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Antimicrobial activity of methanol extract and fractions from *Sarcochlamys pulcherrima*

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Abstract

Antimicrobial evaluation of methanol extract of *Sarcochlamys pulcherrima* leaf and its hexane, ethyl acetate, *n*-butanol and water fractions against 31 strains of microorganisms, using agar well, agar disc diffusion, and broth microdilution methods, revealed the activity of methanol extract against *Trichophyton mentagrophytes*, *Candida albicans*, *Staphylococcus aureus* and *Escheracia coli* (zone of inhibition: 21-40 mm, 200 mg/mL, and MIC: 6.25-50 mg/mL). All fractions also displayed antimicrobial activity (5-20 mg/mL), indeed ethyl acetate and *n*-butanol fractions showed better activity (MIC: 0.156 to 2.5 mg/mL). *C. albicans* was most sensitive to n-butanol fraction (15 mm, 2.5 mg/mL). Ethyl acetate and n-butanol fractions were more active against *T. mentagrophytes* (12 mm at 1.25 mg/mL) and *S. aureus* (ethyl acetate-16 mm, *n*-butanol-14 mm at 0.625 mg/mL). *E. coli* was inhibited by n-butanol fraction (13 mm at 2.5 mg/mL). Further, *n*-butanol fraction (400 µg/disc) exhibited promising activity against 14 bacteria, 2 dermatophytes and 2 yeasts strains.

Introduction

The steadily increasing drug resistant microorganisms such as bacteria, *Candida* species and dermatophytes are now a therapeutic challenge. Bacterial and fungal pathogens have evolved numerous defense mechanisms against antimicrobial agents, and resistance to the existing drugs is on the rise. A study on antibiotic resistance in *Staphylococcus aureus* clinical isolates from diabetic patients showed that 80% of the isolates were multidrug-resistance to more than eight antibiotics and 35% isolates were methicillin resistant *S. aureus* (MRSA) (Raju et al., 2010). Opportunistic fungal infections are common among immunocompromized patients (Martinez-Rossi et al., 2008; Straten et al., 2003). The indiscriminate use of antibiotics also contributes to the worsening of this picture (Straten et al., 2003). Opportunistic fungal infections caused by *Candida* species produce a wide spectrum of diseases, ranging from superficial mucocutaneous disease to systemic candidia -sis, of which *C. albicans* was reported to be the most predominant species (Shokohi et al., 2010; Sharma and Borthakur, 2007).

In India the data on the burden of opportunistic mycoses is not clear though the climate in this country is well suited for of fungal infections. However, increased incidence of invasive candidiasis was reported in India (Chakrabarti et al., 2008). Predominance of dermatophyte infections has also been reported in many countries (Sharma and Borthakur 2007; Jain et al., 2008; Jaiswal, 2002). Thus, the clinical efficacy of many antimicrobial drugs is being threatened by the emergence of multidrug-resistant pathogens. This has led to the search for new antimicrobial substances in natural products particularly medicinal plants, focusing on the basis of their ethnomedicinal uses, which enhance the probability of success in finding new drugs (Svetaz et

al., 2010).

Sarcochlamys pulcherrima (Roxb.) Gaud. (Urticaceae), is a wild medicinal plant and highly consumed by some ethnic tribes and castes of Assam, India. The leaves are used in treatment of boils and fever blisters, eye complications (Rahman et al., 2007), diarrhea and dysentery (Sharma and Pegu, 2011). Young shoots, leaves and fruits are eaten as vegetable (Sawian et al., 2007; Singh et al., 2012). It is believed that eaten with pork facilitate the digestion of fats (Buragohain, 2011). It is also claimed that *S. pulcherrima* leaves damages tape worm egg present in pork when boiled with it (Paul et al., 2010). In spite of its numerous medicinal properties there is no report on *S. pulcherrima* related to antimicrobial activity. In our earlier study, we observed promising antimicrobial and anti-oxidant activity of the methanol extract of *S. pulcherrima* leaves. Encouraging with the findings, the present study was undertaken to fractionate the methanol extract followed by antimicrobial test in order to identify the fractions with better activity.

Materials and Methods

Microorganism and inoculums

The test microorganisms consisted of 9 dermatophytes strains, 7 yeasts, 10 Gram (+ve) bacteria and 5 Gram (-ve) bacteria (Table I). Nutrient agar (NA, Himedia) was used for culturing the bacteria. For dermatophytes and yeasts, sabouraud dextrose agar (SDA, Himedia)) was used. Bacterial inoculum (1 x 10^8 CFU/mL) was prepared in nutrient broth (Himedia) (Teke et al., 2011). Dermatophytes and yeasts were subcultured in sabouraud dextrose broth (Himedia) to obtain the inoculum density of 2.5 x 10^4 CFU/mL (Hammer et al., 2002) and 1 x 10^8 CFU/mL (Parekh and Chanda, 2008) respectively. The bacteria and the yeasts were incubated at 37 ± 2°C and 28 ± 2°C respectively for 48 hours, while the dermatophyte cultures were incubated at 28 ± 2°C for 10 to 15 days in each experiment.

Plant

Fresh leaves of *S. pulcherrima* were collected from the Arun Punjee (*Punjee* means Tribal Village) of Machkhal, Cachar District, Assam (India). The plant was authenticated at Botanical Survey of India, Eastern Regional Centre, Shillong, India. The herbarium was deposited at the herbarium repository of Defence Research Laboratory, Tezpur, Assam.

Extraction and fractionation of plant material

Shade dried powdered leaves were extracted with methanol (Hayet et al., 2008) and dried at 40°C under reduced pressure using rotary evaporator (Heidolph Instruments GmbH and Co. KG, Germany and lyophilised (The Benchtop FreeZone plus Cascade 4.5L Freeze Dry System, Labconco, USA). The methanol extract was

Table I

Test microorganisms

	Dermatophytes	Gram (+ve) bacteria
1	*Epidermophyton floccosum* var. Nigricans (MTCC 613)	*Bacillus subtilis* (MTCC 121) *B. subtilis* (MTCC 441)
2	*Microsporum boullardii* (MTCC 6059)	*B. subtilis* (MTCC 619)
3	*M. fulvum* (MTCC 8478)	*B. subtilis* (MTCC 736)
4	*M. gypseum* (MTCC 2829)	*B. subtilis* (MTCC 2616)
5	*M. gypseum* (MTCC 2830)	*B. subtilis* (Cinical isolate)
6	*M. gypseum* (MTCC 8469)	*Micrococcus luteus* (MTCC 106)
7	*Trichophyton rubrum* (MTCC 8477)	*Staphylococcus aureus* (Cinical isolate)
8	*T. rubrum* (MTCC 296)	*S. aureus* sub sp. *aureus* (MTCC 737)
9	*T. mentagrophytes* (MTCC 8476)	*S. aureus* sub sp. *aureus* (MTCC 96)
	Yeasts	Gram (-ve) bacteria
1	*Candida albicans* (Cinical isolate)	*Enterobacter aerogenes* (MTCC 111)
2	*C. albicans* (MTCC 183)	*Proteus mirabilis* (MTCC 743)
3	*C. albicans* (MTCC 3018)	*Yersinia enterocolitica* (MTCC 4848)
4	*C. glabrata* (MTCC 3019)	*Salmonella enteria* ser. *typhi* (MTCC 733)
5	*C. parapsilosis* (MTCC 4448)	*Escheracia coli* (Cinical isolate)
6	*C. tropicalis* (MTCC 1000)	
7	*Trichosporon beigelii* (Cinical isolate)	

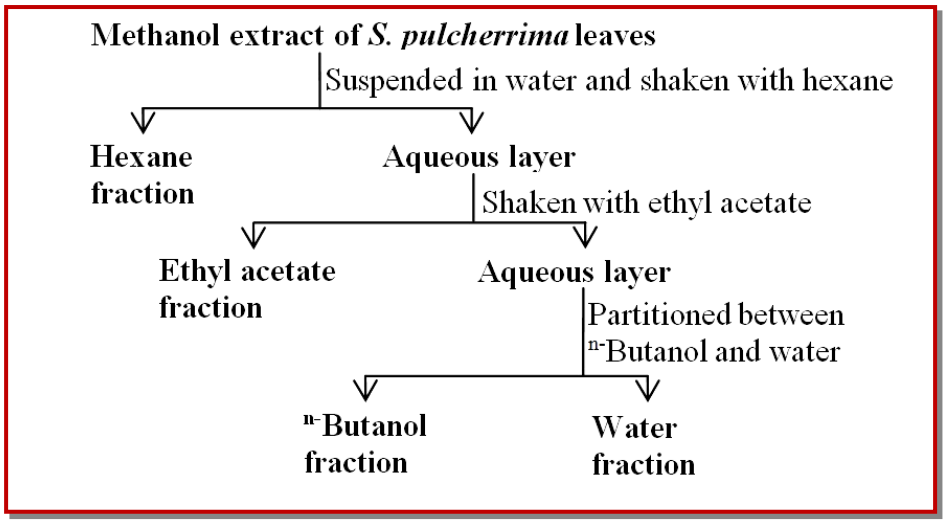

Figure 1: Fractionation of methanol extract from *S. pulcherrima* (leaf)

Table II

Antimicrobial activity of methanol extract from *S. pulcherrma* leaves

Microorganism	Zone of inhibition (mm)				MIC (mg/mL)
	Methanol extract (200 mg/mL)	Clotrimazole (0.1 mg/mL)	Amphotericin B (0.1 mg/mL)	DMSO	
T. mentagrophytes	28	31	No zone	No zone	6.25
C. albicans	21	17	No zone	No zone	12.50
S. aureus	40	No zone	25	No zone	12.50
E. coli	22	No zone	22	No zone	50.00

sequentially fractionated with hexane, ethyl acetate, n-butanol and water as shown in Figure 1. On subsequent evaporation of solvents afforded hexane (7.66 g), ethyl acetate (7.04 g), n-butanol (14.19 g) and water (21.37 g) fractions. Test samples of extract and fractions were prepared in dimethyl sulfoxide (DMSO, w/v) and filtered using 0.22 mm Millipore filter (MILLEX® GP, Ireland).

Antimicrobial assay and determination of minimum inhibitory concentration (MIC)

Antimicrobial activity was tested by agar well diffusion (Kaushik and Goyal, 2008) and agar disc diffusion method (Trakranrungsie et al., 2008). The culture (SDA/NA) plates were swabbed with 150 mL of the inoculum and loaded with 150 mL of the test extract/ fraction into the well of 8 mm diameter, made on the agar plate. DMSO was used as negative control while clotrimazole and amphotericin B were used as positive control for fungi and bacteria respectively. The zone of inhibition was recorded to evaluate the antimicrobial activity. In agar disc diffusion method, sterile paper discs (Whatman No. 1 paper, 5 mm diameter) were impregnated with test sample (400 µg/disc), clotrimazole (10 µg/disc), amphotericin B (10 µg/disc) and methanol separately, dried. The discs were placed on the surface of the agar plate, preswabbed with 150 mL of inoculum and recorded the zones of inhibition. MIC was determined by broth microdilution method, as described earlier (Hammer et al., 2001; Edziri et al., 2012) with some modifications. Test sample was serially diluted in 96-well microtiter plate prepared with NB or SDB to obtain a concentration ranging from 39 to 5,000 µg/mL. Inoculum concentration in each well was adjusted as mentioned above. MIC was interpreted as the lowest concentration of the test sample which showed no visible growth. Five replicates were maintained in each experiment.

Results and Discussion

As per standard protocol a small set of reference microorganisms, which represent common pathogenic species of different classes may be used in primary antimicrobial screening (Paul et al., 2006). Hence, four microorganisms namely, *T. mentagrophytes*, *C. albicans*, *S. aureus* and *E. coli*, which represented dermatophytes, yeast, Gram (+ve) and Gram (-ve) bacteria respectively were used in the initial antimicrobial study by agar well

Table III

Antimicrobial activity of different solvent fractions of *S. pulcherrima* methanol extract

Fraction	Microorganism	Zone of inhibition (mm), Conc.entration- mg/mL						MIC (mg/mL)
		20	10	5	2.5	1.25	0.625	
Hexane	*T. mentagrophytes*	15	12	10	-	-	-	1.25
	C. albicans	24	19	14	-	-	-	2.5
	S. aureus	21	20	18	16	12	-	1.25
	E. coli	13	11	9	-	-	-	5
Ethyl acetate	*T. mentagrophytes*	22	20	18	15	12	-	0.625
	C. albicans	24	20	18	-	-	-	1.25
	S. aureus	28	27	26	25	18	16	0.625
	E. coli	10	9	9	-	-	-	5
n-Butanol	*T. mentagrophytes*	22	19	18	16	12	-	0.156
	C. albicans	30	26	16	15	-	-	0.625
	S. aureus	24	23	20	19	15	14	0.625
	E. coli	21	20	18	13	-	-	2.5
Water	*T. mentagrophytes*	11	9	9	-	-	-	5
	C. albicans	24	20	15	-	-	-	2.5
	S. aureus	18	16	15	11	7	-	1.25
	E. coli	9	-	-	-	-	-	20

diffusion assay. The methanol extract exhibited antimicrobial activity against the test pathogens exhibiting zone of inhibition in between 21 and 40 mm at 200 mg/mL and MIC values of 6.25-50 mg/mL (Table II). The Gram (+ve) bacterium, *S. aureus* was found to be more sensitive than the Gram (-ve) bacterium, *E. coli*, which has been well established earlier (Paul et al., 2006; Jayaraman, 2009; Yagi et al., 2012). Clotrimazole (0.1 mg/mL) produced zone of inhibition of 31 mm against *T. mentagrophytes* and 17 mm against *C. albicans*, while the zone of inhibition caused by amphotericin B against *S. aureus* and *E. coli* were 25 and 22 mm respectively. Activity of clotrimazole and amphotericin B at very low concentration (0.10 mg/mL) as compared to the plant extracts may be attributed to its pure nature. Therefore higher MICs of the crude methanol extract, observed in the present study were considered as effective. DMSO did not inhibit the growth of test pathogens. All fractions (5-20 mg/mL) revealed activity against *T. mentagrophytes*, *C. albicans*, *S. aureus* and *E. coli* (zone of inhibition: 9-30 mm), although ethyl acetate and n-butanol fractions showed better activity (Table III). For *C. albicans*, n-butanol fraction was found to be the most active (15 mm at 2.5 mg/mL). Ethyl acetate and n-butanol fractions were more active against *T. mentagrophytes* (12 mm at 1.25 mg/mL) and *S. aureus* (ethyl acetate-16 mm, *n*-butanol-14 mm at 0.625 mg/mL). Gram (+ve) bacterium, *S. aureus* was found to be more sensitive than Gram (-ve) bacterium *E. coli* towards the test fractions. Only n-butanol fraction showed a bit activity against *E. coli* (13 mm at 2.5 mg/mL). Susceptibility difference between Gram (+ve) and Gram (-ve) bacteria may be due to the cell wall structural differences between them. Outer phospholipids membrane with the structural lipopolysaccharide components in Gram (-ve) bacterium, make the cell wall impenetrable to antimicrobial agents, while the Gram (+ve) bacterium is more susceptible having only an outer peptidoglycan, which is not an effective permeability barrier (Jayaraman 2009; Yagi et al., 2012). Over all, n-butanol and ethyl acetate fraction exhibited better antimicrobial profile with MIC within the range of 0.156-2.5 mg/mL related to the tested bacteria and fungi (Table III). The activity was more pronounced than the hexane and water fractions and methanol extract. The results indicated that the pattern of inhibition depends largely upon the solvent used for fractionation of the plant material and the organisms tested. It can be inferred that antimicrobial compounds are present mostly in ethyl acetate and n-butanol fractions, which might be medium polar to polar in nature. Similar obser-vations were also reported earlier in other medicinal plants (Teke et al., 2011; Das et al., 2010; Mazumder et al., 2012). Earlier in a study, higher range of antidermatophytic activity of hexane fraction of *O. gratissimum* leaves was reported (Silva et al., 2005). Contrary to this, in our study the hexane fraction was found to be less effective. This difference may be due to differences in plant constituents in different plant species, time of sample collection or other geographical factors.

Owing to higher extractive value and the promising activity, n-butanol fraction was further tested against 9 strains of dermatophytes, 7 yeasts, 10 Gram (+ve) and 5 Gram (-ve) bacteria employing agar disc diffusion method to ascertain its maximum potentiality in

combating a wide range of pathogenic microbes. The n-butanol fraction (400 µg/disc) exhibited broad spectrum of activity against majority of the test bacteria, except *B. subtilis* (MTCC 441) and *S. enteria ser. typhi* (MTCC 733). The most sensitive bacterium was *B. subtilis* (MTCC 619) (zone of inhibition: 20 mm), followed by *S. aureus sub sp. aureus* (MTCC 96) (zone of inhibition: 12 mm). The remaining bacteria were moder -ately sensitive (zone of inhibition: 6-12 mm). The dermatophytes and yeasts were observed to be comparatively less sensitive showing inhibition (zone of inhibition: 0.6 mm) of only *E. floccosum var. Nigricans* (MTCC 613) and *M. boullardii* (MTCC 6059) and two yeasts namely *C. albicans* (MTCC 183) and *C. glabrata* (MTCC 3019) (zone of inhibition: 0.8 mm). Clotrimazole (10 mg/disc) was effective against all test dermato-phytes (zone of inhibition: 1.0-1.1 mm). Among the yeasts, *C. albicans* (MTCC 183), *C. glabrata* (MTCC 3019), *C. parapsilosis* (MTCC 4448), *C. tropicalis* (MTCC 1000) were found to be susceptible to n-butanol fraction. Three bacteria namely, *M. luteus* (MTCC 106) *E. aerogenes* (MTCC 111) *S. enteria ser.typhi* (MTCC 733) were not inhibited by amphotericin B (10 mg/disc), whereas for the remaining bacteria were found to be susceptible (zone of inhibition: 6-8 mm). The strongest antibacterial activity and a weak antifungal activity of crude methanol extracts from aerial parts of *Penstemon campanulatus* was reported earlier (Zajdel et al., 2012). Yeasts and dermatophytes are eukaryotic organisms with more complex structural organization compared to the simple prokaryotic bacterial cells. This probably explains the difference in sensitivity of these two groups of microorganisms.

The antimicrobial potential of *S. pulcherrima* (leaf), especially its n-butanol fraction suggested future research on isolation of active molecule to serve either as novel antimicrobial drug or lead compounds.

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