusion assay technique, of various parts of 27 higher plants of Karachi region against 13 bacteria and 10 dermatophytic fungi. The extracts of nine plants showed activity against 2-9; 12 plants against 10-15 and six plants against 16-22 test organisms. The extracts of those plants which showed activity against dermatophytic fungi and skin pathogenic bacteria, *Staphyllococcus aureus* and *Streptococcus pyogens* were also tested *in vitro*. For *in vivo* test, patients with nail, hair and skin infections were taken as volunteers. The extracts of the active plants were either added in a simple ointment or cream base or mixed in a lotion, for external application. Even severe cases were cured to a great extent. Chemical investigations for the isolation and characterization of the active constituents of three plants have been undertaken and results will be reported in separate communications.

Materials and Methods

Collection of the Plant Material. Most of the collection for wild or cultivated plant materials in their flowering or fruiting season have been made from the Council campus, University Area, Drigh Road, Gulshan-e-Iqbal and the nearby areas of Karachi.

Extraction Procedure. Different parts of the fresh plants were soaked in alcohol, and collected after every 48 hr by the percolation method. Collected extract was then concentrated and freed from alcohol at 40° under vacuum. A 4% solution of the extract was made in 90% alcohol (wt/vol.) for antibacterial testing, while a 5% solution was used for antifungal activity. A 2% ethanolic solution (vol/vol) from Jadit (10% of 4-chloro-2-hydroxybenzoic acid-*n*-butylamide), an antimycotic preparation of Farmwerke, Hoechst AG, West Germany was prepared for antifungal tests. Tests were also performed against all the test bacteria with undiluted *Savlon* (0.3% chlorohexidine) antiseptic.

Testing of Antibacterial and Antifungal Activities

Medium. The medium for antibacterial testing contained nutrient agar (Difco), nutrient broth (Difco) and blood agar. For antifungal testing the medium contained Sabouraud's dextrose agar and Sabouraud's dextrose broth.

Procedure. The agar cavity method prescribed by Reddish[5] was adopted without any modification for both the antibacterial and antifungal tests. Its details have also been reported earlier.[4] Results of the tests were noted after 24-48 hr as required by the test organisms.

Test Organisms. Salmonella typhosa, S. para A, S. para B, Shigella dysenteriae, Escherchia coli, Klebsiella pneumoniae, Streptococcus faecalis, S. pyogenes, Vibrio cholera ElTor, V.C. Inaba, Staphylococcus aureus (of coagulase positive type), *Diplococcus pneumoniae* and *Corynebacterium diptheriae* for antibacterial tests were obtained from the Department of Microbiology, University of Karachi. Inocula were made from 24 hr broth cultures or spore suspensions at a rate of 1 ml to 100 ml of the appropriate agar.

The fungi, *Sporotrichum schenckii, Trichophyton gourvilli, T. rubrum, T. schoenleinii, Microsporum audouni, M. gypseum, M. vanbruseghemii, Epidermophyton floccosum, Canadida albicans* and *C. tropicalis*, were received from Microbiology Department of Karachi University. Strains were isolated from the patients and reared in laboratories.

Results and Discussion

An elaborate account of antibacterial and antifungal properties showed by ethanolic extracts of plants,

Jadit and *Savlon* against 23 micro-organisms are summarised in the table.

Six plants showed activity against 16-22 test organisms, 12 plants against 10-15 micro-organisms while nine plants showed inhibition zones of A and B categories against most of the test organisms, and they were selected for extensive chemical investigation.

Ethanolic extracts of *P. glandulosa*, *M. hamata* and *A. indicum* were found effective in the eradication of dermatophytic fungal and pus forming bacterial infection of human beings, when applied externally in a simple ointment base. This trial antimycotic preparation requires improvement and its further studies from chemical and pharmacological point of view are in progress. Similarly many more plants exhibiting a category of antibacterial or antifungal properties could be selected for the therapy of most common infections of this region.

The use of ethanolic extracts as drug cannot be assured at this stage. For the purpose of incorporating them for human use, their toxicity and interaction with blood constituents must be known. Similarly there is much variation in the activity of the plant material. The antibacterial and antifungal principles may change with the age of the plant. Even environmental factors such as excessive rainfall or draught enhance the quantity of an active constituent or diminish it. Other factors, like nature of the soil, temperature and length of the sunny period also count very much. Variation in the relative proportions of various constituents in plants have been observed by many workers. The two main alkaloids, hyoscine and hyoscyamine, of an Australian plant, *Duboisia myoporoides* varied considerably from 0.8 to 3.7. The results presented in this report may, therefore, not be valued as absolute. Studies in the isolation, characterization and toxicology of the active principles are being continued.

TABLE. SHOWING ZONES OF INHIBITION OF FUNGAL AND BACTERIAL GROWTH IN CM. BY 5% AND 4% SOLUTION OF PLANT EXTRACTS IN ALCOHOL (WT./VOL.).

Apocynaceae

Vinca rosea L. ("sada bahar"), B2, C3, A7, C9, C10, C12, A16, B21, B23.

Aristolochiaceae

Aristolochia bracteata Retz (Kirmari), B1, A2, B3, A5, B6, B7, B8, B9, C10, C11, B12, C13, A14, C15, C18, B19, B20, C21, C22, C23.

Boraginaceae

Cordia rothii Rom & Schultz (Gundni) shoot, C3, B4, C6, C9, C10, C16, B19, B21, B22.
Heliotropium ophioglossum stocks, C2, C3, C6, C10, B11, C12, C15, A16, B17, C20, B21.
H. ramosisismum Sieber B2, A3, C4, B9, C10, C11, B12, C13, C15, B16, C19, C20, B22.
H. strigosum Willd, B11, C12, B15, C16, C17, B18, C19, C22.
Trichodesma amplexicaule Roth, A3, B5, C7, C8, B10, B15, B16, B18, B19, B20, C23.

Caesalpiniaceae

Cassia angustifolia Vahl, 9C, 10C.

Chenopodiaceae

Chenopodium album L. ("bathua"), C1, C4, C5, C6, C7, B10.
Salsola baryosma (R & S) Dandy ("lanan"), C3, A4, C5, C6, C7, B12, C13, B14, C15, B16, C17, C18, B19, B20, C21, C22, C23.

Compositae

Blumea balsamifera DC ("kakronda"), C8, C9, B10, C11, C13, C14, C15, B16, B17, B18, B19, C23.
Vernonia cinerascens (Schultz) Bip., A1, B2, B3, A4, A6, B7, A8, A9, A10, B11, A12, B13, C14, B15, C19, A20, B21, B22, B23.

Euphorbiacea

Euphorbia hirta ("dodhi") Forsk, C2, C3, C5, C6, C9, B19, C20.
E. prostrata Ait., C2, C3, C5, C9, C11, C12, B15, B17, B19, B20, C21, C23.
E. pulcherrima Willd., B5, B6, C7, C14, C20, C22.

Malvaceae

Abutilon fruiticosum Guill, C2, A8, B9, B10, C11.
A. glaucum Cav. (Swt.)., C2, C6, C7, C8, C10.
A. indicum Linn. B1, C2, B3, B4, A5, A6, A8, B9, B10, A11, A12, B13, A14, A15, A16, A17, A18, A19, A20, A21, A22, A23.
A. pakistanicum Retz., C2, C5, C6, C7, C8, C9, B10, C11, B16, C20.

Mimosaceae

Mimosa hamata Willd., B3, C4, C5, C6, B7, C8, C9, C10, B11, B12, A13, B14, B15, B16, A17, A18, A19, B20, B21, B22, B23.
Prosopis glandulosa Torr, leaves, A1, B2, C3, B5, B6, B7, B8, B9, B10, B11, B12, A13, B14, A15, B16, B17, B18, B19, A20, B21, B22, B23. inflorescence A1, C2, C3, C5, B6, B11, C12, C13, C14, C18, C22, C23.
Stem bark. B1, C3, B5, B6, A8, C9, C10, B12, B13, A14, B15, B17, B19, A20, B21, B22, B23. dried leg. A1, B2, B3, B6, C9, B11, B12, B13, A14, B15, B16, A17, B19, B20, B21, B22, B23.

Myrtaceae

Psidium guajava L., C1, B2, B3, A5, B6, B8, A11, B12, B13, B14, B15, B16, B17, B18, B19, B20, B21, B22, B23.

Punicaceae

Punica granatum L., roots, C3, C6, C9, (Anar-Ke-Per)

B10, B11, B12, A13, B14, B18, B22, A23.

st. bark C4, C7, B11, B12, B13, B14, B17, B18, B19, B22, B23.

Fr. peel B3, B6, B9, B10, C11, C13, B14, B15, B18, C19, C20, C21, C22, C23.

Rhamnaceae

Ziziphus nummularia ("jhar") Wt. & Arn. (Burm. f.), C2, C3, C5, C7, C9, B10, B12, B14, C19, C22.

Rutaceae

Aegle marmelos ("bel"). Correa. fruit peel, C1, C3, B5, C6, B7, C8, C11, C12, C13, C14, C17, C18, C20, C21, C22, C23.

Averrhoa carambola ("qamrakh") Fr., C3, B5, C7, C9, C10, B12, C13, B14, B15, C16, C18, B20, B21, C22.

Solanaceae

Lycopersicon esculantum (tomato), fruit, C1, C3, C6, C9, C11, B12, C13, C14, C15, B18, C19, B21.

Zones of inhibition of growth showed by undiluted *Savlon* (0.3% chlorohexidine + cetrimide) against test micro-orgnisms, 1C, 2C, 3C, 4B, 5B, 6B, 8B, 9B, 10B, 11B, 12B, 13B, 14B, 15A, 16B, 17B, 18B, 22C.

Zones of inhibition of growth showed by 2% solution of *Jadit* (10% 4-chloro-2-hydroxy benzoic acid *N*-butylamide) in water and 2% in alcohol (vol./vol.):

C1, C2, C3, B4, C5, B6, C7, C8, C9, C10.

Details of the topographical figures used in the Table are as follows :

Activities of plants have been categorized according to the dia. of inhibition zones shown by extracts. Inhibition zones with an average dia of 5.0 to 8.5 cm. are represented by **A.**

Inhibition zones with an average dia of 3.0 to 4.9 cm. are represented by **B.**

Inhibition zones with an average dia of 1.5 to 2.9 cm. are represented by C.

Numbers 1-23 represented the names of organisms as follows:

1. 1. *Sporotrichum schenckii*, 2. *Trichophyton gourvilli*, 3. *T. rubrum*, 4. *T. schoenleinii*, 5. *Microsporum audouni*, 6. *M. gypseum*, 7. *M. vanbruchighemi*, 8. *Epidermophyton floccosum*, 9. *Candida albicans*, 10. *C. tropicalis*, 11. *Salmonella typhosa*, 12. *S. para A*, 13. *S. para B*, 14. *Shigella dysenteriae*, 15. *Escherichia coli*, 16. *Klebsiella pneumoniae*, 17. *Streptococcus faecalis*, 18. *S. pyogenes*, 19. *Vibrio cholera ELTor*, 20. *V.C. inaba*, 21. *Staphylococcus aureus*, 22. *Diplococcus pneumonie*, and 23. *Corynebacterium diphtheriae*.

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