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ANTIMICROBIAL ACTIVITY OF ALKALOIDS OF *TRIDAX PROCUMBENS* L. AGAINST HUMAN PATHOGENS

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ABSTRACT

Tridax procumbens L. is a well known medicinal plant. In the present study alkaloids from pedicle and buds of the plant were extracted and evaluated for antimicrobial activity by 'Disc Diffusion Assay' against selected bacteria (*Escherichia coli*, *Staphylococcus aureus* and *Proteus mirabilis*) and fungi (*Aspergillus flavus, Aspergillus niger*, *Candida albicans* and *Trichophyton mentagrophytes*). Minimum inhibitory concentration, minimum bactericidal/fungicidal concentration and total activity of each active extract were also evaluated. Significant antibacterial activity was recorded by alkaloids of pedicle against *P. mirabilis* (IZ 20mm, AI 0.83 and same MIC, MBC 0.039 mg/ml) and antifungal activity was recorded by buds against *C. albicans* (IZ 9.2mm, AI 0.92, MIC 0.625 mg/ml and MFC 1.25 mg/ml). MIC ranged from 0.039 mg/ml-0.625 mg/ml and MBC/MFC ranged from 0.039 mg/ml-1.25 mg/ml against susceptible pathogens. It is noteworthy that MIC values of active extracts was found very low (0.039 mg/ml-0.625 mg/ml) and was recorded below 1 mg/ml against inhibited pathogens, thus ascertain their strong antimicrobial potential against selected microbes. Thus, the result of the present study advocates the exploitation of alkaloid extracts of *T. procumbens* for future antimicrobial drugs.

INTRODUCTION: Nature has been a source of medicinal agents for thousands of years. Folk medicines of almost all civilizations of the world abound in herbal remedies. Majority of the traditional medicines used in healthcare are obtained from plants [1]. In spite of several advancements in the field of synthetic drug chemistry and antibiotics, plants continue to be one of the major raw materials for drugs treating various ailments of humans.

Clinical and pharmaceutical investigations have in fact elevated the status of medicinal plants by identifying the role of active principles present in them and elaborating on their mode of action in human and animal systems [2].

Tridax procumbens is a pantropical weed, belonging to the family Asteraceae. It has slender taproot, perennial herb and well adapted to coarse-textured soils. It is frequently found in annual crops, roadsides, pastures and waste areas. In Nigeria, plant is used as feed for livestock and stops bleeding [3, 4]. It has been used in traditional Chinese medicine by the native populations to treat bronchitis, dysentery and diarrhea and to prevent hair loss.

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Recently, the chemical components of its essential oils have been also investigated [5, 6]. In the present investigation, alkaloids were extracted from *T. procumbens* and were screened for antimicrobial activity against selected bacteria and fungi .

MATERIALS AND METHODS:

Collection and Identification of Plant Material: *Tridax procumbens* was collected from different localities of Jaipur, Rajasthan in the month of June, 2008 and was identified at Herbarium of Botany Department, University of Rajasthan, Jaipur. A voucher specimen (RUBL- 20389) was also submitted to the Herbarium, UOR.

Extraction of Alkaloids: Pedicle and buds of the plant were separated and washed thoroughly. The parts were shade dried, finely powdered and subjected to the extraction of alkaloids following the well established method [7]. Hundred grams of each finely powdered sample was extracted with 10% acetic acid in ethanol (final volume 500 ml) for 4 h. Extract were then concentrated to ¼ of the original volume and made alkaline by NH_4OH. Precipitates collected after centrifugation were washed with 1% NH_4OH, filtered, dried *in vaccuo* and weighed.

Test Microorganisms: Pathogenic bacterial (*Escherichia coli* MTCC No. 46, *Staphylococcus aureus* MTCC No. 87 and *Proteus mirabilis* MTCC No. 1425) and fungal strains (*Aspergillus flavus* MTCC No. 277, *Aspergillus niger* MTCC No.282, *Candida albicans* MTCC No. 183 and *Trichophyton mentagrophytes* MTCC No. 7687) were procured from IMTECH, Chandigarh, India. Bacterial strains were grown and maintained on 'Muller-Hinton Agar medium' (Beef extract 2.0 g; Peptone 17.5 g; Starch 1.5 g; Agar 17.0 g; in 1000 ml of distilled water; Final pH 7.4±0.2) at 37±2°C while fungal strains were kept 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°C.

Antimicrobial Activity of Alkaloids:

A. **Disc Diffusion Assay-** Antimicrobial activity of alkaloid extracts was carried out by the disc diffusion assay (DDA) method [8]. The culture suspensions of bacteria (1×10^8 cfu/ml) and fungi (1×10^7 cfu/ml) were prepared in sterilized distilled water. Muller-Hinton agar and Sabouraud dextrose agar media for bacteria and fungi respectively were prepared and poured in sterilized Petri plates and cooled for solidification. The solidified media plates were seeded with the prepared culture suspensions. Sterilized filter paper discs of 6 mm diameter (Whatman no.1) were impregnated with 100 µl of extract of 10 mg/ml concentration to give a final concentration of 1 mg/disc. These discs were left to dry in *vaccuo* to remove residual solvent, which might interfere with the determination.

The extract discs were placed on the seeded media plates along with discs impregnated with standard drugs (streptomycin for bacteria, itraconazole for *A. flavus* and *A. niger* and terbinafine for *C. albicans* and *T. mentagrophytes*) in the same (1 mg/disc) concentration. These plates were kept at 4°C for 1 h for the diffusion of extracts into the media and thereafter were incubated at 37°C±2°C for 24 h for bacteria and at 27°C±2°C for 48 h for fungi. However *T. mentagrophytes* was kept at 27°C±2°C for 5-7 days. Zone of inhibition (IZ) was measured in mm and the 'Activity Indix' (AI) was calculated by the well established formula. The experiment was performed three times to minimize the error and the mean values were recorded.

$$AI = \frac{\text{IZ of the sample}}{\text{IZ of the standard}}$$

B. **Minimum Inhibitory Concentration-** Extracts that showed positive results in the disc diffusion assay were further evaluated for minimum inhibitory concentration (MIC). The microbroth dilution method [9] was performed for the determination of MIC values. Alkaloid extracts were resuspended 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. Bacterial and fungal suspensions were used as negative control, while broth containing standard drug was used as positive control.

The microtiter plates were incubated at 37± 2°C for 24 h for bacteria, 27± 2°C for 48 h for fungi and 27± 2°C for 5-7 days for *T. mentagrophytes*. Each extract was assayed in triplicate 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.

C. **Minimum Bactericidal/Fungicidal Concentration-** The 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 calculated by the well established formula [10]. TA value (ml/g) is the volume at which the extract can be diluted retaining the ability to kill microorganisms.

$$TA = \frac{\text{Amount extracted from 1 g dried plant material (mg/g.d.w)}}{\text{MIC of the extract (mg/ml)}}$$

RESULTS: Antimicrobial activity (in terms of inhibition zone and activity index) of alkaloid extracts of pedicle and buds against selected microorganisms were recorded in **Table 1.** Result reveled that both the extracts were active against one or more selected microorganisms except *A. flavus* and *A. niger*. *Proteus mirabilis* and *Candida albicans* were found most susceptible pathogens against which both the extracts showed activity.

Alkaloid extracts from pedicle showed activity against *S. aureus* (IZ 9 mm and AI 0.36), *P. mirabilis* (20 mm and AI 0.83) and *C. albicans* (IZ 8.5 mm and AI 0.85) whereas alkaloids from buds showed activity against *E. coli* (IZ 10 mm and AI 0.5), *P. mirabilis* (IZ 12 mm and AI 0.5), *C. albicans* (IZ 9.2 mm and AI 0.92) and *T. mentagrophytes* (IZ 9 mm and AI 0.25).

MIC and MBC/MFC values of active alkaloid extracts were recorded in **Table 2.** MIC ranged from 0.039 mg/ml-0.625 mg/ml and MBC/MFC ranged from 0.039 mg/ml-1.25 mg/ml against inhibited pathogens. Alkaloids from pedicle and buds showed same MIC, MBC values (0.039 mg/ml and 0.156 mg/ml, respectively) against *P. mirabilis*. Same values of MIC (0.625 mg/ml) and MBC/MFC (1.25 mg/ml) were recorded against other selected pathogens.

Amount of alkaloid extracted from plant parts (pedicle and buds) and Total activity was calculated and recorded in **Table 3.** Buds of the plant was found with significant quantity of alkaloid (92.66 mg/g.d.w) whereas alkaloid quantity of pedicle was recorded 32.25 mg/g.d.w. TA of pedicle and buds was recorded highest (826.92 ml/g and 593.97 ml/g, respectively) against *P. mirabilis*. TA of pedicle recorded against *S. aureus* and *P. mirabilis* was 51.6 ml/g whereas total activity of buds recorded against *E. coli*, *P. mirabilis* and *T. mentagrophytes* was 148.25 ml/g.

TABLE 1: INHIBITION ZONE (IZ) AND ACTIVITY INDEX (AI) OF ALKALOIDS OF *TRIDAX PROCUMBENS*

Part	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	-	-	9±0.577	0.36±0.003	20±0.234	0.83±0.010	-	-	-	-	8.5±0.333	0.85±0.002	-	-
Bud	10±0.333	0.5±0.013	-	-	12±0.276	0.5±0.002	-	-	-	-	9.2±0.167	0.92±0.432	9±0.577	0.25±0.002
Standard	20		25		24		15		10		10		35	

AI: (IZ developed by extract/IZ developed by standard); ± = SEM (Standard Error Mean), Standards: Streptomycin (*E. coli, S. aureus and P. mirabilis*); Itraconazole (*A. flavus* and *A. niger*); Terbinafine (*C. albicans* and *T. mentagrophytes*)

TABLE 2: MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM BACTERICIDAL/FUNGICIDAL (MBC/MFC) CONCENTRATION OF ALKALOIDS OF *TRIDAX PROCUMBENS*

Part	Test microorganism													
	E. coli		*S. aureus*		*P. mirabilis*		*A. flavus*		*A. niger*		*C. albicans*		*T. mentagrophytes*	
	MIC mg/ml	MBC mg/ml	MIC mg/ml	MBC mg/ml	MIC mg/ml	MBC mg/ml	MIC mg/ml	MFC mg/ml	MIC mg/ml	MFC mg/ml	MIC mg/ml	MFC mg/ml	MIC mg/ml	MFC mg/ml
Pedicle	-	-	0.625	1.25	0.039	0.039	-	-	-	-	0.625	1.25	-	-
Bud	0.625	1.25	-	-	0.156	0.156	-	-	-	-	0.625	1.25	0.625	1.25

TABLE 3: QUANTITY AND TOTAL ACTIVITY OF ALKALOIDS OF *TRIDAX PROCUMBENS*

Part		Test microorganism						
	Quantity (mg/gdw)	*E. coli*	*S. aureus*	*P. mirabilis*	*A. flavus*	*A. niger*	*C. albicans*	*T. mentagrophytes*
Pedicle	32.25	-	51.6	826.92	-	-	51.6	-
Bud	92.66	148.25	-	593.97	-	-	148.25	148.25

Total activity (ml/g) =weight of extract (mg/g.d.w)/MIC of extract (mg/ml)

DISCUSSION: 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 [11].

Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections [12]. 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 [13].

In the present investigation, *T. procumbens* showed antimicrobial activity against tested pathogens, except *A. flavus* and *A. niger*. The plant has been studied earlier for antimicrobial activity of crude extracts [14, 15, 16] but without MIC, MBC/MFC and TA determination. Such studies could only indicate their antimicrobial potential but are not helpful in establishing them as an alternative for antibiotic. Therefore, the present study has been carried out for antimicrobial activity of alkaloid extracts of *T. procumbens* with MIC, MBC/MFC and TA determination.

CONCLUSION: *Tridax procumbens* showed significant antimicrobial activity against tested pathogens particularly against *P. mirabilis* and *C. albicans*. Hence, *T. procumbens* may be used as accessible source for preparing herbal drug for treating diseases caused by *P. mirabilis* and *C. albicans*. Further research in the direction of isolation and characterization of the active principles of *T. procumbens* is required so that cost effective and safe drugs can be developed for treatment of diseases.

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