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Antibacterial properties of extracts of Indian medicinal plants: *Syzygium alternifolium, Phyllanthus niruri* and *Rubia cordifolia*

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ABSTRACT

The antibacteria properties of Indian medicinal plant extracts of syzygium alternifolium, phyllanthus niruri and rubia cord folia against six bacteria strains (Escherichia coli, bacillus subtilis, Staphylococcus aureus, Klebsiella Pneumonia, Pseudomonas aeruginosa and proteus vulgaris have been studies, Chloroform, ethanol and aqueous extracts were prepared sequentially from leaves fo Syzygium, phyllanthus and Rubia plants, it was observed that methanol and ethanolic extracts fo Synygium have significant activity against gram positive bacteria. Klebsiella pneumonia and proteus vulgaris were more sensitive to the extracts of phyllanthus niruri and Rubia cordifolia respectively.

Key words : Syzygium alternifolium, phyllanthus niruri and rubia cord folia ethanol gram positive and negative bacteria.

INTRODUCTION

Medicinal plants play an important role in health care. The demand for medicinal plants in health care is about 70-80% Growing recognition of medicinal plants is due to several factors like cultural acceptability, accessibility, affordability and ability to meet psychological needs[2]. the traditional use of low profile and less known medicinal plants should be documented to disseminate the therapeutic efficacy to pave the way for preparation of acceptable medicine and to reduce the pressure on over exploited species[3]. WHO recognized that medicinal plants played an important role in the healthcare of about 80% of the world population in developing countries and depend largely on traditional medicine.

Syzygium alternifolium is an endemic aromatic tree distributed in Assam and Andhra Pradesh states of India. It is locally called as mogi/ move. Plant parts were used to cure various diseases viz tender shoots for dysentery, seeds for diabetes and stem bark was used for gastric ulcers. Several Syzygium species were reported to posses antibacterial. Antifungal and anti-inflammatory activities. Syzygium alternifolium was reported to posses hypoglycemic and anti hyperglycemic activity.

Rubia cordifolia is a climbing plant found in the Himalayas and hill stations in India used as ayurvedic herb. The best herb for blood purification, blood circulation controls bleeding, mends broken bones, amenorrhea, cancer, cleans and regulates liver, spleen, pancreas, and kidneys, diarrhea, dysentery, dysmenorrheal, edema, destroys, kidney and gal stones, heart disease, hepatitis, herpes, jaundice, menopause, menorrhagia, painful menstruation, post partum uterus stimulation paralysis, skin problems tissue healing, traumatic injuries, skeletal disease kapha disorders, joint pain rheumatoid arthritis, improves complexion and voice, helps destroy benign and malignant tumors [11,12,13], Phyllanthus amarus is herb common to central and southern India and can grow to 30 – 60 cm in height. All parts of plant are employed therapeutically. Antibacterial activities and preliminary phytochemcial screening of phyllanthus was reported by Akinjobi et al[14].

The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes Numerous studies have identified compounds within herbal plants that are effective antibiotics. Traditional healing systems around the world that utilize herbal remedies are an important source for the discovery of new atibiotics. Some traditional remedies have already produced compounds that are effective against abtibiotic resistant strains of bacteria. The result of this indicate the need for further research into traditional health systems. It also facilities pharmacological studies leading to synthesis of amore potent drug with reduced toxicity. The need of the hour is to screen a number of medicinal plants for promising biological activity.

Plants used for traditional medicine contain a wide range f substance that can be used to treat chronic as well as infectious disease. A vast knowledge of how to use plants against different illness may be expected to have accumulated in areas where the use of plants is still of great importance[15]. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds are alkaloids, flavonoids tannisns and phenolic compounds[16].

MATERIAL AND METHODS

Collection of plant materials and preparation of extracts

Plants were collected in Tirumala hills of Chittoor dist. A.P. The leaves were washed and air dried at room temperature. The chloroform, ethanaol and aqueous extracts were prepared sequentially in soxhlet extractor using 30gms of dried plant tissue mixed with 150 ml of respective solvents (100%v/v) for 24 hours[17 18].Chloroform and ethanol extracts were evaporated to dryness in rotary evaporator, where as aqueous extracts are lyophilized[19].25 mg of dry weight of each crude extract was further reconstituted in 2.5ml of distilled water and 1:15 dilutions of all these extracts were used for further studies.

Antimicrobial screening

The chloroform and ethanolic extracts fo plants were screened against six bacterial strains. The test organisms were *Bacillus subtilis* (ATCC 441). *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Kelbsiella pneumonia* (ATCC 15380) *Pseudomonas aeruginosa* (ATCC 27853), and *Proteus vulgaris* (MTCC 1771) obtained from IMTECH, Chandigarh.

Preparation of inoculum

Stock cultures were maintained at 4°C on slants of nutrient agar. Active cultures for experiments were prepared by transferring a loop full of culture from the stock cultures to test tubes of Muller – Hinton Broth (MHB) for bacteria that were incubated without agitation for 24hours at 37°C The cultures were diluted with fresh Mueller – Hinton Broth to achieve optical densities corresponding to 2.0x106 colony forming units (CFU/ml) for bacteria.

Antimicrobial susceptibility test

The disc diffusion method Bauer et al[20].was used to screen the antimicrobial activity. Invitro antimicrobial activity was screened by Mueller – Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 20ml of molten media into sterile Petri plates. The plates were allowed to solidify for 10 minutes and 0.1%inoculum suspension was swabbed uniformly and the inoculums was allowed to dry for 5 minutes. The different concentrations of extracts were loaded on 6mm sterile discs. The loaded discs were placed on the surface of the medium and the compounds were allowed to diffuse for 5 minutes and the plates were kept for incubation at 37°C for 24hours. At the end of incubation, inhibition zones were formed around the discs were measured with transparent ruler in millimeters. These studies were performed in triplicate.

RESULTS AND DISCUSSION

The results of the antibacterial properties of extracts on the microorganisms are tabulated as follows

Isolation of chemical compounds from plant material is largely dependent on the type of

solvent used in extraction procedure. In this assay the extracts were prepared using sterile distilled water and solvents like chloroform and Ethanol. We found in this study that none of the aqueous produced any zones of inhibition. The ethanolic and chloroform extracts were found to show consistent antimicrobial activity in comparison to the aqueous extracts. This might have resulted from the lack of solubility of active constituent in aqueous solutions while the extracts showed increased solubility in solvent like chloroform and ethanol.

The tested plant extracts were most active against gram positive microorganisms than gram

Table 1: Sensitivity Pattern of Syzygium alternifolium on test organisms

Organism	Type of Organism	Concentration of extract (in µl) Zone of Inhibition (in mm)			
		20	30	80	110
Bacillus Subtilis	Gram positive	2.7	3.0	3.2	3.5
Pseudomonas aeruginosa	Gram positive	2.1	2.4	2.8	3.1
Staphylococcus aureus	Gram positive	2.4	2.9	3.2	3.7
Proteus vulgaris	Gram positive	1.7	2.1	2.3	2.7
Klebsiella pneumonia	Gram positive	1.3	1.6	2	2.2
Escherichia coli	Gram positive	1.8	2	2.5	2.7

Table 2: Sensitivity Pattern of Phyllanthus niruri on test organisms

Organism	Type of Organism	Concentration of extract (in µl) Zone of Inhibition (in mm)			
		20	30	80	110
Bacillus subtilis	Gram positive	0.6	0.8	1.1	1.4
Pseudomonas aeruginosa	Gram positive	0.5	0.7	1	1.2
Staphylococcus aureus	Gram positive	0.6	0.9	1.3	1.8
Proteus vulgaris	Gram positive	0.5	0.9	1.1	1.3
Klebsiella pneumonia	Gram positive	1.2	1.5	1.7	2.6
Staphylococcus aureus	Gram positive	1	1.4	1.8	2
Escherichia coli	Gram positive	0.7	1	1.6	1.8

Table 3: Sensitivity Pattern of Rubia cordifolia on test organisms

Organism	Type of Organism	Concentration of extract (in µl) Zone of Inhibition (in mm)			
		20	30	80	110
Bacillus subtilis	Gram positive	0.5	0.7	1.5	1.6
Pseudomonas aeruginosa	Gram positive	0.6	1	1.1	1.2
Proteus vulgaris	Gram positive	1.3	1.4	1.9	2.8
Klebsiella pneumonia	Gram positive	0.4	0.6	1.4	1.7
Staphylococcus aureus	Gram positive	1.1	1.3	1.5	1.7
Escherichia coli	Gram positive	1.0	1.2	1.9	2.4

negative microorganisms. This is agreement with the previous reports by several workers. [21 22 23 24 25 26].The antimicrobial assay was carried out on gram positive bacteria i.e. *Bacillus substilis, Staphylococcus aureus* and gram negative bacteria i.e. Escherichia coli, Proteus vulgaris, Klebsiella pneumonia, Pseudomonas aeruginosa.

Bacillus subtilis and *Staphylococcus aureus* were found to be susceptible to ethanolic extracts of Syzygium alternifolium. It was found that methanolic extracts of syzygium inhibited the growth.

Rajakaruan et. al[27]. reported that Syzygium cumini showed good activity against Bacillus subtilis and Staphylococcus aureus. The essential oils from the leaves of Syzygium cumini were most active against *Escherichia coli, Proteus vulgaris, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Staphylococcus aureus*[28].

These difference in the bacterial susceptibility to different extracts may be attributed to the fact that cell wall in gram positive bacteria are of single layer whereas gram negative cell wall is multilayered. Alternatively the passage of the active compound through the gram negative cell wall may be inhibited. It is thought that observed difference may result from the doses used in this study. In addition microorganism show variable sensitivity to chemical substances related to different resistance levels between strains. The microorganism susceptibility to difference extracts did not correlate with the susceptibility or resistance to a particular antibiotic within same species. Further the result obtained by this method may vary as many factors such as microbial growth, exposure of microorganisms to different chemical substances and the quantity of the substance.

The factors responsible for this high susceptibility of gram positive and negative organisms to the extracts are not exactly known but may be attributed to the presence of secondary metabolites[29].

It is worthy to note that the antimicrobial activities of these plants extracts were dependent on the concentration of the extracts as reported by Ekwenye et al[30].also, if the extract has high molecular weight the rate of diffusion is always slow, reduced and also takes longer time, whereas an extract of low molecular weight diffuse faster and at a quicker rate.

The antimicrobial properties exhibited by the extracts may be associated with the presence of tannins, saponins, cardiac glycosides and alkaloids found in the plant extracts. A large number of flavonoids have been reported to posses antimicrobial properties[31,32]. Tsuchiya et al[33]. attributed the antimicrobial activities of flavonoids to their ability to complex with extra cellular and soluble proteins as well as their ability to complex with bacterial cell walls. They suggested that more lipophylic flavonoids exert antimicrobial activity by disrupting microbial cells membranes.

CONCLUSION

In conclusion the negative result does not mean the absence of bioactive compounds nor is the plant inactive. Active compounds may be present in insufficient quantities in the crude extracts to show activity with the dose levels employed. Lack of activity can thus only be proven using higher doses. Alternatively, if the active principle is present in high enough quantities there could be other constituents exerting antagonistic effects or negating the positive effects of bioactive agents.

REFERENCES

1. Fransworth, N.R. Akerele O. Bingel, A.S. Soejarto D.D. Guo, Z.G. “Medicinal plants in therapy” *Bull. World health organization* **63**: 965-981(1985)
2. Kala, C.P.*Ethnobot* Res App. **3:**267(2005).
3. Kala, C.P. *et al. J Ethnobiol Ethnomed* :**2**:32(2006).
4. Bakshu, L,M.D. Ethanomedicobotanical and phytochemical evaluation of certain rare, endemic and endangered medicinal plants

fro eastern ghats, Andhra Pradesh India, Thesis S.K.Univeristy Anantapur (2002.)

5. Shafi, P.M., Rosamma, M.K. Jakil, K., Reddy, P.S. Antibacterial activity of *Syzygium cumuni* and S.Travancoricum leaf essential oils, Fitoterapia, **73**: 414-416 (2002).
6. Corine, D.D. Michel, D., Quetin – Leclercq, J., "Antibacterial activity of bark extracts of *Syzygium jambos," Enthnopharmacololgy,* **71:** 307-313 (2000).
7. Tsakala, T.M.Penge, O, John, K. "Screening of in vitro antibacterial activity from *Syzygium guineense* hydrosoluble dry extract" *Annals pharmaceutiques francaises*, **54:** 276-279 (1996)
8. Chandrasekaran, M., Venkatesalu, V,. "Aantibacterial and antifungal activity of *Syzygium Jambolanum* seeds". *J.Ethnopharm.,* **91:** 105-108 (2004).
9. Murunganandan, S, Srinivasan, K., Chandra, S.K. lal, J., Raviprakash V. "Anti inflammatory activity of *Syzygium* cumini bark". *Fitoterapia,* **72:** 369-375 (2001).
10. Rao, B.K, Rao, C.H. "Hypoglycemic and antihyperglycemic activity of *Syzygium alternifolium* seed extracts in normal and diabetic rats", *Phytomedicine,* **8:**88-93(2001).
11. Ramaiah, N, Thankamma, A. *Plants Med* : **3**: 104 (1982).
12. Tessier, A.M. *et al, Plant Med.* **39**: 194 (1980).
13. Itokawa, H. *et al. Chem, Pharin, Bull.* 2353-2358 (1983).
14. Akinjobi, C.O. Anyanuwu, B.N. Onyeze, G.O.C. and ubekwe, V.I. (2004) "Antibecterial Activities and preliminary phytochemica screening of four medicinal plants" .*J. Applied Sci*. **7:** 4328-4338 (2004).
15. Diallo, D, Hveem B, Mahmoud, M.A. betge G, Paulsen, B.S. Maiga, A, "An ethanobotanical survey of herbal drugs of Gourma district Mail, *"Pharmaceutical Bioloyg,* **37:** 80-91 (1999).
16. Edeoga, H.O. Okwu, D.E. Mbaebie, B.O. "Phytochemical constitutions of some Nigerian medicinal plants" *African J. Biotech* **4**:685-688(2005).
17. Adesegun, S.A. Fajana, A, Orabueze, C.I. Coker H.A.B. "Evaluation of antioxidant properties of phaulopsis fascisepala C.B.Cl (Acanthaceae)". *Evid. Based complement Med,.***6:** 227-231(2009).
18. Punitha, I.S.R Rajendra K, Shiwaikar, A, "Alcoholic stem extract of *Coscinium fenestratum* regulates carbohydrates metabolism and improves antioxidant status in streptozotocin nicotinamide induced diabetic rats". *Cam* **2:** 375-381 (2005).
19. Tafesse, G, Mekonne, Y, Makonnne, E. "Antifertility effect of aqueous and ethanol extracts of the leaves and roots of asparagus africanus in rats" *Afr, Health Sci,* **6:** 81-85 (2006).
20. Bauer, R.W, Kirby, M.D.K., Sherris, J.C. Turck, M. "Antibiotic susceptibility testing by standard single disc diffusion method" *Am. J. Clin, Pathol*, **45:** 493-496 (1996).
21. Buwa, L.V. Staden, J.V. "Antimicrobial and antifungal activity of traditional medicinal plants used against venereal disease in south Africa" *J.Ethno . Pharm,* **103**: 139-142 (2006).
22. Valsaraj, R, Pushpangadan, P, Smitt, U.W. Adsersen, A, Nyman, U." Antimicrobial screening of selected medicinal plants from India". *J.Ethno, Pharm*, **58:** 75-83 (1997).
23. Srinivasan, D, Nathan, S, Suresh T, Lakhsmana perumalsamy, P, "Antimicrobial activity of certain of Indian medicinal plants used in folkloric medicine", *J.Ethno. Pharm.* **74:** 217-220 (2001).
24. Samy, R.P. Ignacimuthu, S, "Antibacterial activity of some folklore medicinal plants used by tribals in Westeran Ghats in India". *J.Ethno pharm*, **69:** 63-71 (2000).
25. Perumalsamy, R, Ignacimuthu S, Raja, D.P. "Preliminary screening of ethanomedical plants from India". *J.Ethno. Pharm.* **66:**235-240(1999).
26. Parekh J. Jadeja, S, Chanda S, "Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity". *Turkish J.Biol*, **29:** 230-210 (2005).
27. Rajakaruan, N, harris, C.S. Towers, G.H.N. "Antimicrobial activity of plants from serpentine out crops in Sri Lanka". *Pharm Biol.* **40**: 235-244 (2002).
28. Saxena, V.K. sharma R.N. "Antimicrobial activity of the essential oil of *Taddalia asiatica". Fitoterapia,* **40:** 235-244 (2002).
29. Nweze, E.I., Okafor, J.I., Njoku, O. "Antimicrobial Activities of Methanolic

extracts of Trema guineesis (Schumm and Thorn) and Morinda lucida Benth used in Nigerian Herbal Medicinal Practice". *J. Biol. Res. Biotech*., **2**: 39-46 (2004).

30. Ekwenye, U.N., Elegalam, N.N. "Antibacterial activity of ginger (Zingiber officinale Roscoe) and garlic (Allium sativum L.) extracts on Escherichia coli and Salmonella typhi". *J. Mol. Med. Advan. Sci*., **1**: 411-416 (2005).
31. Olowosulu, A.K. and Ibrahim, Y.K.E. "Studies on the antimicrobial screening of Aqueous extracts of five plants used in Folk medicine in Nigeria". *West African J. boil. Sc*., **3**: 21-26 (2006).
32. Akujobi, C.O., Ogbulie, J.N. and Uchegbu, U.N. "Antibacterial activities and preliminary phytochemical screening of Vernonia amygdalina and Citrus aurantifolia". *Nigeria Journal of Microbiology* 20: 649-654 (2006).
33. Tsuchiya, H.M.S., M iyazaki, T., Fujiwara, S., Taniyaki, S., Ohyama, M., Tanaka, T. and Inuwa,M.: "Comparative study on the antibacterial activity of bacterial flavonoids against methacillin resistant Staphylococcus aureus". *J. Ethrophamacology.* **50**: 27-34 (1996).