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STUDIES ON THE ANTIBACTERIAL ACTIVITY OF SELECTED MEDICINAL PLANTS AGAINST HUMAN SKIN PATHOGENS

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ABSTRACT

Medicinal plant represents a rich source of antimicrobial agents. The present study was an attempt to analyse the antibacterial potential of ten medicinal plants against four skin diseases, 10% extract of all the plants were prepared by Soxhlet extraction and antibacterial efficiency of the extracts were tested by Agar Well Diffusion assay. Streptomycin was used as the positive control and toluene served as negative control. The study leads to the discovery of a potent antibacterial agent of *Phyllanthus emblica* against *Serratia marcescens.* Among the tested sampling plants, *Phyllanthus emblica*, *Curcuma longa* and *Embelia ribes* were found to be more effective against the tested pathogens when compared to the other plants.

KEYWORDS: Medicinal plants, antibacterial, streptomycin, well diffusion.

INTRODUCTION

Human skin acts as a home for many microorganisms such as aerobic and anaerobic bacteria, non-pathogenic myco-bacteria, pathogenic fungi and yeasts. These microorganisms normally do not cause any disease condition but the integrity of the skin is breached by trauma, burns, foreign body or primary skin diseases cause infection (Badame, 1988). The common bacteria *Staphylococcus aureus*, *Streptococcus pyogenes* and *Pseudomonas aeruginosa* cause various types of skin infections (Walker *et al*., 2004; Bowersox and John, 1999; Ryan and Ray, 2004).

Plants are reserve houses of various antimicrobial compounds and these compounds can be isolated by screening of selected plant extracts for the activity followed by bioassay – guided fractionation of active extracts (Tahir *et al*., 2010). Medicinal plants have been used for the treatment of skin diseases worldwide. *Acalypha wilkesiana* (Alade and Irobi, 1993); *Quisqualis indica*, *Cormelina benghalensis*, *Amaranthus spinosus*, *Ranunculus scleratus*, *Cassia alata* (Sofowora, 1986; Damodaran and Venkataraman, 1994) and fruit extracts of *Xylopia aethiopica* (Asuquo, 1976; Malcom and Sofowara, 1969) have been used as remedy for skin infections. Roots and bark of *Xylopia aethiopica* contain 17% tannin and infusion made from them is traditionally applied to wounds and skin diseases (Thomas, 1965; Irvin, 1961). The present study was mainly conducted to assess the antibacterial potential of ethanol, methanol and hexane extracts of ten medicinal plants which are the major ingredients of many decoctions used for treating skin diseases.

MATERIALS AND METHODS

Collection of plant materials

The plant materials were collected from unpolluted rural areas of Thiruvananthapuram district of Kerala and from ayurvedic shops. The collected materials were properly dried and powdered with the help of a blender. The fine powder of the plant materials were then stored in dark at room temperature in closed containers until use. The list of plants along with the parts used for the present study was presented (Table-1).

Table 1: Catalog of the Sampling Plants

S.No	Name of the plant	Part used
1.	*Acacia catcheu*	Heart wood
2.	*Terminalia chebula*	Seed
3.	*Phyllanthus emblica*	Fruit
4.	*Curcuma longa*	Rhizome
5.	*Alstonia scholaris*	Leaf
6.	*Embelia ribes*	Fruit
7.	*Cassia fistula*	Bark
8.	*Jasminum grandiflorum*	Flower
9.	*Thevitia neerifolia*	Leaf
10.	*Semicarpus anacardium*	Seed

Preparation of extracts

Extraction of the phytochemicals was carried out using a Soxhlet extractor; 5g of each plant powder was weighed out and extracted with the solvents hexane, ethanol and methanol separately at 60^0C for 8 hours. The extracts

were concentrated in a rotary evaporator, then dried and stored at 4^0C in airtight bottles for further use.

Test pathogens

Streptococcus pyogenes is a spherical, Gram – positive bacterium which typically contain group A antigen on its cell wall, produces large zones of beta – hemolysis when cultured on blood agar plates.

The major types of skin infections caused by *S. pyogenes* include impetigo, cellulitis and erysipelas. *Staphylococcus aureus* is a facultative anaerobic Gram – positive bacterium, cause a range of infections from minor skin infections such as pimples, impetigo, boils, cellulitis, carbuncles, scalded skin syndrome and abscesses to life threatening diseases such as pneumonia, meningitis, toxic shock syndrome etc. *Serratia marcescens* is a Gram-negative, facultative anaerobic, rod-shaped bacterium of Enterobacteriaceae family.

Serratia infection is responsible for 2% of nosocomial infections of the bloodstream, lower respiratory tract, urinary tract, surgical wounds, skin and soft tissues in adult patients. *Pseudomonas aeruginosa* is a Gram – negative, aerobic, rod – shaped bacterium with unipolar motility mainly causes pneumonia, dermatitis, urinary tract infection, gastrointestinal infection and skin and soft tissue infections.

Antibacterial activity

Antibacterial activity was determined by Agar well diffusion method (Okeke *et al*., 2001). Nutrient agar plates were prepared and overnight grown different species of bacteria were swabbed. Wells of approximately 10mm was bored using a well cutter and 50µl of 10% extracts (methanol, ethanol and hexane) in toluene were added to the cut wells.

The zone of inhibition was measured after overnight incubation was compared with that of standard antibiotic Streptomycin. Toluene was used as negative control. Each extract was tested in triplicate against 4 bacterial strains and the result is expressed as mean ± standard error.

RESULTS

Antibacterial activity of the plant extract

Ethanolic extract of *Phyllanthus emblica* showed maximum response of *Streptococcus pneumonia, Pseudomonas aeruginosa* and *Staphylococcus aureus*. The activity of *Curcuma longa* and *Thevitia neerifolia* was found to be higher against the pathogen *Serratia marcescens.* Result of the *in vitro* antibacterial activity of ethanolic plant extracts is tabulated (Table 2).

Among the ten methanolic extracts, *Phyllanthus emblica extract* showed maximum zone of inhibition against *Streptococcus pneumonia* and *Serratia marcescens.*

Terminalia chebula extract was found to be more active against *Staphylococcus aureus* and that of *Embelia ribes* was found to be more active against *Pseudomonas aeruginosa* (Table 3). *Jasminum grandiflorum* and *Thevitia neerifolia* showed maximum activity against *Staphylococcus aureus* and *Semicarpus anacardium showed maximum activity against Serratia marcescens*.

Hexane extract of *Embelia ribes* and *Alstonia scholaris* alone showed inhibition capacity against *Pseudomonas aeruginosa* and *streptococcus pneumonia.* Inhibition zone diameter of ten hexane extracts along with the antibiotic streptomycin is given in (Table 4).

Table 2: *In vitro* antibacterial activity of ethanolic extract

Name of the sampling plants	Inhibition zone diameter (mm)			
	Staphylococcus Aureus	***Pseudomonas aeruginosa***	***Streptococcus pneumoniae***	***Serratia marcescens***
Acacia catechu	4.0±0.85	1.5±0.54	2.0±0.64	1.5±0.34
Terminalia chebula	1.1±0.43	1.0±0.01	1.0±0.76	-
Phyllanthus Emblica	4.0±0.09	2.5±0.03	2.9±0.07	2.0±0.89
Curcuma longa	2.0±0.04	2.3±0.19	2.0±0.05	2.2±0.05
Alstonia scholaris	1.4±0.39	1.5±0.00	1.3±1.09	1.3±0.14
Embelia ribes	1.3±0.65	1.6±0.60	-	-
Cassia fistula	1.1±0.04	-	-	-
Jasminum grandiflorum	1.5±0.07	1.2±0.34	1.5±0.9	1.2±0.11
Thevitia neerifolia	1.8±0.67	1.5±0.06	1.5±0.02	2.2±0.61
Semicarpus anacardium	1.2±0.09	-	-	2.0±2.0
Streptomycin	5.0±0.78	2.0±0.00	5.2±0.05	2.4±0.74

Table 3: I*n vitro* antibacterial activity of methanolic extract

Name of the sampling plants	Inhibition zone diameter (mm)			
	Staphylococcus aureus	***Pseudomonas aeruginosa***	***Streptococcus pneumoniae***	***Serratia marcescens***
Acacia catechu	1.4±0.01	1.0±0.48	-	1.2±0.73
Terminalia chebula	2.3±0.53	-	1.8±0.01	1.5±0.08
Phyllanthus Emblica	2.0±0.23	-	2.6±0.03	4.0±0.39
Curcuma longa	1.3±1.04	-	-	3.4±0.57
Alstonia scholaris	1.9±0.04	-	2.3±0.54	-
Embelia ribes	1.8±0.14	1.6±0.74	-	1.0±0.83
Cassia fistula	1.6±0.62	-	-	-
Jasminum grandiflorum	1.2±0.46	-	1.3±0.42	-
Thevitia neerifolia	2.0±0.00	-	1.8±0.90	2.0±0.03
Semicarpus anacardium	1.7±0.02	1.0±0.06	-	2.2±0.09
Streptomycin	5.0±0.09	2.0±0.04	5.2±0.05	2.4±0.66

Table 4: I*n vitro* antibacterial activity of hexane extract

Name of plant	Inhibition zone diameter (mm)			
	Staphylococcus aureus	***Pseudomonas aeruginosa***	***Streptococcus pneumoniae***	***Serratia pyogenes***
Acacia catechu	-	-	-	-
Terminalia chebula	-	-	-	1.0±1.04
Phyllanthus Emblica	1.5±0.04	-	-	-
Curcuma longa	1.0±0.21	-	-	-
Alstonia scholaris	-	-	2.1±0.04	-
Embelia ribes	1.7±0.00	1.6±0.02	-	-
Cassia fistula	1.4±1.01	-	-	-
Jasminum grandiflorum	2.5±0.06	-	1.7±0.09	-
Thevitia neerifolia	2.2±0.04	-	-	1.0±0.06
Semicarpus anacardium	1.0±0.00	-	-	1.5±0.56
Streptomycin	5.0±0.07	2.0±0.01	5.2±0.11	2.4±0.32

DISCUSSION AND CONCLUSION

The present investigation revealed more or less antibacterial activity against the bacterial strains, among the ten sampling medicinal plants *P. emblica*, *C. longa* and *E. ribes* showed more inhibitory potential on the tested Gram –negative pathogens with respect to the positive control, i.e. Streptomycin. The extracts of *P. emblica* was effective against Gram negative organisms i.e., *P.aeruginosa* (ethanol extract alone) and *S. marcescens* (both extracts). The methanolic extract of *P. emblica* act as a strong inhibitor of the pathogen *S. marcescens* than Streptomycin. This is a positive sign for the presence of a strong antibiotic agent in *P. emblica* against *S. marcescens*. The three extracts of *Embelia ribes* also exhibited significant activity against *P. aeruginosa*. The ethanol and methanol extracts of *C. longa* were found to be more effective on both *P. aeruginosa* and *S. marcescens*.

Among the three extracts, hexane extract was very weak in action against all the pathogens tested. Hexane extract of *Acacia catechu* fail to exhibit the tested pathogens. Eventhough hexane extract of *Embelia ribes* could produce an inhibitory action against *P. aeruginosa* and *Terminalia chebula* and *Thevitia neerifolia* against *S. marcescens* which is very near to the inhibitory potential of streptomycin. The variation observed in the inhibitory potential of different plants as well as different solvent extracts suggests the presence of phytochemical variation in the plants and antimicrobial agents eluted in different solvents. So further studies are needed for the understanding and identification of antimicrobial compounds in the tested plant extracts. The present study revealed that the ethanolic and methanolic extracts of *P. emblica*, *C. longa* and *E. ribes* contained potent antibacterial compounds that work strongly against the tested pathogenic bacteria when compared to the other plant extracts.

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