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EFFICACY OF *AZADIRACHTA INDICA* AND *COLEUS AROMATICUS* AGAINST PLANT PATHOGENIC BACTERIA AND FUNGI

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A B S T R A C T

Objectives: To test the efficacy of *Azadirachta indica and Coleus aromaticus* and to compare them with antibiotics, *viz* Chloramphenicol and Ketoconazole against Plant pathogens.

Methods: Methanol extracts from two medicinal plants, *Azadirachta indica and Coleus aromaticus* at 200mg/liter were studied for its biocidal effects on the plant *pathogenic* bacteria such as *Cellulomonas cellulose, Proteus mirabilis and Vibrio vulnificus* and plant pathogenic fungi such as *Aspergillus niger, Rhizopus solonifer, and Mucor circinelloides.* Antimicrobial test was carried out by disc diffusion method. Antibacterial antibiotic, Chloramphenicol at 200mg /liter and antifungal agents, namely ketoconazole at 200 mg/liter were used in the culture susceptibility tests of the identified bacteria and fungi, respectively.

Results: Response varied with plant species and antibiotics. *Proteus mirabilis and Vibrio vulnificus* were resistant to Medicinal plants. Even though effectiveness of the crude extracts of two different Medicinal plants is significantly high when compared to antibiotics. *Cellulomonas cellulose* and all the fungal species tested showed effective inhibition by the plant extracts used.

Conclusion: Further studies are required to investigate the bioactive compounds responsible for the activity of plants against pathogens by chromatographic and other advanced methods. It therefore suggests that constituents of the plant extracts could serve as a good source in agricultural field also.

INTRODUCTION

The increasing incidence of antibiotic resistance among bacterial pathogens necessitates medicinal plants as an alternate therapy in restricting the resistant infectious organisms. For many years the Government of India had, through its Indigenous Drugs Committee, given desultory and rather ineffective attention to the origin and use of indigenous drugs, but it required the stimulus of the war and the consequent scarcity of drugs up to then imported-many, if not most, of which could be made in India from plants grown in that country-to induce an attempt to give more sustained study to the subject of drug production.(1)

The versatility of the Neem tree *Azadirachta indica* A. Juss. is reviewed. This species, native to India, grows in nutrient-poor soils in arid habitats and has tremendous potential for human use. Various derivatives of the tree have potential use in toiletries, pharmaceuticals, the manufacture of agricultural implements and furniture, cattle and poultry feeds, nitrification of soils for various agricultural crops, and pest control. Since Neem is a natural renewal resource producing extensive useful biomass, its propagation and economic exploitation will be beneficial, particularly to the Third World. In recent years, some useful commercial products have been developed from *A. indica*, and mere is considerable scope for future product development. Potentially profitable lines of research on this plant species are suggested.(2)

The word *Coleus* come from the Greek"koleus', meaning sheath. It is believed that there are 150 species of Coleus. Coleus aromaticus Benth. (Fam. Lamiaceae), syn. *Coleus amboinicus Lour. Spreng or Plectranthus ambonicus Lour*, is commonly known as Indian/ country borage and 'Pathorchur' in Hindi and Bengali It is recorded in the Indian system of medicine as one of the sources of Pashanabheda. *Coleus aromaticus* is used for seasoning meat dishes and in food products, while a decoction of its leaves is administered in cases of chronic cough and asthma (Anonymous, 1992). It is considered to be an antispasmodic, stimulant and stomachic and is used for the treatment of headache, fever, epilepsy and dyspepsia. It is used to treat conditions such as indigestion, diarrhea,

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nervous tension, insect bites, toothache, earache, rheumatism, whooping cough, and bronchitis The plant also finds prominent importance in modern medicine .(3) In this present study we have tested the bioactivity of *Azadirachta indica and Coleus aromaticus* against the plant pathogens damaging the agricultural plants an crops.

MATERIALS AND METHODS

Plant collection and pre-extraction preparation

The medicinal plants *Azadirachta indica and Coleus aromaticus* were chosen from agricultural farm of Annamalai University. The leaves, were collected, shade dried for 7 days, powderd using pestle and wooden mortar.

Extraction procedure

100 g of the ground powder of leaf part of *Azadirachta indica* were soaked separately in 250 ml of methanol contained in a 1litre capacity flask for five days. The samples were then strained to remove solids. The solutions were again filtered using Whatman's filter paper No.1 to obtain a solution free of solids. *Coleus aromaticu*s was extracted in a similar way as *A.indica*.

Preparation of bacteria

The bacteria used were *Cellulomonas cellulose, Proteus mirabilis and Vibrio vulnificus.* Isolation and identification of the organisms were done following standard procedures in handling clinical specimens (4). The organisms were maintained on nutrient agar slants at 2 - 8°C. Purity of the organisms was checked at regular intervals by plating and staining (5). The bacterial cultures were standardized using the method of (6). The test organisms were suspended into sterile universal bottles containing nutrient broth and normal saline added gradually to it so as to compare the culture turbidity to that of Mc Farland standard, which corresponded to approximately1.0 x 107cells/ml

Preparation of fungi

The fungal plant pathogen such as *Aspergillus niger, Rhizopus solonifer, Mucor circinelloides*
were used for this study. Fungal culture were isolated from plant samples and identified by microscopic observations. The test organisms were maintained under refrigerated condition.

ANTIMICROBIAL SCREENING

Agar disc diffusion method

This method (Kirby Bauer et al, 1966) is suitable for organism that grows rapidly over night at 35-37°C. The filter paper discs of 0.5 mm in diameter (Whatman paper No. I) was impregnated with the plant extracts of (200 ppm) concentration were placed on the petriplates overlaid with the culture. Impregnated disc absorbs moisture from the agar and antibiotic diffuses in to the agar medium. Zone of inhibition of bacterial growth and fungal growth around each disc was measured and the susceptibility was determined

Medium

3.8g of Muller Hinton Agar was added to 100 ml distilled water and autoclaved at 121° C for 15 minutes at 15 lbs and poured in sterile Petri plates up to a uniform thickness of approximately 4mm and the agar is allowed to set at ambient temperature and used.

Inoculum preparation of bacteria

The microorganisms were inoculated in peptone medium and incubated at 37° C for 3-4 hours and this was used as inoculums. A sterile cotton swab was inserted into the bacterial suspension and then rotated and compressed against the wall of the test tube so as to expel the excess fluid. The culture was aseptically swabbed on the surface of Muller Hinton Agar plates .To ensure that the growth is uniform and confluent (or semi confluent) the swab is passed three times over the entire surface, by repeating the procedure, taking care the second and third time to turn the plate through 60°. Disc impregnated with methanol extract at 200ppm concentration (200mg/l) was then placed on the inoculated agar surface using sterile forceps.

Method for fungi

The prepared fungal cultures were inoculated in the sterilized Muller Hinton agar in a flask. The agar was poured in the petriplates and the filter paper disc impregnated with the methanol extract of two different plants at (200 ppm) concentration were placed on the petriplates and the plates were incubated at 28°C.

Antibiotic sensitivity testing

The test microorganisms were also tested for their sensitivity against the antibiotics of Chloramphenicol for bacteria and Ketoconazole for fungi by the disc diffusion method. The culture was enriched in sterile nutrient broth for 24 hours at 37c using a sterile cotton swabs. The culture was aseptically swabbed on the surface of Muller Hinton Agar plates. Using an ethanol dipped and flamed forceps the antibiotic disc was aseptically placed over the seeded Muller Hinton Agar plates. The plated were incubated at 37 °c for 24 hours and the diameter of the inhibition zones was measured in mm. the fungal plates were incubated at 28°C for 48-72 hours and the diameter of the inhibition zones was measured in mm.

RESULTS AND DISCUSSION

The inhibition zone was measured and observed for plant pathogens. The study focused on the comparison of *Azadirachta indica and Coleus aromaticus*. The comparison is based on their effectiveness. Both medicinal plants were differing in their effects. When compared to *Coleus aromaticus, Azadirachta indica* showed better results. The tested leaf extracts of plant showed potential antimicrobial activity against plant pathogenic Microbes. Methanol leaf extract of *Azadirachta indica and Coleus aromaticus* showed significant activity by disc diffusion method.

Azadirachta indica showed the highest antibacterial activity of 15mm against *Cellulomonas cellulase* and *Coleus aromaticus* showed 14mm. there was no activity for *Vibrio* in case of *Azadirachta indica* whereas *Coleus aromaticus* showed 6mm of inhibition. The above said activity was vice versa in case of *Proteus mirabilis.*

Azadirachta indica showed the highest antifungal activity of 11mm in *Aspergillus niger.* Antifungal activity of *Coleus aromaticus* was low when compared to *Azadirachta indica. Highest activity of* 13mm was recorded for *Mucor circinelloides* followed by *Rhizopus solonifer* (11) mm and for *Coleus aromaticus* its was comparatively low.(Table 2)

Table 1 Activity of *Azadirachta indica and Coleus aromaticus* against plant pathogenic bacteria

Inhibition Zone in Diameters (mm)

Plant pathogenic bacteria	*Azadirachta indica* Methanol extract (200ppm)	*Coleus aromaticus* Methanol extract (200ppm)
Cellulomonas cellulase	15mm	14mm
Vibrio vulnificus	No zone	6mm
Proteus mirabilis	6mm	No zone

Table 2 Activity of *Azadirachta indica and Coleus aromaticus* against plant pathogenic fungi

Plant pathogenic fungi	*Azadirachta indica* Methanol extract (200ppm)	*Coleus aromaticus* Methanol extract (200ppm)
Aspergillus niger\	11mm	No zone
Mucor circinelloides	13mm	11mm
Rhizopus solonifer	11mm	11mm

Table 3 Antibiotic sensitivity testing

Inhibition Zone in Diameters (mm)
Culture susceptibility test of the identified bacterial plant pathogens to Ketoconazole

Plant pathogenic bacteria	Chloramphenicol (200mg/l)
Cellulomonas cellulase	12mm
Vibrio vulnificus	No zone
Proteus mirabilis	No zone

Table 4 Culture susceptibility test of the identified fungal plant pathogens to Ketoconazole

Plant pathogenic fungi	Ketaconozole(200mg/l)
Aspergillus niger	7mm
Mucor circinelloides	14mm
Rhizopus solonifer	12mm

Chloramphenicol, an antibiotic against bacteria was used in this study which showed lowest zone of inhibition when compared with the two medicinal plants. *Cellulomonas cellulase* was recorded as 12mm in diameter whereas *Proteus mirabilis* and *Vibrio vulnificus* were highly resistant. (Table 3)

In case of fungi Ketaconozole was used as antifungal agent. *Aspergillus niger* (7mm) was considered as resistant as per the standard chart. Whereas *Mucor circinelloides* and *Rhizopus solonifer* was sensitive to Ketaconozole. (Table 4)

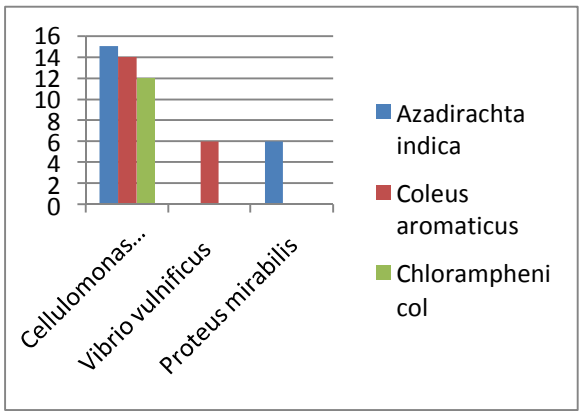
Azadirachta indica and *Coleus aromaticus* showed better results when compared to the antibacterial land antifungal agents used in this study. (Graph 1 and Graph 2)

Evidently (7) reported that hydro-alcohol extract of *Valeriana jatamansi, Coleus barbatus, Berberis aristata, Asparagus racemosus, Andrographis paniculata, Achyranthes aspera, Tinospora cordifolia, Plantago depressa* showed maximum antifungal activity against *Aspergillus niger and Candida albicans.* The antifungal activity of *Senna alata linn.* Crude leaf extract exhibited moderate activity against *Microsporum canis*, *Trichophyton jirrucosum, Trichophyton mentagrophytes* and *Epidermophyton jlorrcosum*

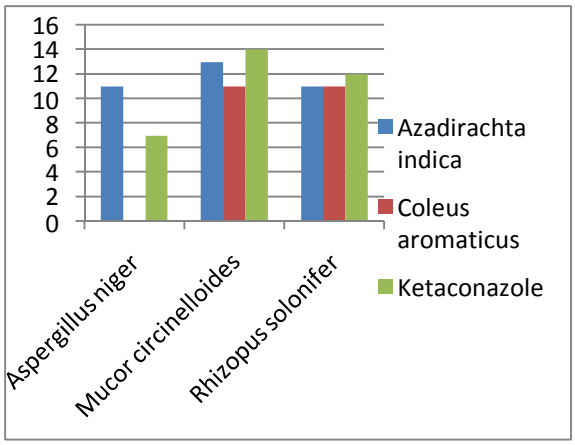
Works (9) were done on the antifungal potential of aqueous and ethanol extracts of eight different plants species done in both *invitro* and *invivo* against *Colletotrichum kahawae* in completely randomized design with three replication. Among all the eight plants extract the chloroform and ethanol extract of *Vitex negundo* exhibited maximum antifungal activity (25 and 25 mm) compared with other plant extract. The ethanol extract of *Calotropis gigantea* (24 mm) and *Centella asiatica* (25 mm) showed prominent (10) antifungal activity against.

Cyrtomium falcatum Coleus aromaticus leaf showed the highest antimicrobial activity against *Shigella* sp., *Salmonella typhi and E.coli.* For instance, methanol crude extracts of *A.ferore and W.somnifera* exhibited inhibitory activity against all the strains of *Neisseria gonorrhoea* (11). On the contrary (12) observation the aqueous extract of *Leucas aspera* along with the eight plants against eleven human pathogenic bacteria showed least antibacterial activity. Chloramphenicol is a broad-spectrum bacteriostatic agent that inhibits protein synthesis and is usually effective against a wide range of gram negative and gram positive bacteria (13). Ketoconazole is a

systemic antifungal agent that interferes with the synthesis of fungal cell membranes as well as certain enzymes' activities (14). Although reports on phytotoxic effects of ketoconazole are scanty, the antifungal agent has been reported to suppress larval development in mussel in vitro culture (15). It is known that many plant pathogenic bacteria have acquired resistance to synthetic pesticides (16). For instance, pathovars of *Xanthomonas campestris* have developed resistance to some antibiotic such as kanamycin, ampicillin, penicillin and streptomycin (17,18,19,20).



Graph 1 Comparison of antibacterial agent with *Azadirachta indica and Coleus aromaticus*



Graph 2 Comparison of antifungal agent with *Azadirachta indica and Coleus aromaticus*

CONCLUSION

Economic losses arising from crop diseases caused by phytopathogenic bacteria are principally associated with yield reductions. However, crop quality and safety may also be adversely affected, undermining both consumer confidence and profitability to the producer. Hence protection of plants from agriculture pest and pathogens is the preoccupation of agricultural scientist around the world and it is the unifying goal of plant pathology to control plant disease, and chemicals play a major role in accomplishing that goal in contemporary agricultural production.

Reference

1) Kirtikar, K. R.; basu, b. D. Indian medicinal plants. pg. 72 pp. Record Number19202900465, 1918
2) Opender Koul, Murray B. Isman, C. M. Ketkar Properties and uses of neem,*Azadirachta indica Canadian Journal of Botany*, 1990, 68(1): 1-11, 10.
3) Om Prakash Rout, Rabinarayan Acharya, Sagar Kumar Mishra Rashmibala Sahoo pathorchur (coleus aromaticus): a review of the medicinal evidence for its phytochemistry and pharmacology properties. International Journal of Applied Biology and Pharmaceutical Technology 2012; (3) issue 4.
4) Cheessbrough M. Biochemical Tests to Identify Bacteria. In: Laboratory Practice in Tropical Countries .Cambridge Edition. 2002; Pp. 63-70.
5) Acheampong YB, El-Mahmood AM, Olurinola PF . The Antibacterial properties of the liquid antiseptic TCP. Indian J. Pharm. Sci.1988; (3):183-186.
6) Baker CN, Thornsberg CH. Inoculum standardization in antimicrobial susceptibility tests: Evaluation of overnight age culture. J. Chin. Microbial.1983; (17):140-457.
7) Mathur A, Singh R, Yousuf S, Bhardwaj A, Verma S, Babu P, Gupta V, Prasad GBKS, Dua VK, Advances in Applied Science Research, 2011, 2, 260-264.
8) Sule WF, Okonko IO, Joseph TA, Ojezele MO, Nwanze JC, Alli JA, Adewale OG, Ojezele OJ, Advances in Applied Science Research, 2010, 2, 14-2
9) Abera A, Lemessa F, Muleta D, International journals of agricultural research, 2011, 3, 268-279.
10) L. Prince and P. Prabakaran., Antifungal activity of medicinal plants against plant pathogenic fungus *Colletotrichum falcatum* PG and Research Department of Microbiology, Prist University (East Campus),Thanjavur, Tamilnadu (India). Asian J. Plant Sci. Res 2011, 1 (1):84-87
11) Kambizi, L. and A.J. Afolayan.. Extracts from *Aloe ferox* and *Withania somnifera* inhibits *Candida albicans* and *Neisseria gonorrhoea*. *African J.biotechnol.*, 2008; 7: 12- 15
12) Mohana, D.C., S. Satish. and K.A. Raveesha. Antibacterial evaluation of Some Plant Extracts against some human pathogenic bacteria. *Advances in Biological Research,* 2008; 2(3-4): 49-55
13) Gholamreza, A., Salehi, H. and Khosh-Khui, M. Nano silver: a nanomaterial for removal of bacterial contaminants in valerian (*Valeriana officinalis L.*) tissue culture. Acta Physiolog Plant 2008; 30: 709 -714.
14) Shepp, DH., Klosterman, A., Stegel, MS. and Meyers, JA. Comparative trial of ketoconazole and nystatin for prevention of fungal infection in neutropenic patients treated in a protective

environment. The Journal of Infectious Diseases 1985; 152 (6): 1257 – 1260.
15) Owen, CT., Alexander, JE. and McGregor, M. Control of microbial contamination during in vitro culture of larval unionid mussels. Invertebrate Reproduction and Development 2010; 54 (4): 187 – 193.
16) White, D.G., Zhao, S., Simjee, S., Wagner, D.D. and McDermott, P.F. Antimicrobial resistance of food-borne pathogens. Microbes and Infections 2002; 4:405-412.
17) Cooksey, D.A. Characterization of a copper resistance plasmid conserved in copperresistant strains of Pseudomonas syringae of tomato. Applied and Environmental Microbiology1987; 53: 454-456
18) Bender, C.L., Malvick, D.K., Conway, K.E., George, S. and Pratt, P. Characterization of pXV10A, a copper resistance plasmid in *Xanthomonas campestris pv. vesicatoria.* Applied and Environmental Microbiology 1990;56: 170-175.
19) Rodriguez, H., Aguilar, L. and Lao, M. Variations in Xanthan production by antibiotic resistant mutants of *Xanthomonas campestris.* Applied Microbiology and Biotechnology, 1997 ;48: 626-629.
20) McManus1, P. S., Stockwell, V. O., Sundin, G. W. and Jones, A. L. Antibiotic use in plant agriculture. Annual reviews of phytopathology 2003; 40:443–65

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