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Full Length Research Paper

Antimicrobial activity of ten common herbs, commonly known as 'Dashapushpam' from Kerala, India

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Ten herbs which are widely used in Ayurvedic system of medicine and are collectively known as "Dashapushpam "in Kerala, (India), were screened for their antimicrobial properties against nine spp. of pathogenic fungi and seven spp. of pathogenic bacteria. In the preparation of extracts, the entire shoot systems were used for *Cardiospermum halicacabum* and *Evolvulus alsinoides* and only leaves for others. Crude plant extracts were prepared by cold extraction with acetone. Two sets of pathogenic fungi-Set-1 and Set- 2-were used with Nystatin and Amphotericin as standards, respectively. From Set-1, all the extracts showed antimicrobial properties at least with two fungal species, the most commendable being the extract of *Vernonia cinerea* which was effective against all the fungal strains, outscoring the standard Nystatin. Only *Ipomoea sepiaria* and *V. cinerea* could inhibit the growth of *Rhodotorula* sp. Among the six extracts tested with Set- 2, only *I. sepiaria* and *V. cinerea* were effective which inhibited the growth of only one strain, in sharp contrast to the activity of the standard, Amphotericin which was highly effective against all other spp. The extracts of *Aerva lanata*, *C. halicacabum* and *V. cinerea* inhibited the growth of six, seven and four species of bacteria respectively. Results show that the herbal extracts involved are more effective against pathogenic fungi than pathogenic bacteria and throws light on the future prospects of plants as sources of potent antibiotics.

Key words: Antimicrobial, pathogenic fungi, pathogenic bacteria, crude plant extract, dashapushpam.

INTRODUCTION

Dashapushpam literally means "ten flowers" ('Dasham' refers to'Ten' and 'Pushpam' refers to 'flowers'). In the present context, the word refers to' ten species of plants'. They are considered auspicious in Kerala (India) and each herb is associated with a Deity in Hindu Mythology. All are used as ingredients in various Ayurvedic formulations (Sindhu et al., 2009). They are therapeutically active against fever, dysentery, haemorrhage, constipation etc (Radhamani and Muralidharan, 2000).

Herbs play a significant role in Pharmaceutical industries as natural sources of life saving drugs (Trease and Evans, 1983; Khanna et al., 2008). Healthcare sectors around the world, more frequently than ever are facing the problems of combating the entry of novel, mutant pathogenic strains of microorganisms and their resistance against synthetic drugs. This calls for the discovery of new antimicrobial compounds with diverse chemical structures and novel mechanisms of action. Natural products either as pure compounds or as standardized plant extracts are the right solutions because of their unmatched display of chemical diversity (Parekh and Chanda, 2007). Essential oils from some plants like Allium sp. having antimicrobial properties can be used as natural antimicrobial additives in food production (Benkeblia, 2004). But for the development of safe and effective antimicrobials based on indigenous knowledge and ethnomedicine, the screening of enormous, untapped plant wealth is inevitable (Amit and Shailendra, 2006). Joy et al. (2008) have done extensive research on the native and naturalized plants of Minnesota and neighbouring areas. The wild flora of Peninsular India, one among the mega diversity centres in the world still remains to be exploited for its medicinal properties. With this background, "Dashapushpam" herbs

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Table 1. List of "Dashapushpa" herbs.

S/no	Botanical name of the herb	Sanskrit name	Popular english name	Some popular medicinal uses	Family	Deity in Hindu Religion
01.	*Aerva lanata* (L) Juss.	Bhadra	-	Anti diabetic, diuretic.	Amaranthaceae	Yama Dev
02.	*Biophytum sensitivum* (L.) DC.	Peethapushpa	-	For curing chest and stomach complaints	Oxalidaceae	Shree Parvathy
03.	*Cardiospermum halicacabum* (Linn.)	Chakralatha	Baloon vine	For curing stomach disorders, fever, jaundice.	Menispermaceae	Lord Indra
04.	*Curculigo orchioides* Gaertn.	Varaahi	Black musali	For curing leucorrhoea, asthma, diarrhoea.	Amaryllidaceae	Bhumi Devi
05.	*Cynodon dactylon* (Pers.)	Durva	Bermuda grass	For treating Haemorrhage, piles, eczema.	Poaceae	Sun
06.	*Eclipta alba* (L.) Hassk.	Kesharaja	-	For curing cough, sinus, eye diseases, for lustrous hair.	Asteraceae	Lord Shiva
07.	*Emilia sonchifolia* (L.) DC.	Jaraayu priya	Canada Flea-bane	For curing pulmonary disorders, sore throat.	Convolvulaceae	Kamadeva
08.	*Evolvulus alsinoides* (Linn.) Linn.	Harikranthi	-	For curing stomach disorders, haemorrhage.	Convolvulaceae	Lord Vishnu
09.	*Ipomoea sepiaria* Roxb.	Nagini	-	A uterine tonic	Convolvulaceae	Shree Bhagavathy
10.	*Vernonia cinerea* L.	Sahadevi	Ash-coloured Flea-bane	To cure eruptive boils, leprosy.	Asteraceae	Brahma

that grow wild in Kerala (India) are screened for their antimicrobial properties.

MATERIALS AND METHODS

Collection of herbs

All plants were collected from the college campus except *C. halicacabum* and *E. alsinoides* the dry specimens of which were provided by Kottakkal Arya Vaidhyasala Herbal Garden authorities, Kerala, India .The specimens were identified in the college laboratory, Dept of Botany. Photo documentation of all plants was done and herbaria prepared on sheet size 45 x 30 cm (Table 1).

Preparation of plant extract

Except for *C.halicacabum and E. alsinoides,* leaves were used for testing. Because of the scarcity of specimens, entire shoot systems were used for assay in the case of *C. halicacabum and E. alsinoides*. Specimens were shade dried, cut into small pieces, weighed and powdered in an electric mixer.

Powdered plant materials were kept in separate conical flasks and labeled. 250 ml of Acetone was added, shaken well, plugged with cotton and kept at room temperature (27°C) for seven days. On the eighth day, contents were shaken well and filtered through tea strainer. The acetone solutions thus obtained were concentrated in Rotavapor (Laborota 4000-Heidolph).

Nutrient media, standard antibiotics and pathogenic microorganisms involved

Potato dextrose agar and nutrient agar media were prepared for culturing fungal and bacterial pathogens respectively (Aneja, 1996). Pure cultures of fungal and bacterial pathogens were obtained from Goa medical college, Goa, India.

Standard antibiotics used: Amphotericin- 10 mcg/ ml, Nystatin - 100 units or 100 mcg / disc and Streptomycin- 10 mcg / disc.

Microorganism: For investigations, two sets of fungal pathogens were used -Set - 1 and Set–2 with two different reference drugs namely Nystatin and Amphotericin respectively and one set of bacterial pathogens with the reference drug, Streptomycin.

Table 2. Zone of inhibition for "Dashapushpam" extracts compared to reference drugs against pathogenic fungi from Set 1.

S/no.	Name of the herb	Name of the pathogenic fungi						
		***A. fumigatus* (mm)**	***A. niger* (mm)**	***C. albicans* (mm)**	***C. neoformans* (mm)**	***Fusarium* sp. (mm)**	***Nocardia* sp. (mm)**	***Rhodotorula* sp. (mm)**
01.	*Aerva lanata*	-	4	-	4-5	2	2	-
02.	*Biophytum sensitivum*	2	3	-	2 static	-	2 static	-
03.	*Cardiospermum halicacabum*	-	3-4	-	4	1-2	1-2	-
04.	*Curculigo orchioides*	-	-	-	-	-	-	-
05.	*Cynodon dactylon*	6	4-5	2	4	-	2 static	-
06.	*Eclipta alba*	-	1-2	-	2	0-1	1-2	-
07.	*Emilia sonchifolia*	5	4	-	3	-	3	-
08.	*Evolvulus alsinoides*	-	1-2	-	1-2	-	-	-
09.	*Ipomoea sepiaria*	-	1	-	1 static	-	-	1 static
10.	*Vernonia cinerea*	6	5-6	6-7	5	5	6-7	4 static
11.	Std. Nystatin	6	-	-	-	-	-	-

Table 3. Zone of inhibition for "Dashapushpam" extracts compared to reference drugs against pathogenic fungi from Set 2.

S/No.	Name of the herb	Name of the pathogenic fungi						
		A. fumigatus	***C. albicans***	***C. albicans* SC09**	***C. glabrata* H05 fluc R**	***C. glabrata* H05 fluc S**	***G. krusei* G03**	***C. krusei* G06**
01.	*Aerva lanata*	----------	----------	----------	----------	----------	----------	----------
02.	*Biophytum sensitivum*	-	-	-	-	-	-	-
03.	*Cardiospermum halicacabum*	----------	----------	----------	----------	----------	----------	----------
04.	*Curculigo orchioides*	-	-	-	-	-	-	-
05.	*Cynodon dactylon*	-	-	-	-	-	-	-
06.	*Eclipta alba*	----------	----------	----------	----------	----------	----------	----------
07.	*Emilia sonchifolia*	-	-	-	-	-	-	-
08.	*Evolvulus alsinoides*	----------	----------	----------	----------	----------	----------	----------
09.	*Ipomoea sepiaria*	-	-	-	12 mm vh	-	-	-
10.	*Vernonia cinerea*	-	-	-	11 mm vh	-	-	-
11.	Std. Amphotericin	11 mm	16 mm	15 mm	18 mm	15 mm	12 mm	15 mm

vh- very hazy, - No zone of inhibition developed, ---------- Extract not used in testing.

Fungal pathogens from Set–1 with Nystatin as standard antibiotic: *Aspergillus fumigatus, Aspergillus niger, Candida albicans*, *Cryptococcus neoformans, Fusarium* sp., *Nocardia* sp. and *Rhodotorula* sp. (Table 2).

Fungal pathogens from Set-2 with Amphotericin as standard antibiotic: *Aspergillus fumigatus, Candida albicans*, *Candida albicans* S CO9, *Candida glabrata* HO5 fluc R, *Candida glabrata* HO5 fluc S, *Candida krusei* GO3 fluc R and *Candida krusei* GO3 fluc S. Only six plant sps. were used here namely *B. sensitivum*, *C. orchioides*, *C. dactylon*, *E. sonchifolia*, *I. sepiaria* and *V. cinerea* (Table 3).

Bacterial pathogens with Streptomycin as standard antibiotic were: *Escherichia coli, Klebsiella* sp. *Pseudomonas aeruginosa, Salmonella typhii, Shigella flexinerii, Staphylococcus aureus and Vibrio cholerae* (Table 4).

Disc diffusion and Agar well method were followed when Nystatin and Amphotericin were used as the standards respectively with pathogenic fungi. For pathogenic bacteria, disc method was followed. Results were recorded by measuring the zone of inhibition around the discs or wells and compared with the standards (Casida, 1997).

RESULTS

With pathogenic fungi from Set- 1 when Nystatin was used as the standard - extract of *A. lanata* was effective against *A. niger*, *C. neoformans, Fusarium* and *Nocardia* sps. *B.sensitivum* inhibited the growth of *A. fumigatus*, *A. niger*, *C. neoformans* and *Nocardia* sp. *C. halicacabum* was effective against *A. niger*, *C. neoformans*, *Fusarium* and *Nocardia* sps. Extract of *C. orchioides* was ineffective against all pathogenic fungi. Extract of *C. dactylon* was

Table 4. Zone of inhibition for "Dashapushpam" extracts compared to reference drugs against pathogenic bacteria.

S/no.	Name of the herb	Name of the pathogenic bacteria						
		E. coli (mm)	*Klebsiella* sp. (mm)	*P. aeruginosa* (mm)	*S. typhii* (mm)	*S. flexineri* (mm)	*S. aureus* (mm)	*V. cholerae* (mm)
01	*Aerva lanata*	2	-	0-1	1-2	1	0-1	1-2
02	*Biophytum sensitivum*	-	-	-	-	-	-	-
03	*Cardiospermum halicacabum*	1	2	+	0-1	1	0-1	1-2
04	*Curculigo orchioides*	-	-	-	-	-	-	-
05	*Cynodon dactylon*	-	-	-	-	-	-	-
06	*Eclipta alba*	-	-	-	-	-	-	-
07	*Emilia sonchifolia*	-	-	-	-	-	-	-
08	*Evolvulus alsinoides*	-	-	-	-	-	-	-
09	*Ipomoea sepiaria*	-	-	-	-	-	-	-
10	*Vernonia cinerea*	2-3	-	2-1	-	3-4	-	3-4
11	Std. Streptomycin	6-7	6-7	6-7	4-5	5-6	5-6	6

effective against all fungi except *Fusarium* and *Rhodotorula* sps. Extract of *E. alba* was effective against *A. niger, C. neoformans*, *Fusarium* and *Nocardia* sp. Extract of *E. sonchifolia* inhibited the growth of *A. fumigatus*, *A. niger*, *C. neoformans* and *Nocardia* sp. Extract of *E. alsinoides* was effective against *A. niger* and *C. neoformans* .Extracts of *I. sepiaria* was effective against *A. niger*, *C. neoformans* and *Rhodotorula* sp. Extract of *V. cinerea* was effective against all pathogens. The standard antibiotic Nystatin was found to be ineffective against all pathogenic fungi except *A. fumigatus* and *Nocardia* sp. (Table 2).

With pathogenic fungi from Set 2 when Amphotericin was used as the standard, only six plant extracts were used for the assay. Extract *of I. sepiaria* was effective against *C. glabrata* HO5 fluc R with a zone of inhibition as 12 mm compared to 18 mm size of the standard, Amphotericin. Extract of *V. cinerea* also inhibited the growth of *C. glabrata* HO5 fluc R and size of zone of inhibition was 11 mm. All other extracts were ineffective (Table 3).

With pathogenic bacteria and Streptomycin as standard, extract of *A. lanata* was effective against all bacteria except *Klebsiella* sp. Extract of *V. cinerea* inhibited the growth of *E. coli*, *P. aeruginosa*, *S. flexineri* and *V. cholerae.* All other extracts were ineffective. Streptomycin showed antibacterial activity against all pathogenic bacteria (Table 4).

DISCUSSION

A critical analysis of the results of bioassays shows that all the ten herbs inhibited the proliferation of one pathogen or the other with which they are tested with.

A. lanata was active against four fungal species and was effective against all bacterial sp., except *Klebsiella*. *B. sensitivum* showed mild inhibition against four species of fungi. *C. halicacabum* inhibited the growth of four species of fungal pathogens. *C. orchioides* was ineffective against all pathogenic fungi and bacteria. *C. dactylon* was equivalent to the standard Nystatin against *A. fumigatus* and was effective against four more species. Extract of *E. alba* showed mild antimicrobial properties with four sps. of fungi. *E. sonchifolia* inhibited the growth of four sps. of pathogenic fungi. *E. alsinoides* exhibited antimicrobial properties against two fungal pathogens only. *I. sepiaria* showed mild activity preventing the spread of three species of fungi and was successful against *C. glabrata* HO5 fluc R when all others were ineffective except *V. cinerea. V. cinerea* showed mild antibacterial properties and were effective against four bacterial sps.

Earlier researches show that ethyl acetate and methanol extracts of *A. lanata* have some interesting antimicrobial properties (Chowdhury et al., 2002).

Flavonoids, triterpenes and tannins are present in this herb and the antimicrobial properties are due to this (Soundararajan et al., 2007). A comparative study of the antimicrobial properties of Cardiospermum grandiflorum and C. halicacabum showed that C. grandiflorum is more effective than the latter against C.albicans, and S. aureus (Banso, 2007). Seeds of *C. halicacabum* contain myristic acid, ricinoleic acid etc. and can act against *A. niger*, *C. albicans*, *E. coli*, *P. aeruginosa*, *S. typhi* (Neogi et al., 2008). Steam distilled aqueous extract of rhizomes of *C. orchioides* is effective against pathogenic bacteria like *S. aureus*, *E. coli* and *P. aeruginosa* (Nagesh and Shanthamma, 2009) and the roots exhibit antimicrobial properties when fractionated with different solvents. It is observed that it acts against pathogenic fungi like *A. flavus* and *A. niger* and pathogenic bacteria like

P. aeruginosa, K. pneumonieae and *S. aureus* (Rajesh and Gupta, 2008). Active compounds like flavones, steroids, triterpenoides and other secondary metabolites (Misra et al., 1984; Misra et al., 1990; Xu et al., 1992) are reported from the herb and the antimicrobial properties of *C. orchioides* can be attributed to the above mentioned compounds.

Methanolic leaf extracts of *E. alba* is active against *Plasmodium berghei* ANKA strain which cause malaria in mice (Bapna et al., 2007). *E. alsinoides* is known to demonstrate only mild activity against *B. subtilis*, *P. aeruginosa* and *S. aureus* when acetone extract was used wheras methanolic extract is found to be highly effective (Tharan et al., 2003).

A study by Muko and Ohiri (2000) brings to light, the antimicrobial and anti-inflammatory properties of *E. sonchifolia* leaves. *E. alsinoides* contains alkaloids like betaine shakhupushpaine and evolvine (Singh, 2008) which may be responsible for this. Earlier researches show that Benzene extract of *V. cinerea* shows a wide spectrum of antibacterial activity (Gupta et al., 2003). The herb contains compounds like sitosterol, stigmasterol, spinasterol and phenolic resins (Satish, 1970). *In vitro* studies with callus, cell suspension and root culture of *V. cinerea* record alkaloid production (Priti Maheshwari et al., 2007). This justifies the wide spectrum antibiotic properties of *V. cinerea*.

Conclusion

An overall assessment of the antimicrobial qualities of “Dashapushpam“ herbs show that they are more effective as antifungal agents than as antibacterial agents. It has to be noted that *V. cinerea* was effective against all pathogenic fungi when Nystatin was the standard and the inhibition zones were of equal dimensions with *A. fumigatus*. The latter was true even in the case of *C. dactylon*. *A. lanata, C. halicacabum*, and *V. cinerea* were the only herbs that showed antibacterial properties. Investigations using various plant parts of the above mentioned herbs with different solvents and varying concentrations of extracts are needed for a better understanding of their potential as antibiotics. A deeper research into the antimicrobial properties of all the plants of the “Dashapushpa” group solvents will be rewarding.

As exemplified by the present and previous investigations, the future perspectives of phytoantibiotics hold invaluable promises in the field of medicine. Antimicrobial properties of herbs are due to the presence of secondary metabolites and are bactericidal or bacteriostatic depending upon their concentrations (Rasooli et al., 2002) or may be fungistatic or fungicidal. The success of future pharmaceutical research depends on the maximum utilization of hitherto untapped bioresources. Scientists are exploring into the relevance of folk medicines, searching for new plant sources for drugs having exceptional powers against mutant forms. Such investigations will definitely enrich, equip and empower the clinical world for the challenges ahead. Traditional wisdom combined with rational scientific temperament, utilizing the modern technology can surely bring in revolutionary changes in healthcare segments the world over in this era of globalization.

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