Screening of selected single and polyherbal Ayurvedic medicines for Antibacterial and Antifungal activity

Prashith Kekuda T.R , Kavya R, Shrungashree R.M, Suchitra S.V

ABSTRACT

The present study deals with antimicrobial activity of ayurvedic drugs containing single herb (Amalaki Choorna and Yastimadhu Choorna) and combination of herbs (DN-90 and Asanadi Kwatha Choorna). Disc diffusion method was used to assess antibacterial activity and antifungal activity was tested using Poison food technique. Absence of bacterial growth around the discs impregnated with the aqueous extracts of drugs and reduction of fungal growth in poisoned plates indicated antimicrobial activity. Further, the results of antibacterial activity of Amalaki choorna were comparable with standard drug Streptomycin. Asanadi Kwatha Choorna inhibited bacteria to more extent than Yastimadhu choorna and DN-90. Among fungi tested, more antifungal activity was observed against Mucor sp. The antimicrobial activity of drugs tested could be due to active principles present in them.

Key words: *Ayurvedic drugs, antimicrobial activity, inhibition zone, Disc diffusion method, Poison food technique.*

INTRODUCTION

Traditional medicine relies on many plants, and many current medicines have been developed from plants. Medicinal plants are important elements of traditional medicine in virtually all cultures. 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 constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds. Phytomedicines derived from plants have shown great promise in the treatment of various diseases including viral infections. Single and poly herbal preparations have been used throughout history for the treatment of various types of illness. Interest in plants with antimicrobial properties has revived as a result of current problems associated with the use of antibiotics (Kavya *et al*, 2007). In India, a number of plant extracts are used against diseases in various systems of medicine such as Ayurveda, Unani and Siddha. Only a few of them have been scientifically explored. Plant derived natural products have received considerable attention in recent years due to their diverse pharmacological activities. The ayurvedic approach to the prevention and treatment of microbial infection recognizes the emergency use of modern drugs, but recommends traditional herbal combinations and extracts known to balance the individual and improve health, as well as herbs that help to combat or prevent microbial infections. The Indian plants possessing significant antimicrobial activity are neem, long pepper fruit, *Tinospora cordifolia* (Willd.) Hook. f. & Thomson and *Emblica officinalis* Gaertn., among others (Treadway, 1998). The present work is a preliminary study on antibacterial and antifungal activity of powders of single herbs (*Amalaki* and *Yastimadhu choorna*) and two ayurvedic formulations containing combination of herbs (DN-90 and *Asanadi Kwatha Choorna*) against bacteria and fungi.

MATERIALS AND METHODS

Ayurvedic drugs tested

Four drugs have been chosen for the study. *Yastimadhu choorna, Amalaki choorna* and *Asanadi kwatha choorna* manufactured by S.D.M Ayurveda pharmacy, Udupi, Karnataka, *DN-90* manufactured by *Acharya Sushruta* Ayurvedic pharmacy, Sringeri, Karnataka were used against test organisms.

Extraction: Preparation of aqueous extract

10g of drug powder was added to 100ml of distilled water taken in a beaker and boiled for about half an hour. The contents were filtered and the filtrate was divided into two parts. One part was further reduced to 50% of the filtered volume and used for antifungal studies. Another part was condensed to almost pasty mass and used for antibacterial studies.

Screening for antibacterial activity (disc diffusion method)

The test bacteria were obtained from National Chemical Laboratory, Pune. Gram positive bacteria namely *Bacillus subtilis NCIM 2063, Staphylococcus aureus NCIM 2079* and Gram negative bacteria namely *Escherichia coli NCIM 2065, Enterobacter aerogenes NCIM 2340* were used. Test tubes containing sterile Nutrient broth were aseptically inoculated with the pure cultures of test bacteria maintained on slants and incubated at 37°C for 18 hours to get standard bacterial load. The broth cultures of test bacteria obtained after incubation were used for swab inoculation on agar media.

Dept. of Microbiology, S.R.N.M.N College of Applied Sciences, Shivamogga-01, Karnataka, India

The antibacterial activity was assessed using the simple disc diffusion method (Gnanamani *et al*, 2003). The test bacterial suspensions were spread over the plates containing Nutrient agar using a sterile cotton swab dipped in broth culture in order to get a uniform bacterial lawn growth. Sterile Whatman filter paper discs of 0.5 cm diameter were impregnated with condensed drug extract (5mg), dried and placed on medium inoculated with test bacteria. Streptomycin disc (10 mcg/disc) was used as standard. The plates were left for 30 min at room temperature to allow the diffusion of the drug, and then incubated at 37°C for 18 hours. After incubation, the inhibition zone was measured with a ruler. Experiment was performed in triplicate, and mean value was calculated and compared with standard (Streptomycin).

Screening for antifungal activity (poison food technique)

In the present study, we have selected two species of the genus *Aspergillus* (namely *Aspergillus niger, Aspergillus oryzae) and Mucor sp.* Some species of the genus *Aspergillus* are known to cause called Aspergillosis and *Mucor sp*. causes Mucorosis, the opportunistic mycotic infection. The fungal inoculum was prepared by making the suspension of the spores of the test fungi in a test tube containing 0.85% sterile normal saline containing 0.01% Tween 80 detergent (Rihakova *et al*, 2002). The fungal spores were taken from well grown cultures of test fungi using sterile inoculation loop under aseptic conditions and placed in sterile saline solution and mixed well using vortex mixer. The fungal spore suspension was used for inoculation on plates poisoned with the test drugs.

The antifungal activity was assessed using Poison food technique (Singh *et al*, 2005). The test fungus was allowed to grow in Sabouraud's dextrose agar plate poisoned with test drugs (10% aqueous extract of test drugs). The test fungi were inoculated by Point inoculation method where the spore suspension of test fungus was taken using inoculation needle and touched at the centre of the medium. The plates were incubated at room temperature for 72 hours. The effect of test drug on fungal growth was determined by measuring the diameter of the colony obtained on poisoned plate and comparing with colony diameter in control plates. The experiment was carried in triplicates and average reading was recorded.

RESULT AND DISCUSSION

Table 1: *In vitro* antibacterial activity of *Ayurvedic* drugs

Extract	Zone of inhibition in cm			
	E. coli	***S. aureus***	***E. aerogenes***	***B. subtilis***
Amalaki choorna	1.8	1.5	2.0	1.9
Yastimadhu choorna	0.7	1.0	1.0	0.8
DN-90	0.7	1.0	0.9	0.8
Asanadi Kwatha Choorna	1.2	1.3	1.3	1.3
Streptomycin	1.9	1.6	2.1	2.0

Table 2: *In vitro* antifungal activity of *Ayurvedic* drugs

Test fungus	Colony diameter in cm				
	Control	***Amalaki choorna***	***Yastimadhu choorna***	**DN-90**	***Asanadi kwatha choorna***
A. niger	5.6	5.0	2.7	5.4	3.8
A. oryzae	4.3	3.0	3.0	3.6	2.4
Mucor sp.	7.0	4.8	3.5	1.1	2.4

Table 3: Percentage reduction in growth of test fungi (as compared to control) by ayurvedic drugs

Test fungus	*Amalaki choorna*	*Yastimadhu choorna*	DN-90	*Asanadi kwatha choorna*
A. niger	10.71	51.80	03.58	32.14
A. oryzae	30.23	30.23	16.28	44.20
Mucor sp.	31.43	50.00	82.30	65.71

Antibacterial activity of test drugs is depicted in Table-1. *Amalaki choorna* and *Asanadi Kwatha choorna* were found to inhibit test bacteria to more extent when compared to DN-90 and *Yastimadhu choorna*. The test bacteria were more inhibited by *Amalaki choorna* and the results are almost comparable to the standard drug. Less activity was observed in case of DN-90 followed by *Yastimadhu choorna*. Table-2 reveals reduction in colony diameter of test fungi in poisoned plates. Percentage reduction in fungal growth in poisoned plates compared to control was depicted in Table-3. Medium poisoned with 10% aqueous *Amalaki choorna* extract showed good antifungal activity against test fungi. Growth retardation of about 30% was recorded in case of *A. oryzae* (30.5%) and *Mucor sp.* (31.5%). *A. niger* was least affected by the extract of *Amalaki choorna*. 10% aqueous extract of *Yastimadhu choorna* significantly reduced the growth of test fungi. Percentage inhibition of *A. niger*, *A. oryzae* and *Mucor sp.* was found to be 51.8, 30.2 and 50.0 respectively. *A. niger* was more inhibited followed by *Mucor* sp. and *A. oryzae*. Percent growth inhibition of *A. niger*, *A. oryzae* and *Mucor sp.* by the aqueous extract of DN-90 was found to be 3.6, 16.3 and 84.3 respectively. Maximum activity was observed against *Mucor sp.* The effect on *A. niger* and *A. oryzae* were not much appreciable. Aqueous extract of *Asanadi kwatha choorna* inhibited *A. niger*, *A. oryzae* and *Mucor sp.* and percentage inhibition was found to be 29.6%, 46.7% and 68.0% respectively. *Mucor sp*. was retarded to maximum extent followed by *A. oryzae* and *A. niger*.

The plant may be considered as a biosynthetic laboratory, not only for the chemical compounds such as carbohydrates, proteins, and lipids that are utilized by man, but also for a multitude of compounds like glycosides, alkaloids, volatile essential oils, tannins, flavones, terpenes etc., that exert a physiological effect (Kokate, 2004; Manjunatha *et al*, 2006; Sridhar *et al*, 2003). Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen derivatives. Most are secondary metabolites, of which at least 12000 have been isolated, a number estimated to be less than 10% of the total. In many cases, these substances serve as plant defense mechanism against predation by microbes, insects and herbivores. Some, such as terpenoids, give plants their odors while quinones and tannins are responsible for plant pigments. Many compounds are responsible for plant flavor as in case of terpenoid capsaicin from chili peppers. Some of the same herbs and spices used by humans to season food yield useful medicinal compounds (Cowan, 1999). One of the best approaches in search for antimicrobial agents from plant resources is the selection of plants based on ethno medical leads and testing the selected plants efficacy and safety in light of modern science (Gupta *et al*, 2004).

CONCLUSION

Single and poly herbal preparations have been used throughout history for the treatment of various types of illness. *Ayurvedic* drugs (either singly or in herbal combination) have been proven promising in inhibiting microbes that have developed drug resistance. All the drugs tested were found to possess good antimicrobial activity. The two drugs (having combination of drugs) namely *Asanadi Kwatha Choorna* and DN-90 were found to possess antimicrobial activity in addition to the activity (Antidiabetic) for which they are prescribed. Thus consumption of such herbal medicine could bring multifold beneficial effects. This is the first report on antimicrobial activity of two ayurvedic drugs namely DN-90 and *Asanadi Kwatha Choorna* recommended primarily in Diabetes mellitus. The antimicrobial activity of drugs tested could be possibly due to the presence of various phytochemical constituents present in them. *In vivo* experiments have to be carried in animal models which could reveal similar results as that of *in vitro* trials.

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