A COMPARATIVE STUDY ON THE EFFECT OF PLANT EXTRACTS WITH THE ANTIBIOTICS ON ORGANISMS OF HOSPITAL ORIGIN

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ABSTRACT

Thirty five plants belonging to twenty families were studied for their antimicrobial activity. Among the plants tested, 43 % showed antimicrobial activity. Fifteen plants belonging to 10 families exhibited activity against gram positive bacteria and gram negative bacteria. Four plants namely *Azadirachta indica, Garadenia jasminoides, Magnifera indica,* and *Wrightia tinctora* showed an appreciable activity against the gram positive bacteria and seven plants against gram negative organisms. Leaf extract of *Tabermontana coronaria* showed a maximum zone of inhibition (24 mm) against *Staphylococcus aureus* and the leaf extract of *Sida cordifolia* showed a maximum zone (20 mm) against *Corynebacteriun diphtheriae*. *Mentha piperanta* gave a maximum zone size against *E,coli* (22 mm) and *Vibrio cholerae* (20mm). The inhibitory percentage of the leaf extracts against various pathogens were observed to be *Staphylococcus aureus* (40%), *E.coli* (28%), *Shigella* sp (25%), *Salmonella* sp (22%), *Pseudomonas aeruginosa* and *Bacillus subtilis* (20%), *Klebsiella pneumoniae* and *Proteus vulgaris* (17%), *Vibrio cholera* (14%) and *Corynebacterium diphtheriae* (11%). The results suggested that the leaf extracts of various plants has significant antibacterial activity against the tested microorganisms. The present study is done to compare the activity of the plant extracts with the activity of currently used antibiotics against the selected organisms.

INTRODUCTION

Scientific interest in medicinal plants has burgeoned due to an increased efficiency of new plant derived drugs, growing interest in natural products and rising concerns about the side effects of conventional medicine. The use of various herbal remedies and preparations are described throughout human history representing the origin of modern medicine. Many conventional drugs originates from plant sources. It is estimated that around 80% of the world population uses herbal medicines, mainly as self described products. Dorman *et al*., (2000) studied the antibacterial activity of some volatile oils like clove and pepper. Herbal medicine is also called botanical

medicine or phytomedicine, and is defined as the use of whole plant or parts of plants to prevent or treat illness. Herbal medicines appear relatively safe but there is limited human research or perspective data concerning adverse effect and herbal drug interaction. They are generally less potent than their pure drug relatives because they contain mixture of many chemicals in small quantities. Plants also have considerable potential for the production of biopharmaceutical proteins and peptides. As the demand for biopharmaceutical is expected to increase, it would be wise to ensure that they will be available in significantly larger amounts on a cost effective basis. Currently the cost of biopharmaceuticals limits their availability. Plant derived biopharmaceuticals are cheap to produce and store, easy to scale up for mass production and safer than that derived from animals and microorganisms. Use of various antibiotics may lead to development of drug resistance which is an extremely serious public health problem and much of the difficulty arises form drug misuse. Due to the various problems encountered with the handling of infections and treatment with antibiotics there is a usage for the antimicrobial compounds of natural effects. Many published reports show the effectiveness of traditional herbs against microbes and as a result plants are one of the bedrocks for modern medicine to attain new principles (Evans *et al*., 2002). Plants have been used empirically long before the concept and identification of etiological infectious agents had been developed. Indian Medicinal plants and their products are used to control diverse diseases such as bronchitis, pneumonia, ulcers, liver diseases and diarrhoea. Caffeic acid found in some common herbs **tarragon and thyme** is effective again like st bacteria (Branter *et al*., 1996). Hence more studies pertaining to the use of plants as therapeutic agents should be emphasized; especially those related to the control of antibiotic resistant microbes. The objective of this study is to evaluate and compare the potential of the crude leaf extract on selected pathogen against the standard antibiotics currently used.

MATERIAL AND METHODS

Sampling area

Fresh plants belonging to various families were collected from different localities in and around Coimbatore and Palghat districts.

Preparation of plant extracts

Fresh matured leaves (25 g) was collected. Leaves were washed with tap water and sterile distilled water respectively. The sterile leaves were minced to pieces and crushed using a mortar and pestle with 15 ml of sterile distilled water. The extract was then filtered using a Whatman No.1. filter paper. The extract was then transferred into a sterile container and stored at 4^{o} C until its use.

Innoculum

The test organisms were obtained from the patients admitted at PSG institute of Medical Sciences and Research. The three organisms selected for the study are the causative agents of diverse number of diseases. All the isolated organisms were identified and confirmed by gram's staining, culturing

on appropriate selective media and by performing various biochemical tests and sugar fermentation tests..

Media

Mueller Hinton Agar (pH – 7.4) was prepared for testing the antibacterial activity and for the comparison of the efficiency with the standard antibiotics.

Evaluation of antibacterial activity

Well diffusion method (Perez *et al*., 1990) was followed. Onto the prepared sterile Mueller Hinton agar plates, the selected broth cultures of different bacteria were seeded and incubated at 37^0 C for 18 h, to obtain a lawn culture. In each of these plates 2 wells of 8 mm in diameter was cut out using sterile well cutter. By using a micropipette, 75 l of the plant extract was added aseptically into each of the appropriate wells and allowed to diffuse at room temperature for 30 mins. These plates were then incubated in an upright position at 37^0 C for 18 h. The antibiotic activity was determined by measuring the diameter of the zone of inhibition in millimeters. The experiments were carried out in triplicates and the mean of the diameter of the zone of inhibition was calculated.

Comparison with the standard antibiotics

Ten commercially available current antibiotic discs were selected and tested the antibacterial efficiency against the isolated bacterial pathogens following disc diffusion method (Baurer *et al*., 1966). Broth culture of 18 h was swabbed onto the Mueller Hinton agar using a sterile cotton swabs. Using a sterile forceps, the antibiotic discs of appropriate concentrations were placed on the agar surfaces in such a way that no overlapping of the zones are got. The plates were than incubated at 37^0 C for 18 h in an inverted manner. After the incubation, the diameter of the zones of inhibition was measured. The results are subsequently tabulated and compared with the efficiency of the plant extracts used.

RESULTS AND DISCUSSION

Antibacterial activity of plant extract against gram positive bacteria

Out of the 35 plant extracts used for the stud, only 15 extracts (43 %) were found to have antibacterial effect against the selected gram positive bacteria. The plants that showed the antibacterial activity are *Achyranthus aspera, Caesalpinia pulcherrima, Calatropis gigantia, Carcia papaya, Citrus limon, Coreus ammonificans, Corriandum sativum, Emblica officinalis, Medntha piperita, Murraya roenigi, Psidium guajava, Rauwolfia serpentina, Sida cardifolia, Tabermortana coronaria and Vinca rosea.* (Table. 1).

Acalphya indica showed no activity against any of the three bacteria used. Whereas, *Achyranthes aspera* showed a minium zone of 13 mm against *Bacillus subtilis* similar to the effect of amikacin antibiotic. Also it gave a zone size of 15 mm against *Corynebacterium diphtheriae.* The leaf extracts of *Acalypha indica* showed acitivty against *E.coli, Pseudomonas aeruginosa* and *Salmonella citrus.* (Hiremath *et al*., 1993). *Corriandum sativum* gave no

zone against *Bacillus subtilis* and *Staphylococcus aureus*, but a zone size of 17 mm was found proving the susceptibility to *Corynebacterium diphtheriae* which is more effective than amikacin. *Emblica officinalis* gave an activity of 17 mm against *Bacillus subtilis* and 16 mm against *Corynebacterium diphtheriae* which is more effective than amikacin. No response to *Staphyloccus aureus.* Caumarins are phenolic substances made of fused benzene and L-pyrone rings. They are found invitro to inhibit *Candida albicans* (Piller.,1975).The extracts of *Allium sativum* and *Pithecellobium dulce* exhibited activity against the rice brown leaf spot pathogen *Drechslera oryzae*. (Raju, *et al.*, 2004).

Mentha piperata was found to have more efficiency on *Bacillus sp* giving 17 mm which is more sensitive to amikacin. Also showed high resistance by *Corynebacterium diphtheriae and Staphylococcus aureus.*. Leaf extracts of *Mentha piperata* showed inhibitory activity against *Bacillus subtilis*, *E.coli* and *Vibrio cholerae*. (Diaze *et al*., 1988). *Piper nigrum* was resisted by all the strains used for the study. *Psidium guajava* was susceptible only to C*orynebacterium diphtheriae* with 14 mm of zone size. It was resisted by *Bacillus subtilis* and *Staphylococcus aureus. Tabermontana coronaria* found to be the highly efficient extract showing 24 mm that controlled *Corynebacterium diphtheriae* even more than amikacin and piperacillin. It also produced a zone size of 17 mm against *Staphylococcus aureus* which equally effective like that of amikacin and chloramphenicol. Osato *et al*.,(1993) proved that extracts of *Carica papay*a is bacteriostatic to *E.coli* and *Staphylococcus aureus*. Of the plant extracts used, maximum percentage (40%) was susceptible to *Staphylococcus aureus*, 20 % were active against *Bacillus sp* and 11 % were effective against *Corynebacterium diphtheriae*. The result also revealed that most of the extracts were having similar effect to that of amikacin. (Table.2.).

Antibacterial activity of plant extract against gram negative bacteria

Seven plant extracts showed activity against gram negative bacteria like *E.coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella sp, Shigella sp* and *Vibrio cholerae. Acalpha indica* showed moderate susceptibility to *E,coli* (12 mm) and *Pseudomonas aeruginosa* (14 mm) and was found to be less effective than the modern antibiotics used. *Achranthus aspera* gave a zone of 18 mm against *E.coli* and found to be more effective than ampicillin and cefotaxime. *Citrus limon* gave 17 mm zone size against *E.coli* which is more sensitive than ampicillin. Also gave a zone of 18 mm against *Pseudomonas aeruginosa*, 20 mm for *Salmonella* sp which is more effective than gentamycin and 20 mm for *Shigella* sp. *Emblica officinalis* gave a weaker zones against *E.coli, K.pneumoniae* and *Proteus vulgaris* similarly *Coriandrum sativum* showed against *Salmonella* sp, *Shigella* sp and *V.cholerae*. *Mentha piperita* was observed to give a larger zone size of 22 mm against *E.coli* which is highly inhibitory than ampicillin, cefotaxime, chloramphenicol, piperacillin and tetracycline. It also produced a zone of 20 mm which is similar to gentamycin. *Piper nigrum* responded well against

E.coli (17 mm) and *Salmonella* sp (21 mm) and found to be more effective than ampicillin and chloramphenicol against *E.coli* and *gentamycin* against *Salmonella* sp (19 mm). *Psidium guajava* found to have more inhibitory action against *P. aeruginosa* and lesser pattern of sensitivity to *Salmonella* sp and *V.cholerae.* (Table. 3.).

Table 1. Plants showing activity against Gram positive bacteria.

S. No	Name of the Plant	Name of the Family
01	*Achyranthus aspera*	Amarantiacea
02	*Caesalpinia pulcherrima*	Caesalpinaceae
03	*Calotropis gigantica*	Asclepidaceae
04	*Carica papaya*	Caricaceae
05	*Citrus limon*	Rutaceae
06	*Coleus ammonificans*	Labiaceae
07	*Coriandrum sativum*	Apiaceae
08	*Emblica officinalis*	Euphorbiaceae
09	*Mentha piperita*	Labiaceae
10	*Murraya koenigi*	Rutaceae
11	*Psidium guajava*	Myrtaceae
12	*Rauwolfia serpentina*	Apocynaceae
13	*Sida cordifolia*	Malvacea
14	*Tabermontana coronaria*	Apocynaceae

Table 2. Comparative study of Plant extract activity with antibiotics against gram positive bacteria

S. No	Name of the Plant/Antibiotics	Antimicrobial activity (mm)		
		B. subtilis	***C.diphtheriae***	***Staph. aureus***
01	*Acalpha indica*	-	-	-
02	*Achyranthus aspera*	13	15	-
03	*Citrus limon*	20	18	16
04	*Coriandrum sativum*	-	17	-
05	*Emblica officinalis*	17	16	-
06	*Mentha piperata*	17	-	-
07	*Piper nigrum*	-	-	-
08	*Psidium guajava*	-	14	-
09	*Tabermontana coronaria*	-	24	17
10	Amikacin	20	15	18
11	Ampicillin	-	-	-
12	Cephotaxime	-	-	26
13	Chloramphenicol	22	-	17
14	Erythromycin	-	-	20
15	Gentamycin	25	-	-
16	Nalidixic acid	18	-	-
17	Piperacillin	-	21	-
18	Tetracycline	-	-	-
19	Vancomycin	-	-	30

(-) indicates no zone formation (resistant)

Table 3. Comparative study of Plant extract activity with antibiotics against gram negative bacteria

		Antibiotic activity (mm)						
S. No	Plant Extract/ Antibiotics used	*E.coli*	*Klbesiella*	*Proteus*	*Pseudomonas*	*Salmonella*	*Shigells*	*V.cholerae*
01	*Acalpha indica*	12	-	-	14	-	-	-
02	*Achyranthus aspera*	18	-	11	-	15	-	-
03	*Citrus limon*	17	12	-	18	20	20	-
04	*Coriandrum sativum*	-	-	-	-	12	13	15
05	*Emblica officinalis*	13	12	17	-	-	-	-
06	*Mentha piperita*	22	-	-	-	-	-	20
07	*Piper nigrum*	17	-	-	-	21	14	-
08	*Psidium guajava*	-	-	-	17	14	-	11
09	*T.coronaria*	-	-	-	19	-	-	18
10	Amikacin	-	15	19	20	-	-	21
11	Ampicillin	16	-	22	21	-	21	-
12	Cefotaxime	20	18	30	-	21	30	-
13	Chloramphenicol	17	-	-	-	21	-	-
14	Erythromycin	-	-	-	-	-	-	-
15	Gentamycin	-	-	21	20	19	21	20
16	Nalidixic acid	-	-	-	-	-	-	-
17	Piperacillin	19	-	-	20	-	-	-
18	Tetracycline	18	-	-	-	-	-	-
19	Vancomycin	-	-	-	-	-	-	-

(-) indicates no zone formation (resistant)

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