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Research Article

ANTIMICROBIAL POTENTIAL OF FLAVONOIDS OF *TRIDAX PROCUMBENS* L. AGAINST PATHOGENIC MICROORGANISMS

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ABSTRACT

The present study was conducted to assess the antimicrobial potential of free and bound flavonoid extracts of pedicle and buds of *Tridax procumbens* L. *T. procumbens* (Family: Asteraceae) is an important medicinal plant, is used traditionally for the treatment of many diseases. In the present study, disc diffusion assay was performed against three bacteria (*Escherichia coli, Staphylococcus aureus* and *Proteus mirabilis*) and four fungi (*Aspergillus flavus, Aspergillus niger, Candida albicans* and *Trichophyton mentagrophytes*). Minimum inhibitory concentrations, minimum bactericidal/fungicidal concentrations were evaluated for determination of antibiotic potential of the active extracts. Total activity of the extracts, against each sensitive test pathogen was also evaluated. The flavonoid extracts showed good antimicrobial activity against all the test pathogens except *A. flavus* against which none of the test extract showed activity. Free flavonoids from pedicle (active against 5 out of 7 test pathogens) and bound flavonoids from bud (active against 4 out of 7 pathogens) exhibited remarkable antimicrobial activity. *S. aureus* was the most susceptible microorganism which was sensitive towards all extracts. Result of the present study indicates that the antimicrobial flavonoids from *T. procumbens* could be used in developing noval antibacterial and antifungal drugs.

KEYWORDS: Antimicrobial potential, Flavonoids, *Tridax procumbens*, Disc diffusion assay, Minimum inhibitory concentration, Minimum bactericidal concentration, Minimum fungicidal concentration

INTRODUCTION

Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases [1]. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections [2]. The systematic screening of antimicrobial plant extracts represents a continuous effort to find new compounds with potential to act against multi-resistant pathogenic bacteria and fungi [3].

Flavonoids are known to have medicinal properties and play a major role in the successful medicinal treatments from ancient times [4]. It was reported that flavonoids can improve the blood circulation and lower the blood pressure [5]. *Tridax procumbens* is well known for its wound healing activities [6, 7]. Crude extracts of *T. procumbens* have been reported to have anti-inflammatory [8], antiprotozoal and antimicrobial activity [9-11]. Thus in the present study flavonoids of pedicle and buds of *T. procumbens* have been extracted and evaluated for their antimicrobial potential.

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.

Extraction of Flavonoids

Pedicle and buds of *T. procumbens* were separately shade dried and were milled to a fine powder using a grinder. Powdered parts were subjected for flavonoids extraction, following the well established method [12]. Hundred grams of finely powdered plant part was 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.

Test Microorganisms

Pathogenic bacteria (*Escherichia coli* MTCC 46, *Staphylococcus aureus* MTCC 87 and *Proteus mirabilis* MTCC 1425) and fungi (*Aspergillus flavus* MTCC 277, *Aspergillus niger* MTCC 282, *Candida albicans* MTCC 183 and *Trichophyton mentagrophytes* MTCC 7687) were procured from IMTECH, Chandigarh, India. Bacterial strains were grown and maintained on Muller-Hinton Agar medium while fungal strains were kept on Sabouraud Dextrose Agar medium.

Antimicrobial Activity of Flavonoids

A. Disc Diffusion Assay

Antimicrobial activity of flavonoid extracts was performed by disc diffusion assay (DDA) method [13]. Standard size of microbial inoculums (1×10^8 CFU/ml for bacteria and 1×10^7 CFU/ml for fungi) were used with 1 mg/disc concentration of both the test extracts and standards (streptomycin for bacteria, itraconazole for *A. flavus* and *A. niger*, and terbinafine for *C. albicans* and *T. mentagrophytes*) was tested in triplicate. Antimicrobial activity was determined by measuring zone of inhibition (IZ) in mm. Activity Index (AI) for each extract was also calculated by using following formula:

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

B. Minimum Inhibitory Concentration

Minimum inhibitory concentration (MIC) was determined for each extract showing activity against test pathogens in disc diffusion assay. Micro-broth dilution method [14] was followed for determination of MIC values. Experiments were conducted three times and the mean values were recorded.

C. Minimum Bactericidal/Fungicidal Concentration

Minimum bactericidal/fungicidal concentration (MBC/MFC) 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 MBC/MFC.

D. Total Activity

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 [15]. 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.

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

Plant part	Flavonoids (mg/g.d.w)		
	Free	Bound	Total
Pedicle	2.3	2	4.3
Bud	2.8	1.8	4.6

Table 2: Inhibition zone and activity index of flavonoids of *T. procumbens*

Plant part	Extract	Test microorganism													
		E. coli		*S. aureus*		*P. mirabilis*		*A. flavus*		*A. niger*		*C. albicans*		*T. mentagrophytes*	
		IZ mm	AI	IZ mm	AI	IZ mm	AI	IZ mm	AI	IZ mm	AI	IZ mm	AI	IZ mm	AI
Pedicle	Free	10± 0.333	0.5± 0.013	11.7± 0.123	0.46± 0.002	9.6± 0.333	0.4± 0.029	-	-	-	-	10± 0.333	1± 0.000	14.8± 0.167	0.42± 0.029
	Bound	13.5± 0.167	0.67± 0.001	11± 0.120	0.44± 0.003	-	-	-	-	-	-	-	-	-	-
Bud	Free	-	-	10± 0.333	0.4± 0.001	-	-	-	-	-	-	9.5± 0.332	0.95± 0.333	-	-
	Bound	-	-	14.6± 0.167	0.58± 0.001	12± 0.273	0.5± 0.003	-	-	9± 0.577	0.9± 0.577	-	-	10.8± 0.333	0.3± 0.001
Standard		20		25		24		15		10		10		35	

±: SEM (Standard error mean); (–): No inhibition; Standards: Streptomycin (*E. coli, S. aureus* and *P. mirabilis*); Itraconazole (*A. flavus* and *A. niger*); Terbinafine (*C. albicans* and *T. mentagrophytes*); IZ: Inhibition zone; AI: Activity index

Table 3: Minimum inhibitory concentration and Minimum bactericidal/fungicidal concentration of flavonoids of *T. procumbens*

Plant part	Extract	Test microorganism													
		E. coli		*S. aureus*		*P. mirabilis*		*A. flavus*		*A. niger*		*C. albicans*		*T. mentagrophytes*	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Pedicle	Free	0.312	0.625	0.312	0.625	0.625	1.25	-	-	-	-	0.312	0.625	0.078	0.078
	Bound	0.156	0.156	0.312	0.312	-	-	-	-	-	-	-	-	-	-
Bud	Free	-	-	0.625	1.25	-	-	-	-	-	-	0.312	0.312	-	-
	Bound	-	-	0.078	0.078	0.156	0.312	-	-	0.625	1.25	-	-	0.312	0.312

All figures are in mg/ml unit; (–): Not determined since there was no activity; MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration; MFC: Minimum fungicidal concentration

Table 4: Total activity of flavonoids of *T. procumbens*

Plant part	Extract	Test microorganisms						
		E. coli	*S. aureus*	*P. mirabilis*	*A. flavus*	*A. niger*	*C. albicans*	*T. mentagrophytes*
Pedicle	Free	7.37	7.37	3.68	-	-	7.37	29.48
	Bound	12.82	6.41	-	-	-	-	-
Bud	Free	-	4.48	-	-	-	8.97	-
	Bound	-	23.07	11.53	-	2.88	-	5.76

(–): Not determined since there was no activity

RESULTS

Amount of free and bound flavonoid extracted from pedicle and buds were calculated and recorded in Table 1. Antimicrobial potential evaluated in terms of IZ, AI, MIC and MBC/MFC of the flavonoids, against selected pathogenic microorganisms was recorded in Table 2 and 3. Result revealed the flavonoid extracts of both the parts were active against one or more selected microorganisms except *A. flavus*. Maximum activity against *E. coli* (IZ 13.5 mm ± 0.167 and AI 0.67 ± 0.001) was observed for bound flavonoids of pedicle whereas maximum activity against *S. aureus* (IZ 14.6 mm ± 0.167 and AI 0.58 ± 0.001) and *P. mirabilis* (IZ 12 mm ± 0.273 and AI 0.5 ± 0.003) was observed for bound flavonoids of buds. Most resistant pathogen was *A. niger* against which only bound flavonoids of bud showed activity (IZ 9 mm ± 0.577 and AI 0.9 ± 0.577). Maximum activity against *C. albicans* (IZ 10 mm ± 0.333 and AI 1 ± 0.000) and *T. mentagrophytes* (IZ 14.8 mm ± 0.167 and AI 0.42 ± 0.029) was observed for free flavonoids of pedicle. MIC ranged from 0.078 to 0.625 mg/ml and MBC/MFC ranged from 0.078 to 1.25 mg/ml against sensitive pathogens. Most of the extracts showed MIC values less than 0.5 mg/ml indicate strong antimicrobial potential. All the extracts were found microbicidal against one or more test pathogen as their MIC and MBC/MFC values were recorded same. MIC and MBC/MFC were recorded same against *E. coli* (0.156 mg/ml), *S. aureus* (0.312 and 0.078 mg/ml), *C. albicans* (0.312 mg/ml) and *T. mentagrophytes* (0.312 and 0.078 mg/ml). Total activity was also calculated and tabulated in Table 4. Maximum TA values calculated were 12.82, 23.07, 11.53, 8.97 and 29.48 ml/g against *E. coli*, *S. aureus P. mirabilis* and *T. mentagrophytes*, respectively.

DISCUSSION

Plants have been used traditionally in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeasts. Most of their properties are due to extracts produced by their secondary metabolism [16, 17]. Plants extracts inhibit the growth of various microorganisms at different concentrations have also been reported [18-20] and have been extensively investigated as a source of medicinal agents [21]. Literature indicates that flavonoids have antimicrobial properties [22-24]. Alkaloids, tannins, saponins and flavonoids of *T. procumbes* have been reported [25]. Antimicrobial activity of crude extracts of *T. procumbens* have also been reported [26] but without AI, MIC, MBC/MFC and TA determination. Such studies could only indicate their antimicrobial activity but are not helpful in establishing them as an alternative for antibiotic. Therefore the present study has been carried out for evaluation of antimicrobial potential of flavonoid extracts of *T. procumbens* with AI, MIC, MBC/MFC and TA determination.

CONCLUSION

Flavonoid extracts of pedicle and bud of *T. procumbens* exhibited remarkable antimicrobial potential against tested pathogens particularly against *E. coli*, *S. aureus*, *C. albicans* and *T. mentagrophytes*. Both the flavonoid extracts were found bactericidal and fungicidal against these four pathogens. Hence, *T. procumbens* could be a source of new antibiotic compound for preparing herbal drug for the treatment of diseases caused by these pathogenic microorganisms.

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