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Antimicrobial screening of Trikatu and Sitopladi Churnas

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Abstract

Ayurvedic system of medicine prescribes a number of crude drugs for longevity of life and for curing various ailments. In the present study we have selected two most commonly used ayurvedic formulations i.e. Trikatu and Sitopladi churnas. which are used against respiratory tract infections. The petroleum ether, acetone, methanolic and aqueous extracts of each plant was tested against *Staphylococcus aureus, Streptococcus pyogenes, S. pneumoniae, S. sanguis, S. salivarius, S. mutans, Lactobacillus acidophillus, E.coli, Pseudomonas aeruginosa, Staphylococcus epidermidis, Salmonella typhi, Micrococcus luteus, Bacillus megneterium, Bacillus subtilis, Proteus vulgaris, Candida albicans* and it was found that methanolic extracts exhibited the highest degree of activity. *Zingiber officinales* (tuber) and *Piper longum* (fruits) produced outstanding antibacterial effect with inhibition zone greater than 20 mm against most of the pathogens. Minimum inhibitory concentration of most effective extract (methanolic) was performed against *E. coli, B. subtilis, S. pneumoniae* and *S. pyogenes* whereas *P. longum* showed best results against *E.coli* (0.0391 mg/ml) and *Z. officinales* against *B. subtilis* (0.0391mg/ml).

Keywords: *Minimum inhibitory concentration, Respiratory tract infection concentration, Sitopladi, Trikatu*

Introduction

Medicinal plants are the local heritage with global importance, world is endowed with a rich wealth of medicinal plants. Herbs have always been the principle form of medicine in India and presently they are becoming popular throughout the world, as people strive to stay healthy in the face of chronic stress and pollution and to treat illness with medicines that work in concert with the body's own defense (Chopra *et al*., 1992).

Medicinal plants play an important role in the lives of rural people, particularly in remote parts of developing countries with few health facilities. The ayurvedic system of medicine prescribes a number of crude drugs for longevity of life and for curing various ailments (Holetz *et al*., 2002 and Prabhat *et al*., 2005). These crude drugs are processed in different forms to produce specific therapeutic effect, one of the important forms is churna. The improvement of disease conditions after herbal treatment, without any harmful side effects and the high cost of the other forms of treatment (Ahmad *et al*., 1998) compelled a large population of the world to use medicinal plants. In the present study we have selected two most commonly used ayurvedic formulations i.e. Trikatu and Sitopladi churnas which are known to cure respiratory tract infections.

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Materials and Method

Plant material

The ingredients of both churnas were collected separately from M/s Vijay Herbal Automation, Haridwar and were further confirmed in the Department of Botany, Gurukul Kangri University, Haridwar (Uttarakhand). Trikatu churna was prepared from dry ginger (33.33 gm), long pepper (33.33 gm), black pepper (33.33 gm) in equal quantities while Sitopladi churna comprises of long pepper (12.90 gm), cinnamon (03.23 gm), cardamom (06.45 gm), thorny bamboo (25.81 gm) and Mishri (51.61 gm).

Preparation of extracts

The method of Alade and Irobi (1993) was adopted for the preparation of plant extracts separately. 100 gm of the powdered plant materials and 50 gm of Trikatu and Sitopladi churnas were loaded in Soxhlet assembly and extracted in four different solvents (Petroleum ether, acetone, methanol and aqueous) for 72 hours. After extraction it was passed through filter paper. The filtrate then obtained was concentrated using vacuum-rotator evaporator at 30^0C.

Culture media

Muller Hinton Agar Media No.173 (Hi media Pvt. Ltd., Mumbai, India) was used for screening of antimicrobial activity.

Microorganisms

A total of sixteen different pathogenic microorganisms (12 undesignated and 4 designated) strains or serotypes were isolated from infected patients in Ravi diagnostic laboratory and Aggarwal dental clinic, Haridwar. Out of these *Staphylococcus aureus, Streptococcus mutans, S. salivarius, S. sanguis, S. pneumoniae, S. pyogenes, Lactobacillus acidophillus* were isolated from patients having dental and respiratory tract infection while *E. coli, Micrococcus luteus, Pseudomonas aeruginosa, Candida albicans* and *Salmonella typhii* were isolated from the urinary tract infection (UTI) patients. The isolates were identified according to guidelines given by Burneti *et al.* (1994).The rest of the strains *i.e. Proteus vulgaris* (MTCC-742), *Bacillus subtilis* (MTCC-441), *B. megneterium* (MTCC-428) and *Staphylococcus epidermidis* (MTCC-435) were procured from IMTECH, Chandigarh.

Antimicrobial assay

Antibacterial activity was carried out using Cup-Plate method during the process 0.1 ml of diluted inoculum (10^5 CFU/ml) of test organism was mixed in Muller Hinton Agar media, it was then shaken and poured in sterilized petridishes. Wells of 8 mm diameter were punched into the agar medium and filled with 45 µl of plant extracts. All the solvents were served as negative control. Each extract was assayed in triplicate. The plates were then incubated at 37^0C for 24 hrs. The antibacterial activity was interpreted from the size of the diameter of zone of inhibition measured in millimeters (mm) (Prabhat *et al.*, 2008).

Determination of MIC

The minimum inhibitory concentration of the most effective extract (methanolic) was determined for *B. subtilis*, *E. coli*, *S. pneumoniae* and *S. pyogenes* by using the serial dilution method at a final concentration starting from 10 mg/ml. The extracts were added to sterile Muller Hinton Broth into microtiter plates. Each extract was assayed in triplicate. The turbidity of the wells in the microtiter plate was interpreted as visible growth of the microorganisms. The MIC values were taken as the lowest concentration of the extracts in the wells of the microtiter plate that showed no turbidity after 24 hours of inoculation at 37^0C.

Results and Discussion

The present study was designated to obtain preliminary information on the antimicrobial effect of medicinal plants used in Trikatu and Sitopladi churnas against the disease causing microorganisms. The cup-plate diffusion method was used in this study since it was found to be better than the disc diffusion method (Essawi and Srour, 2000).

A total of six medicinal plants were used for the formulation of Trikatu and Sitopladi churnas, belonging to 4 families of Angiosperms. The petroleum ether, acetone, methanolic and aqueous extracts of each plant at the concentration of 200 mg/ml was tested against the microorganisms (Table 1). Antimicrobial activity was found in all plants and churnas but *Zingiber officinales* (tuber) and *Piper longum* (fruits) produced outstanding antibacterial effect with inhibition zone greater than 20 mm (Table 1). Other plant extracts were found to have moderate antimicrobial activity. Trikatu and Sitoladi churnas showed best antibacterial activity against *B. subtilis* with 15 and 14 mm of zone of inhibition (Table 2). In general methanolic extracts exhibited the highest degree of antimicrobial activity as compared to aqueous, acetone and petroleum ether extracts

Minimum inhibitory concentration of most effective extract (methanolic) was performed against *E. coli, B. subtilis, S. pneumoniae* and *S. pyogenes* (Table 3). *P. longum* showed best results against *E.coli* (0.0391 mg/ml) and *Z. officinales* against *B. subtilis* (0.0391 mg/ml).

Table 1: Antimicrobial activity of plants, Trikatu and Sitopladi churnas

S. No	Family Botanical name	Part used	Fractions Extracts	Antimicrobial activity														
				Sa	Sp	Sp*	Ss	Ss†	Sm	La	Ec	Pa	Se	St	Ml	Bm	Bs	Pv	Ca
1.	Poaceae *Bambusa arundinacea*	Vanslochan	I	9	10	9	-	-	-	-	-	-	-	-	-	11	12	9	8
			II	12	11		-	-	-	-	-	-	12	-	-	-	-	-	-
			III	12	10	10	12	14	16	8	-	-	-	11	12	9	9	11	13
			IV	12	15	13	8	11	9	-	-	-	-	-	-	-	9	10	8
2.	Zingiberaceae *Elettaria cardamomum*	Fruits	I	8	9	8	-	-	-	-	-	-	-	-	-	11	9	8	-
			II	11	12	9	17	9	11	8	12	-	-	-	8	12	-	-	11
			III	10	12	14	8	9	16	13	8	8	10	9	13	17	18	16	13
			IV	9	11	10	8	8	9	-	-	-	-	-	9	17	9	11	12
3.	Lauraceae *Cinnamomum zeylanicum*	Stem Bark	I	9	10	8	9	11	10	8	-	-	11	-	9	8	10	11	-
			II	16	12	16	14	15	9	13	-	-	10	9	14	13	12	14	9
			III	14	17	16	13	17	16	18	10	11	10	9	19	19	20	20	9
			IV	10	9	11	8	9	11	10	-	-	-	11	8	9	-	-	10
4.	Piperaceae *Piper longum*	Fruits ,bark	I	10	9	8	11	8	9	11	10	11	9	12	8	9	11	8	10
			II	9	11	8	12	9	11	8	9	11	9	12	10	10	11	9	8
			III	22	20	21	16	20	11	15	22	14	17	15	16	17	22	22	9
			IV	10	11	9	8	12	9	-	-	-	-	-	-	-	12	9	10
5.	Piperaceae *Piper nigrum*	Fruits	I	9	10	11	8	12	-	-	-	-	-	8	10	12	11	9	8
			II	9	8	11	10	9	10	9	11	8	9	11	9	8	10	9	10
			III	17	20	21	17	18	15	13	21	21	21	20	20	11	10	9	8
			IV	10	17	8	11	17	16	11	9	17	16	9	12	9	10	11	-
6.	Zingiberaceae *Zingber officinale*	Rhizome	I	9	10	11	8	10	11	9	8	11	10	9	8	10	11	9	-
			II	12	9	10	11	9	8	12	9	10	11	8	11	12	8	9	18
			III	22	19	18	13	15	16	17	16	17	15	22	15	21	23	21	16
			IV	13	11	10	-	-	-	-	-	9	11	17	11	9	8	9	12

All values are in mm = millimeter

Sa-*Staphylococcus aureus, Sp-Streptococcus pyogenes, Sp*- S. pneumoniae, Ss- S. sanguis, Ss†- S. salivarius, Sm- S. mutans, La- Lactobacillus acidophillus, Ec- E.coli, Pa-Pseudomonas aeruginosa, Se- Staphylococcus epidermidis, St- Salmonella typhi, Ml- Micrococcus luteus, Bm- Bacillus megneterium, Bs- Bacillus subtilis, Pv- Proteus vulgaris, Ca- Candida albicans I-* Petroleum ether, II-Acetone, III-Methanolic, IV-Aqueous

Table 2: Antimicrobial activity of Churnas (mm) with 100 mg/ml of extracts

S. No	Methanolic extract of Churnas	Antimicrobial activity														
		Sa	Sp	Sp*	Ss	Ss†	Sm	La	Ec	Pa	Se	St	Ml	Bm	Bs	Pv	Ca
1.	Trikatu Churna	15	12	11	11	-	13	-	13	-	-	-	-	-	15	-	-
2.	Sitopladi Churna	12	12	12	11	-	11	-	13	-	-	-	-	-	14	-	-

All values are in mm = millimeters

Sa-*Staphylococcus aureus, Sp-Streptococcus pyogenes, Sp*- S. pneumoniae, Ss- S. sanguis, Ss†- S. salivarius, Sm- S. mutans, La- Lactobacillus acidophillus, Ec- E.coli, Pa-Pseudomonas aeruginosa, Se- Staphylococcus epidermidis, St- Salmonella typhi, Ml- Micrococcus luteus, Bm- Bacillus megneterium, Bs- Bacillus subtilis, Pv- Proteus vulgaris, Ca- Candida albicans* I-Petroleum ether, II-Acetone, III-Methanolic, IV-Aqueous

Table 3:- Showing MIC values against bacteria by two-fold serial dilution method

S. No	Plants (mg/ml)	Bacteria			
		B. subtilis	***E.coli***	***S. pneumoniae***	***S. pyogenes***
1	*P. longum*	0.0781	0.0391	0.0781	0.1593
2	*Z. officinales*	0.0391	0.3125	0.1593	0.1593

All values are in mg/ml

The results of the present study were encouraging and all the six plants and churnas appeared to contain substances that have antimicrobial properties. This correlates with the observations of previous workers made in different parts of the world (El Astal *et al.*, 2005, Ahmad *et al*., 1999, Okemo *et al.*, 2001).

All extracts showed the exhibited inhibitory activity against the pathogens that are not conventially incriminated with the diseases. The methanolic and aqueous extracts showed broad spectrum antimicrobial effects against tested pathogens because more organic components were leached in it. The antibacterial activities of the plants are particularly note worthy, considering the importance of these organisms in dental, respiratory and urinary tract infections. *Staphylococcus aureus* and *Streptococcus pyogenes* are more susceptible to lot of extracts obtained from the studied plants and churnas as also reported by Madamombe and Afotayan, (2003). MIC values of both the extracts showed that *B. subtilis* is the most susceptible bacteria among all tested organisms. Our findings have

validated the use of these medicinal plants and formulations for the treatment of respiratory tract infections as well as dental and urinary tract infections.

Further work is needed to locate the active principle from the various extracts and their phyto-pharmaceutical studies. Research into the effect of local medicinal plants is expected to boost the use of these plants in the therapy against diseases caused by the test microorganisms. It is possible that better therapy for microbial diseases can be found in the fruits, bark, leaves etc. of the plants.

The traditional therapeutic indication of the plant studied appears to have a fairly good degree of correlation with their specific antibacterial activity.

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