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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITIES OF PLANT EXTRACT OF *TRIDAX PROCUMBENS*

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ABSTRACT
Tridax procumbens Linn. (Compositae) is a weed found throughout India. The plant is native of tropical America and naturalized in tropical Africa, Asia, and Australia. Local people known it as "Ghamara", in English popularly called 'coat buttons' and is dispensed for "Bhringraj" by some of the practitioners of Ayurveda. The phytochemical screening revealed the presence of alkaloids, carotenoids, flavonoids (catechins and flavones), fumaric acid, fl-sitosterol, saponins and tannins. Natural products continue to play an important role in the discovery and development of new pharmaceuticals, as clinically useful drugs, as starting materials to produce synthetic drugs, or as lead compounds from which a totally synthetic drug can be designed. The present study is designed for phytochemical analysis of *Tridax procumbens* and HPLC analysis. This also includes the antimicrobial activity of the bio active compound obtained by crude extract and the column chromatography.

INTRODUCTION

The essential values of some plants have long been published, however, a large number of them remain unexplored as yet. *Tridax procumbens* is traditionally used in the treatment of fever, typhoid fever, cough, asthma, epilepsy and diarrhoea (Mann *et al.,* 2003). The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds (Hill, 1952; Ali *et al.,* 2001). The chemical constituents of *Tridax procumbens* were reported by many scientists. The occurrence of fumaric acid is reported.The β –sitosterol and tannins were reported (Kasture *et al.,* 1971). The water soluble novel polysaccharides were reported (Raju and Davidson, 1994). Lutelin and glucoluteolin from the flowers were isolated from *Tridax procumbens* (Subramanian *et al.,* 1968). The sterols, hydrocarbons, saturated and unsaturated fatty acids were reported (Gadre and Gabhe, 1992). Polyphenols especially TF exert cancer chemo preventive activity of inducing apoptic signals. (Lu *et al.,* 1997, Yang *et al.,* 2000, Javed *et al.,* 1998).The lipid constituents were isolated from the flowers of *Tridax procumbens* and reported and the steroidal saponins which were characterized as β-sitosterol 3-O-b-D-xylopyranoside were also isolated and identified (Verma *et al.,* 1988).

In the present investigation, we report our findings on the total extractions of chemical components of the plant by HPLC method.

MATERIALS AND METHODS

Plant materials are collected from university botanical garden and the identified plant was confirmed by plant taxonomist. The preliminary phytochemical screening tests were carried out on the aqueous leaf crude extract of *Tridax procumbens* using standard procedures to identify the constituents.

Extraction of the Compound: Extraction of bioactive compound by HPLC

For crude extraction fresh plant material were washed with tap water ,air dried and homogenized to fine powder and stored in air tight bottles. 10 g air dried powder mixed with 100 mL water at 37ºC for 48 hr. filtered in muslin cloth centrifuged at 500g for 10 min. The supernatant was stored at 4ºC

The compound can be extracted from above supernatant by passing through the column and is first fitted with Cotton and then silica gel, activated charcoal and again silica gel in ratio 1:2:1 and the crystals of plant extract are collected.

HPLC Analysis

The *Tridax procumbens* leaf extracted and purified compound was tested for the compound conformity and purity. The compounds extracted from leaf, stem, root and flower were set up for HPLC (High performance liquid chromatography) to test the purity of the compounds. In the HPLC mobile as

well as stationary phases are used to test the purity of the compound. Usually a single gradient or a binary gradient are used as mobile phase. From the already available data it is known that the mobile phase used are shown in the figures itself. The detector used here is D_2 lamp as the measurement of the sample is < 400 nm. The run time was set to 1 mL/1min. An injection volume of 20 uL is injected in to the stationary phase column. The gradient program was set up and the peak analysis was estimated by observing the graph and comparing the obtained chromatogram with that of the already available data (Jane and Plumb, 2004).

Anti microbial activity

Preparation of nutrient agar medium

All the components were taken into a conical flask and 100 mL of distilled water was added and pH was adjusted to 7.2 and 2g of agar was added. Then the nutrient agar medium was sterilized in an autoclave at 121ºC under 15 lbs pressure for 15-20 minutes.

Preparation of microbial cultures

The following bacterial strains for the antimicrobial assay have been collected from microbial type culture collection (MTCC) of IMTECH, Chandigarh. The microorganisms that were used in the antimicrobial assay are *Escherichia coli*, *Pseudomonas aeruginosa, Bacillus subtilis* and *Escherichia fecalis*.

Preparation of pure cultures

Pure cultures nutrient agar plates were prepared by taking a loopful of culture from stock cultures and it was streaked on Petri plates by streak plate method to obtain fine isolated colonies. The petriplates were incubated at 37ºC for 24 hr.

Pour plate technique

Pour plates allow for the growth of isolated colonies on the surface of the agar. An isolated colony is a colony that is not touching any other colonies and is assumed to be a pure culture. These colonies were easily accessible for performing staining and identification procedures. The materials used for the experimentation are culture tubes, inoculating loop, incubator, sterile petri plates and nutrient agar.

The standard procedure was followed to test the antimicrobial activity by pour plate method. A loopful of innoculum containing the microorganism from the broth was taken using a sterile agar medium plate and poured in to the plate. The loop was sterilized on the flame and continued to pour the bacteria again. The plate was rotated for about 90 degrees and spread the bacteria. Repeat the process till it is required. Later the plates were incubated. The crude and column cleaned up compounds were used to test the antibacterial activity by disc method. The plates were observed for inhibition of culture growth by the tested compounds. The results were photographed and discussed in the section results and discussion.

RESULTS AND DISCUSSION

Anti microbial activity

The plant materials showed good anti microbial activity. As most of the microorganisms are showing resistance to various

Table 1: Phyto Chemical Screening of *Tridax Procumbens*

Qualitative Test	*Tridax Procumbens*
Alkaloids	+ + +
Glycoside	+ +
Sterols	+
PhenolsandTannins	+ + +
Sterols	+
Flavonoids	+
Gums and Mucilage	+ +
Saponins	+
Terpenes	+ +
Fixed Oils	+

+: Low Concentration, + +: Medium Concentration and + + +: High concentration

Table 2: Anti Bacterial activity of crude and column extracts *Tridax Procumbens*

Microbes	Zone of Inhibition in mmMean +/- SD Crude	Coloumn
E.Coli	8.1+/-0.6	4.0+/-0.6
E.Fecailis	7.2+/-0.6	4.0+/-0.2
B.Subtilis	6.8+/-1.2	5.2+/-0.8
P.Aerugens	8.0+/-0.4	5.2+/-0.8

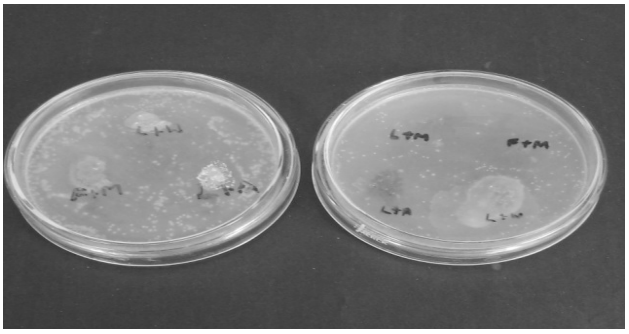


Figure 1: Photographs showing the antibacterial activity of *E.Coli* on Crude and Column Extracts

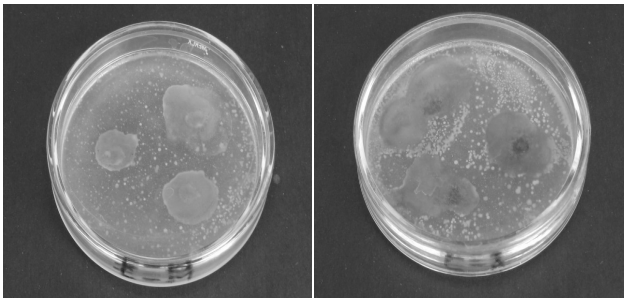


Figure 2: Photographs showing the antibacterial activity of *E.Fecalis* on Crude and column extract

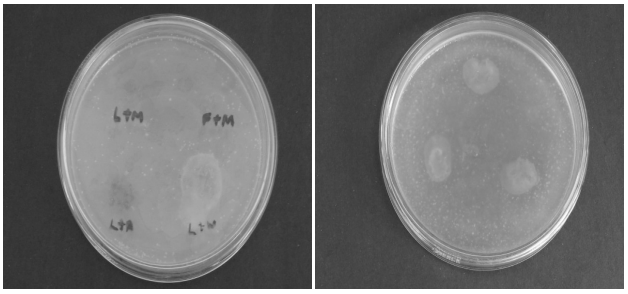


Figure 3 : Photographs showing the antibacterial activity of *Bacillus Subtilis* on crude and coloumn extracts

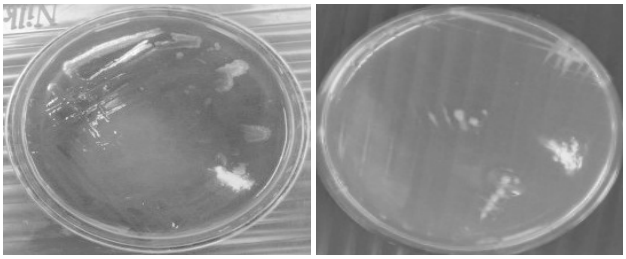
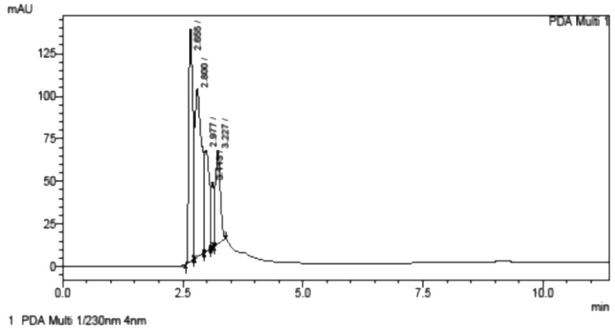


Figure 4: Photographs showing the antibacterial activity of *Pseudomonas aeruginosa* on Crude and column Extracts

Centre for biotechnology

Sample Name : Leaf-MeOH (Tridax)1:10
Sample ID : Tridax Procumbens
Injection Volume : 20μL
Data Acquired : 8/9/2009 2:36:14PM



PDA Ch 1230nm 4nm

Peak#	Ret. Time	Area	Height	Area%	Height%
1	2.655	876510	136898	31.442	35.195
2	2.800	954971	99393	34.257	25.553
3	2.977	416934	59741	14.956	15.359
4	3.113	163104	38165	5.851	9.812
5	3.227	376157	54775	13.494	14.082
Total		2787677	388971	100.000	100.000

Figure 5: HPLC Analysis of Leaf extract showing peaks Phenols, Tannins and Flavonoids at 230nm

compounds there is a need for going naturally occurring compounds for the use as anti microbial agent. The antimicrobial activity were represented in Figs. 1- 4.

The antimicrobial activity is greater in crude extracts than coloumn extracts (Table 2). Hence we can say the active components which are present *T.procumbens* are exbhiting antimicrobial activity from the above results.

The order of susceptibility of tridax procumbens on microorganisms is as follows *P.aeruginosa* > *B.subtilis* > *E.fecalis* > *E. coli*.

Phenolic compounds are reported to the active quenching oxygen –derived free radicals by donating hydrogen atom or an electron to the free radical .It has been well known that plant materials have been well known that plant materials have shown to neutralize free radicals in various invitro model systems (Zhang and Reith, 1996). Earlier the poly phenolic compounds have protective effects on mutagenesis and carcinogenesis in human when ingested 1g daily from a diet rich in fruits and vegetables (Tanaka *et al.,* 1989). The phenolic and flavonoid contents results of phytochemical analysis were carried out by HPLC method.

Bioactive potential of Flavonoids has been reported. (Ilic *et al.,* 2004; Cushner and Lamb, 2005). The present results comparable with comparable with that of Samy *et al.,* (1999). Sanchez *et al.,* (2005).who reported the antimicrobial activity of *T. procumbens*. In light of the fact that micro organism are becoming resistant against the drugs in use, present investigation is of great importance in pharmaceutical industries for preparing plant based antimicrobial drugs.

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