International Journal of Scientific Engineering and Technology (ISSN : 2277-1581)
Volume No.3 Issue No.6, pp : 789-792 1 June 2014

Antimicrobial Activities Of Certain South Indian Medicinal Plants

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Abstract : In the present study antimicrobial activity of ethanolic extracts of six medicinal plants viz. Gynodropsis Pentaphyllum (Capparidaceae family), Solanum nigrum (Solanaceae family), Merremia gangitica (Convolvulaceae family), Cicca acida (Euphorbiaceae family), Erythrina variegata (Leguminaceae family) and Asparagus fysoxii (Asparagaceae family) were carried out against five pathogenic Bacteria. Out of which three Bacteria are Gram positive – (Staphylococcus aureus NCIM 2079, Streptococcus mutans NCIM 2611, Bacillus cerus NCIM 2106) and two Gram negative bacteria – (Escherichia coli NCIM 2005, Solmonella abony NCIM 2257) using disc diffusion method. The respective bacteria were inoculated in a nutrient broth for overnight incubation. In the comparative study of plant extracts with standard drugs, Sterile disc's (HIMEDIA) obtained and respective volume of each extract was dispensed over the disc to attain 10 mcg concentrations each of the disc's were dried and impregnated over the pre-inoculated plates. Finally the zone of inhibition was observed and the inhibitory zone was measured using zone inhibitory scale (HIMEDIA) and the values are noted in mm.

Keywords: Antibacterial study: Gp, Sn, Mg, Ca, Ev and Af.

Introduction

Bacteria are microscopic, prokaryotic organisms. They were omnipresent. They inhabit every conceivable habitat. They live in tropical temperate and snow covered alpine regions of the world they lead their life in terrestrial and aquatic habitats – ponds, lakes, pools, streams, lagoons, etc contain them. Seas, oceans, estuaries have millions of bacteria. No place on earth is without them.

Bacteria are deadly parasites of plants, animals and human being, yet others live peacefully with plants as symbionts. Harmful pathogenic bacteria can be removed, inhibited or killed by physical processes (Heating, low temperature treatment, radiation method etc.) or chemical agents. Treatment of infectious bacterial disease with chemicals is known as chemotherapy. Generally chemotherapeutic agents are prepared in the chemical laboratory or obtained from plants or animals or micro organisms.

The naturally occurring substances are distinguished from synthetic compound by the name antibiotics. In general, the selective toxicity for the parasite is higher for antibiotics than synthetic chemicals. Many different species of pathogens are readily destroyed by antibiotics and thus they are **"Broad – Spectrum"** in their antibacterial action. The antibiotics do not produce undesirable side effect in hosts, when compared to synthetic chemotherapeutic agents. Again repeated use of antibiotics may not lead to resistance development in parasitic bacteria for longer periods.

Penicillin (Waksman, 1960) revolutionized the treatment of staphylococcal and streptococcal infections. It is important to note that a significant fraction of all human infections are caused by these two Gram-positive bacteria. Streptomycin (Swatz, 2000) was the first effective treatment of tuberculosis and saved countless lives. The above discovery was followed in the 1950s and 1960s by the finding of many further antibacterial drugs, including cephalosporin from a fungus, but the majority from the actinomycetes, such as tetracycline, erythromycin, kanamycin and vancomycin and antifungal agents like candicidin and belomycin. This period was considered as 'Golden Age' of antibiotic discovery.

The development of resistance to antibiotics in bacteria led to a discussion about the careful use of antimicrobial agents, especially in veterinary medicines, nutrition and agriculture. In animals the antimicrobial agents are not used only for therapy and prevention of bacterial infections but also as growth promoters. Application of antibiotics brings about an increase in the resistance to antibiotics not only in pathogenic bacterial strains but also in strains forming a part of the endogenous flora of humans and animals. Multi-resistant bacterial strains of animal origin may spread into human population by direct contacts and through food from animal sources. These resistant strains colonize the human intestine and the genus coding. Resistance to antibiotics can be transferred to bacterial strains that belong to natural micro flora.

As the pathogens develop resistance, not only is there a problem in finding new antibiotics to fight old disease, there is a parallel problem to find new antibiotics to fight new disease.

Phytochemicals have been reported to be target specific, therefore less harmful to the non – target and beneficial organisms. Since natural products seldom accumulate in the ecosystem, they do not lead to development of resistance and therefore have better prospects especially under the present ecological awareness (Mahadevan, 1982). Several works have already emphasized the importance and role of plant products

and bio agents in future plant production (Benner, 1993, Porter and Fox 1993, Cannell, 1993, Pillmoor et al, 1993).

Recently screening of plant crude extracts, partially purified and pure phytochemicals against important pathogenic microbes became the subject of different publications.

Antimicrobial agents are undeniably one of the most important therapeutic discoveries of the 20th century. However with the "antibiotic era", barley five decades old, mankind is now faced with the global problem of emerging resistance in virtually all pathogens.

It is believed that plants which are rich in a wide variety of secondary metabolites belonging to chemical classes such as saponins, flavonoids, phenolic acids, alkaloids and terpinoids are generally superior in their antimicrobial activities (Sharma et al, 1985: Mukherji Kakalisaha, 1995). Leaves and flowers of medicinal plants have been used for treating many diseases in traditional system medicines.

Materials and methods

Collection of plant materials

Fresh aerial parts of six medicinal plants viz. *Gynodropsis Pentaphyllum* , *Solanum nigrum, Merimia gangitica, Cicca acida, Erythrina variegata* and *Asparagus fysoxii* were collected in and around of Puducherry during the month of April 2013 and authenticated by the department of botany KM Centre for PG Studies, Puducherry, where a voucher specimens of each plants were deposited.

Preparation of plant extracts

The air dried aerial parts of the six medicinal plants each of 500g collected from in and around of Puducherry were extracted thrice with hot 95% ethanol under reflux (3 X 5 litre). These extracts were concentrated in vacuo. 10ml of the concentrated ethanolic extract of each plant was used for the antimicrobial studies. Three different concentrations of the ethanolic extract were used in the study viz. 25µl, 50µl and 100µl for each disc.

Test organisms

The following microbial strains obtained from Microbial type culture collection, Chandigarh were used in the screening programme of ethanolic extract of these six medicinal plants. Organism used in the study are – three Gram positive bacteria (*Staphylococcus aureus* NCIM 2079, *Streptococcus Mutans* WCIM 2611 and *Bacillus cerus* NCIM 2106) and two Gram – negative bacteria (*Escherichia coli* NCIM 2005 and *Salmonella abony* NCIM 2257). Bacteria were maintained on nutrient agar.

The composition of the media used in this study is

nutrient agar

- Peptone - 10.0 g
- Beef extract - 5.0 g
- Sodium chloride - 5.0 g
- Agar - 15.0 g
- pH - 7±0.1
- Distilled water - 100 ml

Preparation

All the three components were dissolved in 100ml distilled water. The volume was made up to 1000 ml with distilled water . pH of the broth was adjusted to 7.0 using 1N HCl/1N NaOH, required volume of broth was distributed to conical flasks to this was added agar and sterilized in an autoclave at 15 psi for 20 minutes.

Screening of Antimicrobial activity

Disc diffusion assay

Disc Diffusion assay was used to determine the antimicrobial activities of ethanolic extract of all the six medicinal plants. The test organisms were grown in 10ml of liquid nutrient medium for 24 hours. A 100 µl of the 24 hour old broth culture of each type were aseptically transferred to 50ml molten (40°C) nutrient agar. After thorough mixing the contents were dispensed into five sterilized 90 mm dia. Petri plates and allowed to cool. Four × Four 6 mm dia. Sterile disc (HIMEDIA) were loaded with 25µl, 50µl and 100µl test solutions of each plants and a control disc with only solvent respectively. Using a sterile forceps the four discs were placed at equidistance on the nutrient agar seeded with test bacteria. The plates were immediately kept at to 4°C for at least 4 hour to allow the diffusion of the metabolites in different concentrations of ethanolic extract of each plants and were then incubated at 37°c for 24 hrs. Control plates contained discs dipped in the respective solvents. The observations were made after the second day of inoculation and the zone of the inhibition were measured and recorded in mm after 24 hours for all bacteria.

Results and Discussion

Anti – bacterial activities of ethanolic extracts of the aerial parts of six medicinal plants *Gynodropsis Pentaphyllum* , *Solanum nigrum, Merremia gangitica, Cicca acida, Erythrina variegata* and *Asparagus fysoxii* were tested against five bacteria. Out of which three Gram positive bacteria (*Staphylococcus aureus* NCIM 2079,*Streptococcus Mutans* WCIM 2611 and *Bacillus cerus* NCIM 2106) and two gram – negative bacteria (*Escherichia coli* NCIM 2005 and *Salmonella abony* NCIM 2257).

Antibacterial Activity of the ethanolic extracts

Sample	Zone of measurement(mm) on different volume of extract (mg/ml)											
	Staphylococcus aureus				Streptococcus mutans				Bacillus cerus			
	Control	25µl	50µl	100µl	Control	25µl	50µl	100µl	Control	25µl	50µl	100µl
Gp	*	0	0	<10	*	0	0	0	*	0	<10	10
Sn	*	0	0	0	*	0	0	0	*	<10	10	11
Mg	*	0	0	0	*	0	0	0	*	0	<10	0
Ca	*	0	<10	11	*	0	0	12	*	<10	10	12
Ev	*	0	0	0	*	0	0	0	*	0	0	0
Af	*	0	0	0	*	0	0	0	*	0	0	0

Table(i). Gram-positive bacteria

Sample	Escherichia coli				Salmonella abony			
	Control	25µl	50µl	100µl	Control	25µl	50µl	100µl
Gp	*	0	0	0	*	0	0	<10
Sn	*	0	0	0	*	0	0	0
Mg	*	0	0	0	*	0	0	0
Ca	*	0	<10	10	*	0	0	<10
Ev	*	0	0	0	*	0	0	0
Af	*	0	0	<10	*	0	0	0

Table(ii). Gram-negative bacteria

The present study revealed that as the concentration of the ethanolic extracts increased the inhibitory zones also increased. The ethanolic extracts of all plants showed an inhibitory zone of 10mm and above 10mm against Gram-positive bacteria. Only *Solanum nigrum* and *Gynodropsis pentaphyllum* showed above 10mm inhibitory zone in the case of Gram-negative bacteria. Among the Gram-positive bacteria, *Bacillus cerus* was found to be sensitive to ethanolic extract of *Gynodropsis Pentaphyllum*, *Solanum nigrum* and *Cicca acida*. *Streptococcus mutans, Staphylococcus aureus, Escherichia coli* were found to be sensitive to *Cicca acida* in 100ml concentration

.**Comparative study of extracts with standard drugs (10mcg concentration)**

Sample	Zone of measurement(mm) with concentration of each extract								
	Staphylococcus aureus			Streptococcus mutans			Bacillus cerus		
	Genta	Tetra	Extract	Amphic	Chlora	Extract	Amox	Tetra	Extract
Gp	<10	19	0	11	22	<10	0	21	0
Sn	11	21	10	10	25	0	10	24	10
Mg	<10	19	0	10	24	0	<10	23	<10
Ca	<10	18	10	10	22	10	0	23	15
Ev	10	20	<10	11	26	0	<10	23	0
Af	11	20	<10	11	27	0	10	24	10

Table(iii). Gram positive bacteria

Sample	Escherichia coli			Salmonella abony		
	Genta	Amoxy	Extract	Genta	Amoxy	Extract
Gp	17	20	0	16	20	<10
Sn	18	21	<10	17	20	<10
Mg	18	20	10	14	20	10
Ca	18	20	11	17	18	10
Ev	16	19	0	14	19	0
Af	17	20	<10	16	20	<10

Table(iv).Gram negative bacteria

G positive bacteria

Gynodropsis Pentaphyllum extract was found to be active against *streptococcus mutans*, its activity was found to be comparable with Amphicillin. *Solanum nigrum* extract was found to be active on par with Gentamycin and Amoxcillin against *Staphylococcus aureus* and *Bacillus cerus*

respectively. *Cicca acida* was found to be better than Gentamycin and Amoxcillin against *Staphylococcus aureus* and *Bacillus cerus* respectively. *Merremia gangitica and Cicca acida* were found to be effective against the Gram negative bacteria under study but their inhibitory zones were lesser than standard antibiotics in use. Among the antibiotics Tetracyclin was very effective against all the Gram positive bacteria as well as gram negative bacteria under study.

Conclusion

The present study revealed the effective antibacterial activities of the ethanolic extracts of six medicinal plants. They exhibited effective activities against Gram positive bacteria comparable to that of standard drugs Gentamycin, Amoxicillin and Amphicillin The concentration used is 10mcg for standard drugs well as the ethanolic extracts. The lesser activities of the ethanolic extracts compared to standard drugs against Gram negative bacteria may be attributed to lesser concentration of active compounds present in 10mcg of ethanolic extracts. The present study suggests that if the active compounds are isolated and used in 10mcg concentration it would lead to better activity than the drugs available. It also suggests that the use of the ethanolic extracts would not produce any side effects and would be eco-friendly and bio-friendly.

Acknowledgement

The authors are very thankful to department of Botany KMPGS Puducherry for having authenticated the plant materials. We also thank Dr. T. Ganesan for helping in the antibacterial studies.

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