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Research Article

IN VITRO ANTIFUNGAL POTENTIAL OF TRIDAX PROCUMBENS L. AGAINST ASPERGILLUS FLAVUS AND ASPERGILLUS NIGER

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ABSTRACT

Human mycoses, especially in immunocompromised patients are not always successfully treated due to the ineffectiveness or toxicity of the available antifungal drugs. The present study was designed to evaluate the antifungal potential of alkaloids and flavonoids of different parts (root, stem, leaf and flower) of *Tridax procumbens* L. Disc diffusion assay was performed against two pathogenic fungal strains (*Aspergillus flavus* and *Aspergillus niger*). Minimum inhibitory concentrations (MIC), minimum fungicidal concentrations (MFC) and total activity were also evaluated for determination of antifungal potential of each active extract. The flavonoid extracts showed remarkable activity against *A. niger* whereas alkaloid extracts were found inactive against both the test fungi. Excellent antifungal potential was recorded for free flavonoid of stem (IZ 12 mm, AI 1.2, with same MIC and MFC 0.156 mg/ml), bound flavonoid of stem (IZ 10 mm, AI 1, MIC 0.312 and MFC 0.625 mg/ml) and flower (IZ 10.2 mm, AI 1.02, with same MIC and MFC 0.312 mg/ml) against *A. niger*. Our study indicated that *T. procumbens* can be used as a source of formulations of antifungal drug for treatment of diseases caused by *A. niger*.

Keywords: Alkaloids, disc diffusion assay, flavonoids, MFC, MIC, *tridax procumbens*

INTRODUCTION

In India thousands of species of plants are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times. Today it is estimated that more than two thirds of the world's population relays on plant derived drugs. About 7000 medicinal compounds used in the Pharmacopoeia are derived from plants. Hence, ethno-pharmacologists, botanists, microbiologists, and natural-product chemists are searching for phytochemicals which could be developed for the treatment of infectious diseases especially in light of the emergence of drug-resistant microorganisms and the need to produce more effective antimicrobial agents[1].

Tridax procumbens (Family: Asteraceae) is a medicinal plant. It is found perennially in various tropical and subtropical regions as well as mildly temperate regions worldwide [2]. The plant is also used for diarrhea, malaria, cough and asthma, boils, epilepsy, liquid purging, wounds, toothache and stomachache. Leaves are applied on cuts and wounds and its paste used for the treatment of dysentery. Roots are mixed with castor oil and applied on paralytic part [3]. Crude extracts have been investigated for wound healing, anti-inflammatory, immunomodulatory and antimicrobial properties earlier [4-8]. The main objective of this study was to investigate the antifungal activity of alkaloids and flavonoids of different parts (root, stem, leaf and flower) of *T. procumbens* against *A. flavus* and *A. niger*.

MATERIALS AND METHODS

Plant material

Tridax procumbens was collected from different localities of Jaipur, Rajasthan in the month of June, 2008. The plant was identified at Herbarium, Department of Botany, University of Rajasthan, Jaipur. A voucher specimen (RUBL-20389) was also submitted to the Herbarium, UOR. All the parts (root, stem, leaf and flower) of *T. procumbens* were separately shade dried and were milled to a fine powder using a grinder.

Extraction of alkaloids and flavonoids

Alkaloids and flavonoids were extracted from root, stem, leaf and flower of *T. procumbens* by the well established methods [9, 10]. For alkaloid extraction powdered plant parts (100 g) were extracted with 10% acetic acid in ethanol (500 ml) for four hours. The extracts were concentrated and were made alkaline by NH_4OH. Precipitates thus obtained were collected by centrifugation, washed with 1% NH_4OH, filtered, dried in *vaccuo* and weighed. For flavonoids extraction powdered plant parts (100 gm) were Soxhlet extracted with hot 80% methanol (500 ml) and filtered. Filtrate was re-extracted successively with petroleum ether (fraction I), ethyl ether (fraction II) and ethyl acetate (fraction III) using separating funnel. Petroleum ether fraction was discarded due to being rich in fatty substances, whereas ethyl ether and ethyl acetate fractions were analysed for free and bound flavonoids, respectively. Ethyl acetate fraction was hydrolyzed by refluxing with 7% H_2SO_4 for 2 h (for removal of bound sugars from the flavonoids). Resulting mixture was filtered and filtrate was extracted with ethyl acetate in separating funnel. Ethyl acetate extract thus obtained was washed with distilled water till neutrality. Ethyl ether (free flavonoids) and ethyl acetate fraction (bound flavonoids) were dried in *vaccuo* and weighed. Alkaloid and flavonoid extracts were then stored in glass vials at 4^0C for further use.

Antifungal activity

Two fungal strains (*Aspergillus flavus* MTCC 277 and *Aspergillus niger* MTCC 282) were procured from IMTECH, Chandigarh, India and maintained on Sabouraud Dextrose Agar medium. Antimicrobial activity of extracts was performed by disc diffusion test [11]. Standard size of microbial inoculum (1×10^7 CFU/ml) was used with 1 mg/disc concentration of both the test extracts and standard drug itraconazole. Each extract was tested in triplicate. Antifungal activity was determined by measuring zone of inhibition (IZ) in mm. Activity Index (AI) for each extract was also calculated by dividing the IZ of the extract by the IZ of the standard. All the values of the IZ and AI were expressed as means of three replicates ± standard error means.

AI=IZ of the extract/IZ of the standard.

Minimum inhibitory concentration (MIC) was determined for each plant extract showing activity against test fungi in disc diffusion assay. Micro-broth dilution method [12] was followed for determination of MIC values. Experiments were conducted three times and the mean values were recorded. Minimum fungicidal concentration (MFC) was also 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) for each active extract was also calculated, which is the volume at which the test extract can be diluted without loosing the ability to kill microorganisms [13]. It is calculated by dividing the amount of extract from 1 g plant material by the MIC of the same extract.

Total activity=Amount extracted from 1 g plant material/MIC of the extract.

RESULTS AND DISCUSSION

The alkaloid and flavonoid content estimated in each gram of dried plant material was recorded [Table 1]. Content of alkaloids were recorded maximum in leaves (82.41 mg/g.d.w) whereas toal flavonoids were recorded maximum in flowers (8.1 mg/g.d.w).

Twelve extracts of alkaloids and flavonoid of root, stem, leaf and flower of *T. procumbens* were screened for antifungal activity (assessed in terms of inhibition zone and activity index) against *A. flavus* and *A. niger* [Table 2]. Results revealed that all the extracts were found inactive against *A. flavus* whereas only four flavonoid extracts were found active against *A. niger*. Free flavonoids of stem demonstrated significant activity against *A. niger* by showing IZ (12 mm) AI (1.2) more than the standard itraconazole drug. Bound flavonoids of stem and flower also showed remarkable activity against *A. niger* where IZ was observed equal (IZ 10 mm, AI 1) and more (IZ 10.2 mm, AI 1.02), respectively than the standard. MIC, MFC and TA values [Table 3] were evaluated for those extracts which showed activity in diffusion assay. Range of MIC and MFC evaluated was 0.312 mg/ml-1.25 mg/ml. Among four active extracts two extracts were recorded fungicidal, as their MIC and MFC were recorded same (0.156 mg/ml for free flavonoids of stem and 0.312 mg/ml for bound flavonoids of flower) whereas two other extracts were found fungistatic because their MFC values were recorded higher than MIC values (MIC 0.312, MFC 0.625 mg/ml for bound flavonoids of stem and MIC 0.625, MFC 1.25 mg/ml for free flavonoids of leaf). TA was calculated maximum for free flavonoids of stem (18.26 ml/g) whereas TA calculated minimum for bound flavonoids of stem (2.08 ml/g).

Table 1: Quantitative estimation of alkaloids and flavonoids of *Tridax procumbens*

Plant Part	Alkaloid (mg/g.d.w)	Flavonoid (mg/g.d.w)		
		Free	Bound	Total
Root	11.72	1.6	0.66	2.26
Stem	62.33	2.85	0.65	3.5
Leaf	82.41	3.5	3.75	7.2
Flower	32.22	5.45	2.65	8.1

Table 2: Inhibition zone and activity index of alkaloids and flavonoids of *Tridax procumbens*

Plant part	Extract	Test microorganism			
		A. flavus		*A. niger*	
		IZ mm	AI	IZ mm	AI
	A	-	-	-	-
Root	F	-	-	-	-
	B	-	-	-	-
	A	-	-	-	-
Stem	F	-	-	12±0.167	1.2±0.000
	B	-	-	10±0.333	1±0.001
	A	-	-	-	-
Leaf	F	-	-	8.6±0.882	0.86±0.002
	B	-	-	-	-
	A	-	-	-	-
Flower	F	-	-	-	-
	B	-	-	10.2±0.123	1.02±0.002
Itraconazole		15		10	

Table 3: Minimum inhibitory concentration, minimum fungicidal concentration and total activity of alkaloids and flavonoids of *Tridax procumbens*

Plant Part	Extract	Test fungi					
		A. flavus			*A. niger*		
		MIC mg/ml)	MFC (mg/ml)	TA (ml/g)	MIC (mg/ml)	MFC (mg/ml)	TA (ml/g)
	A	-	-	-	-	-	
Root	F	-	-	-	-	-	
	B	-	-	-	-	-	
	A	-	-	-	-	-	
Stem	F	-	-	-	0.156	0.156	18.26
	B	-	-	-	0.312	0.625	2.08
	A	-	-	-	-	-	
Leaf	F	-	-	-	0.625	1.25	5.6
	B	-	-	-	-	-	
	A	-	-	-	-	-	-
Flower	F	-	-	-	-	-	-
	B	-	-	-	0.312	0.312	8.49

A: Alkaloids; F: Free flavonoids; B: Bound flavonoids; (–): = Not determined since there was no activity

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