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Antifungal activity of flavonoids of *Sida acuta* Burm f. against *Candida albicans*

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Abstract

Sida acuta, is a common weed plant belonging to the family Malvaceae. In the present study free and bound flavonoids of different parts of *S. acuta* have been evaluated for their antifungal activities using disc diffusion assay, against *Candida albicans* (MTCC No. 183). Minimum inhibitory concentration (MIC) of the extract was evaluated by microbroth dilution method, while minimum fungicidal concentration determined by subculturing the relevant samples. The plant exhibited good antifungal activity against tested fungi. Among 8 extracts tested 7 were found to be active at tested concentration. Free flavonoids were found to be more potent than bound flavonoids. Total activity (TA) was calculated for the extracts, to relate MIC of the extracts with its amount isolated from 1 g dried plant part. The study showed that flavonoids of *S. acuta* can be exploited for future anticanadidal drug.

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Sida acuta, Flavonoids, Antifungal activity, Disc diffusion assay, Total activity.

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Introduction

Natural products provide a rich source of bioactive molecules used for treating a wide range of different human diseases [1]. These products provide a large number of lead compounds used for developing new drugs [2]. Natural product drugs include aromatic polyketides, polyethers, coumarins, flavonoids, terpenoids, alkaloids and aminoglycosides [3].

Flavonoids are known to have medicinal properties and play a major role in the successful medical treatments from ancient times and their use has

persevered till date [4]. They are potent water-soluble antioxidants and free radical scavengers, which prevent oxidative cell damage and have strong anti-cancer activity [5, 6 and 7]. They are used to improve aquaresis and as anti-inflammatory, anti-spasmodic, and anti-allergic, anti-microbial agents [8, 9 and 10].

Sida acuta was selected for antifungal activity which is found throughout the hotter parts of India. The plant is used for various medicinal purposes such as malaria, ulcer, fever, gonorrhea, abortion, breast cancer following inflammation and wound infections [11 and 12]. The antimicrobial activity of the 90% ethanol extract of *S. acuta* was tested against standard strains and clinical isolates of some aerobic bacteria and a fungus [13].

Fungal infections remain a significant cause of morbiddity and mortality despite advances in medicine and the emergence of new antifungal agents [14]. *Candida albicans*, the agent of candidiasis, is an increasingly important disease that has a world wide distribution due to the fact that it is a frequent opportunistic pathogen in AIDS patients [15]. It is a common commensal of the gastrointestinal and urogenital tracts of human [16].

Since strains of *C. albicans* with multiple antibiotic resistance is increasing worldwide, it is of great importance to find effective treatments for this pathogen. Therefore, researchers are increasingly turning their attention to herbal medicine to develop drugs against microbial infections [17]. Hence, the present study was carried out to evaluate the anticandidal potential of free and bound flavonoids of different parts (root, stem, leaves and buds) of *S. acuta*. The study was performed with the standard drug terbinafine.

Materials and Methods

Collection and identification of plant material

Sida acuta Burm f. was collected from different localities of Jaipur in the month of June, 2008 and was identified at Department of Botany, UOR. A voucher specimen (RUBL-20428) was also submitted to the Herbarium of Botany Department, UOR.

Extraction of flavonoids

Flavonoids were extracted from different parts of the plant (root, stem, leaves and buds) following the well established method of Subramanian and Nagarajan [18]. Hundred grams of finely powdered plant parts were Soxhlet extracted with hot 80% methanol (500 ml) on a water bath for 24 h and filtered. Filtrate was re-extracted successively with petroleum ether, ethyl ether and ethyl acetate. Each step was carried out three times to ensure complete extraction. Petroleum ether fraction was discarded due to being rich in fatty substances and ethyl ether fractions (free flavonoids) were collected. Ethyl acetate fractions were analyzed for bound flavonoids. Each fraction was hydrolyzed in 7% H_2SO_4 for 2 h. Resulting mixture was filtered and filtrate was again extracted with ethyl acetate. The ethyl acetate extract was washed with distilled water till neutrality and collected. The ethyl ether (free flavonoids) and ethyl acetate fractions (bound flavonoids) were dried in *vaccuo*, weighed and stored in glass vials at 4^{o}C till used.

Microbial culture and growth conditions

Fungal strain of *Candida albicans* (MTCC No. 183) was procured from IMTECH, Chandigarh, India. The fungus was grown on 'Sabouraud Dextrose Agar' medium (Peptone 10 g; Dextrose 20 g; Agar 20 g in 1000 ml of distilled water; pH adjusted to 6.8-7.0) at 27±2^{o} C and maintained with periodic sub-culturing after every 15 days.

Antifungal screening of extracts

Disc diffusion assay (DDA)

Disc diffusion assay was performed for antifungal screening [19, 20 and 21]. SD agar base plates were seeded with the standard inoculum size (1×10^{7} CFU/ml). Sterile filter paper discs (6 mm in diameter) were impregnated with 100 µl of each of the extract (10

mg/ml concentration) to give a final concentration of 1 mg/disc, left to dry *in vaccuo* to remove residual solvent, which might interfere with the determination. Extract discs were then placed on the seeded agar plates. Each extract was tested in triplicate along with standard Terbinafine (1 mg/disc). The plates were kept at 4°C for 1 h for diffusion of extract, thereafter were incubated at 27± 2°C for 48 h. Zone of inhibition (IZ) was measured in mm and the 'Activity Index' (AI) for each extract was also calculated.

AI = IZ of the sample/IZ of the standard

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration was determined for each plant extract showing antifungal activity against test fungi in disc diffusion assay. Broth microdilution method [22] was followed for determination of MIC values. Plant extracts were re-suspended in acetone (which has no activity against test microorganisms) to make 10 mg/ml final concentration and then was added to broth media of 96-wells of microtiter plates using two fold serial dilution. Thereafter 100 µl inoculum of standard size was added to each well. Fungal suspension was used as negative control, while broth containing standard drug was used as positive control. The microtiter plates were incubated at 27±2°C. Each extract was assayed in duplicate and each time two sets of microtiter plates were prepared, one was kept for incubation while another set was kept at 4°C for comparing the turbidity in the wells of microtiter plate. The MIC values were taken as the lowest concentration of the extracts in the well of the microtiter plate that showed no turbidity after incubation. The turbidity of the wells in the microtiter plate was interpreted as visible growth of microorganisms.

Minimum fungicidal concentration (MFC)

Minimum fungicidal concentration was determined by subculturing 50 µl from each well showing no apparent growth. Least concentration of extract showing no visible growth on subculturing was taken as MFC.

Total Activity (TA)

Total activity for each active extract was calculated by the well established formula [23]. TA value (ml/g) is the volume to which the extract can be diluted retaining the ability to inhibit the growth of microorganisms.

TA =Amount extracted from 1 g plant material/MIC of the extract

Results and Discusssion

Antifungal activity (assed in terms of inhibition zone and activity index) of the flavonoid extracts of *S. acuta* were recorded (Table 1). In the present study total 8 extracts of different parts of the plant were tested for their bioactivity against *C. albicans*, among which 7 extracts showed significant activity against test fungi. Free flavonoids were observed with more anticandidal potency than bound flavonoids. Out of eight extracts, four extracts showed significant inhibition zone (equal or more than the standard) and best activity was observed by free flavonoids of stem (IZ 14±0.167 mm and AI 1.4± 0.001). Bound flavonoids of leaf (IZ 12.2±0.167 mm and AI 1.22±0.000) also showed good activity against tested fungi.

MIC and MFC values (Table 2) were evaluated for those extracts, which were showing activity in diffusion assay. The range of MIC and MFC of extracts recorded was 0.078 mg/ml-0.625 mg/ml and 0.078 mg/ml-1.25 mg/ml, respectively. Most of the flavonoid extracts showed MIC values less than 0.5 mg/ml indicate strong antifungal efficacy of the extracts against inhibited fungi. Among 7 active extracts (out of total 8 extracts tested) one extract was recorded with fungicidal activity, as its MIC and

MFC values were same whereas remaining extracts were found fungistatic because their MFC values were higher than MIC values of extract.

Amount of flavonoids extracted from plant parts and total activity was calculated and tabulated (Table 3). Maximum TA value of free flavonoid was calculated in stem (64.1 ml/g) and maximum TA of bounds flavonoid was calculated in roots (3.2 ml/g).

Conclusions

All the flavonoid extracts showed varying degrees of antifungal activity on *C. albicans*. Some of these extracts were more effective than traditional antibiotic terbinafine to combat the pathogenic fungi. The chance to find antifungal activity was more apparent in free flavonoid extracts of the plant. Three extracts (free flavonoids of stem, bound flavonoids of root and leaf) presented the larger IZ compared to the terbinafine. Thus *S. acuta* could be a source of new antibiotic compounds.

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Table 1: Inhibition zone (IZ) and activity index (AI) of free and bound flavonoids of *S. acuta*.

Plant part	Extract	*C. albicans*	
		IZ (mm)	AI
Root	F	-	-
	B	12±0.278	1.2±0.000
Stem	F	14±0.167	1.4±0.001
	B	8.2±0.882	0.82±0.003
Leaf	F	10±0.333	1±0.001
	B	12.2±0.167	1.22±0.000
Bud	F	8.8±0.333	0.88±0.002
	B	9±0.577	0.9±0.001
Terbinafine		**10**	

F = Free flavonoids; B = Bound flavonoids; AI: (IZ developed by extract/IZ developed by standard), ± = SEM (Standard Error Mean), (–) = No activity

Table 2: Minimum inhibitory concentration (MIC) and Minimum fungicidal concentration (MFC) of free and bound flavonoids of *S. acuta*.

Plant part	Extract	*C. albicans*	
		MIC mg/ml	MFC mg/ml
Root	F	-	-
	B	0.156	0.312
Stem	F	0.078	0.078
	B	0.625	1.25
Leaf	F	0.312	0.625
	B	0.156	0.312
Bud	F	0.312	0.625
	B	0.625	1.25

F = Free flavonoids; B = Bound flavonoids; (–) = No activity

Table 3: Quantity and Total activity (TA) of free and bound flavonoids of *S. acuta*.

Plant part	Extract	Qunatity (mg/gdw)	TA (ml/g)
Root	F	4.25	-
	B	5	3.2
Stem	F	8.15	64.1
	B	4.1	0.72
Leaf	F	4.25	26.12
	B	5	2.24
Bud	F	8.15	13.14
	B	4.1	2.8

F = Free flavonoids; B = Bound flavonoids; TA (ml/g) =weight of extract (mg/g plant material) / MIC (mg/ml) of extract

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