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A STUDY ON ANTIMICROBIAL POTENTIAL OF *TRIDAX PROCUMBENS* (L.) AGAINST CLINICAL ISOLATES

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ABSTRACT: *In-vitro* antibacterial and antifungal activity of *Tridax procumbens* plant parts (leaf and stem) were investigated by agar well diffusion method. The petroleum ether, methanol and aqueous extracts of the plant parts were tested against two bacterial strains *i.e., Bacillus subtilis* and *Escherichia coli* and two fungal strains *i.e., Trichoderma reesei* and *Fusarium oxysporium*. The results of antibacterial activity showed that methanol extract was effective and aqueous extract was devoid of any significant activity. Methanol extract of *Tridax procumbens* showed maximum antifungal activity. The results lend credence to the folkloric use of this plant in treating microbial infection and showed that *Tridax procumbens* leaves and stem could be exploited for new potent antimicrobial agents.

INTRODUCTION: In recent years attention has been devoted to novel molecules derived from plant sources which has replaced vast chemically broad spectrum antibiotics. The demand of plant based therapeutics seems to be increasing due to their incredible ventures, being non narcotic, having no side effects, easily available at affordable prices. Plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases [1]. Over the years, these herbal drugs have been shown to be effective [2]. The antimicrobial activities and the results obtained from these scientific studies have aided in the rationalization of medicinal use of these plants [3].

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Plants have limitless ability to synthesize a vast array of bioactive compounds which possess some bioefficacy. These substances serve as plant defense mechanisms against predation of microbes, insects, herbivores. Phytochemicals from medicinal plants showing antimicrobial activities have the potential of filling this need, because their structure are different from those of the earlier studied microbial sources and therefore, their mode of action may likely to differ [4].

Living organisms are prone to infection by several microorganisms, especially bacteria and fungi [5, 6]. In general, bacteria have genetic ability to transmit and acquire resistance against the drugs used as therapeutic agents. One way to prevent antibiotic resistance is by using new compounds which are not based on existing synthetic antimicrobial agents[7]. Researchers are now in search of effects of various plant extracts of bacteria [8, 9]. Reports are available on *in-vitro* and *in-vivo* efficacy of plant extracts against plant and human pathogens causing

fungal infections [10]. Keeping this in view, the present study has been undertaken to evaluate the antimicrobial effects of various extracts of *Tridax procumbens* (L.) plant parts.

T. procumbens is a semi prostate annual or short-lived perennial herb. *Tridax procumbens* (Linn.) locally known as 'Sadahari' or 'Hari Ghass'. Flower heads are creamy or white and long-peduncled. Its leaf extract is applied on the spot of cut to stop bleeding and pain. It has anticoagulant, antifungal, antidiarrhoel and insect repellent properties [11-12]. It has hepatoprotective activity [13], anti-inflammatory [14], and wound healing [15, 16], antidiabetic activity [17], hypotensive effect, immunomodulating property [18], anticancer activity [19], and antioxidant activity [20-22].

MATERIALS AND METHODS:

Plant material

Tridax procumbens leaves and stem parts were collected from Rajasthan University, Jaipur, Rajasthan, (RUBL 211384), India. The plant material were taxonomically identified and authenticated by Herbarium, Department of Botany, Rajasthan University, Jaipur, Rajasthan, India.

Collection of Microbes:

Bacterial strains of *Bacillus subtilis* and *Eschereschia coli* and fungal isolates of *Trichoderma reesei* and *Fusarium oxysporium* were used for the study and were collected from stock cultures of microbiology lab, SMS medical college, Jaipur, India.

Bacterial strains:

Microorganisms used for the determination of antibacterial activities of isolated compounds were gram positive *Bacillus subtilis* -ATCC 25922 and gram negative *E. coli*- ATCC 6633.Different bacterial strains were maintained on nutrient agar and subcultures were freshly prepared before use. Bacterial cultures were prepared by transferring 2-3 colonies into tube containing 20ml nutrient broth and grown overnight at 37ºC.

Fungal inoculums preparation:

The fungal cultures of *Trichoderma reesi*-NCIM 27853 *and Fusarium oxysporium* NCIM 10259 were maintained in Potato dextrose agar plates and slants which were further subcultured before use. The mother inoculum was maintained at 37ºC for about 48-72 hours. The fungal spores were scooped out by adding 1ml of sterile distilled water. The fungal spores were collected to about 1 ml and it was serially diluted from 10^{-1} to 10^{-6} and plating was done using master agar plate dilution.

Preparation of plant extract:

The plant was freshly collected about 5 kg and were shade dried until all the water molecules were evaporated (15-30 days). After drying, the plant leaves and stem ground well using mechanical grinder into fine powder and then transferred into airtight container for further studies. The fine powder (100gm/1000ml of respective solvent i.e 1:10 ratio) was then subjected to mortar and pestle for the extraction of pure form of plant part (leaf and stem) extract.

The extract was filtered and filtrate was concentrated at 30ºC under reduced pressure in a rotary evaporator. The crude extract was them dissolved in respective solvents viz petroleum ether, aqueous and methanolic extracts.

Determination of antibacterial assay

In-vitro anti bacterial activity, samples of gram positive and gram negative bacterial strains by agar well diffusion method [23].

Agar disc diffusion assay

The Mueller Hinton agar was melted and cooled to 48 - 50ºC and a standardized inoculums (1.5×108 CFU/mL, 0.5 McFarland) was then added aseptically to the molten agar and poured into sterile petridishes to give a solid plate. Wells were prepared in the seeded agar plates. The test compound (100 µl) was introduced in the well (6 mm in diameter). The plates were incubated overnight at 37ºC.

The antimicrobial spectrum of the chemical compounds was determined for the bacterial species in terms of zone sizes around each well. The diameters of zone of inhibition produced by the agent were compared with those produced by the commercial control antibiotics, streptomycin. The control zones were subtracted from the test zones and the resulting zone diameter was measured with antibiotic zone reader to nearest

mm. The experiment was performed three times to minimize the error and the mean values are presented in Table.

Determination of Antifungal Assay

Anti fungal activity of the experimental plant was investigated by agar well diffusion method [24]. The yeasts and saprophytic fungi were subcultured onto Sabouraud's dextrose agar, SDA (Merck, Germany), and incubated at 37°C for 24 h and 25°C for 2 - 5 days. Suspensions of fungal spores were prepared in sterile PBS and adjusted to a concentration of 106cells/ml. The plates were dried at room temperature for 15 min. Wells of 10 mm in diameter and about 7 mm apart were punctured in the culture media using sterile glass tube. 0.1 ml of serial dilutions of fresh chemical compounds was administered to fill up each well. Plates were incubated at 37°C. After incubation of 24 h bioactivities were determined by measuring the diameter of inhibition zone (in mm). All experiments were made in triplicate and means were calculated. Results are shown in **Table 1**.

RESULTS:

In present study, two different plant parts from *Tridax procumbens* (leaves and stem) in different solvents were investigated for their antimicrobial potential. Results of antimicrobial studies using agar disc diffusion method have been presented in **Table 1**.

TABLE: 1. ANTIMICROBIAL ACTIVITY (ZONE OF INHIBITION IN MM) OF VARIOUS PLANTS EXTRACTS OF *TRIDAX PROCUMBENS* AGAINST CLINICAL PATHOGENS.

Organism	Plant Part	Diameter of Inhibition Zone in mm		
		Aq.Extract	Pet ether	Methanol Extract
Bacteria				
Bacillus subtilis	Leaf	-	-	-
	Stem	-	-	-
E.coli	Leaf	-	-	IZ=10 AI=0.2
	Stem	-	IZ=12 AI=0.6	IZ=8 AI=0.4
Fungi				
Fusarium oxysporium	Leaf	-	-	IZ=16 AI=0.7
	Stem	-	-	IZ=10 AI=0.4
Trichoderma reesei	Leaf	-	-	IZ=22 AI=1
	Stem	-	-	-

*IZ=Inhibition zone (in mm) includes the diameter of disc (6mm); Standards: Streptomycin (1.0mg/disc); Ketokenazole (1.0mg/disc); AI-Activity Index=IZ of sample/IZ of standard. Values are mean of triplicate readings.

The plant material extracts showed good antimicrobial activity. In order to extract the important phytochemical categories such as alkaloids, glycosides, proteins, terpenoids, flavanoids etc three different solvents with varying polarity were used in the present study. The antibacterial activity of three extracts were evaluated using streptomycin at a concentration 1mg/ml as reference standard of Inhibition zone 20mm, while for antifungal, ketokenazole at same concentration was used of inhibition zone 22mm.

Among three extracts, methanol extract of *Tridax procumbens* showed good activity against *E.coli*. Methanol extract of *Tridax procumbens* leaf exhibited inhibition activity against *E.coli* (ZOI-10mm) while other two extracts i.e, distilled water, petroleum ether did show any significant inhibition zone. Whereas petroleum ether extract of *Tridax procumbens* stem was more promising against *E.coli* when compared to other two solvents as it showed higher activity index in petroleum ether (ZOI-12mm). It is found that *Bacillus subtilis* showed no significant zone of inhibition (ZOI) in any of the three solvents of *Tridax procumbens* plant parts under study.

Methanolic extract of *Tridax* leaf exhibited significant zone of inhibition against both *Fusarium oxysporium* and *Trichoderma reesei* when compared to petroleum ether and aqueous solvents. Activity index of *Trichoderma reesei* is higher in methanolic extract of leaf (ZOI-22mm), while activity index of *Fusarium oxysporium* was found higher in methanol extract of stem (ZOI-10mm). The other two solvents were devoid of any

significant activity index against antifungal activity.

In light of the fact that microorganisms are becoming resistant against the drugs in use, present investigation is of great importance in pharmaceutical industries for preparing plant based antimicrobial drugs.

DISCUSSIONS: The activity of plant extracts against bacteria and fungi have been studied for years, but in a more intensified way during last 3 decades. During this period, numerous antimicrobial screening evaluations have been published on traditional use of Chinese, African and Asian plant based drugs [25]. Plants remain the most common source of antimicrobial agents [26-27]. Many aromatic plants have been used traditionally in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi, and yeast [28]. Biologically active compounds from natural sources have always been a great interest for scientists working in infectious diseases. Plant based anti microbial have enormous therapeutic potential as they can serve the purpose with no or lesser side effects due to an array of secondary metabolites [29].

In present investigation, methanolic, petroleum ether and aqueous extract of *in-vivo* plant parts (leaf and stem) of *Tridax procumbens* were screened for their antibacterial and anti fungal activities. Among the 4 microbes which (*E.coli*, *B.subtilis*, *F.oxysporium* and *T.reesei*), extract of various plant parts tested for antimicrobial activity.

In the present study, stem of *Tridax procumbens* showed largest zone of inhibition in bacterial cultures of *E.coli* (12mm) in petroleum ether extract, while no significant zone was observed in aqueous extracts of any plant part and also no zone was found in pet. ether extract of leaf. However, Monika *et al*., 2013 found (agar well diffusion method) maximum zone of inhibition in methanolic extract against *Klebsiella pneumonia* (1.9±0.7 cm), while minimum in same extract but against *S.aureus* [30]. During the study, it was found that, pet. ether extract showed activity against *E.coli*, while Christudas *et al.,* 2012, studies showed pet ether and ethanolic extract activity against *B.faecalis* which may be due to the presence of alkaloids [31]. Alkaloids are commonly found to have antimicrobial properties. Moreover, Bharathi *et al.*, 2012 also demonstrated that the methanolic extract of *Tridax procumbens* stem was effective against *E.coli* [32].

During the study, as we found inhibition zone of 10 mm at 1mg/ml in methanolic extract of leaves of *Tridax procumbens* , similarly, Razia *et al.,* 2013, showed similar results against *E.coli* (ZOI-12mm) at 75µg/ml concentration [33]. While Tejaswini *et al.*, 2011 reported more ZOI (>12mm) against *E. coli* and *Bacillus subtilis* in same solvent. Petroleum ether and aqueous extract were devoid of any activity against all selected organisms[34]. In similarity with the study of Dhasarathan *et al.*, 2011, the average size of ZOI for *E. coli* was 10 mm in all extract [35] except aqueous extract. Different solvents have been reported to have the capacity to extract different phytoconstituents depending on their solubility or polarity in the solvent, Aniel and Naidu, 2011 have reported antimicrobial activity of ethanol and methanol extracts of root, stem, flower, leaf and whole plant of *T. procumbens* against *E.coli, Klebsiella pneumonia, Proteus vulgaris* and *B.subtilis and Staphylococcus aureus* [36].

They reported higher solubility for more phytoconstituents, consequently the highest antibacterial activity and three extracts (ethanol, methanol and aqueous) were more active against the Gram-positive bacterial strains (*Staphylococcus aureus* and *Bacillus subtilis*) than the Gram-negative bacterial strains, which is in conformity with earlier studies [37-40], whereas in contrast, in our study petroleum ether, aqueous and methanol extract showed no antimicrobial activity against any *Bacillus subtilis*. Only methanolic and petroleum ether extract were found to be effective against *E.coli.*

However, in present investigation maximum zone of inhibition was found in methanolic extract of plant leaf against *T. reesei* (22mm), strong antifungal activity was reported by Dangi *et al.*, 2013 against *Aspergillus niger* [41]. The zone of inhibition varied suggesting the varying degree of efficacy and different phytocontituents of *T. procumbens* on target organisms. The antibacterial

activity of leaves may be due to presence of various active principles.

According to this study, plant based antibacterial drug have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobial agents. The present results revealed that the extract of *Tridax procumbens* L. was effective against both Gram-positive and Gram-negative bacteria. Presence of chemical compounds viz. alkaloids, tannins, flavonoid and saponins of *Tridax procumbens* L. may inhibit the bacterial growth. Traditionally, *Tridax procumbens* L. was employed using/mixing with aqueous for treating the antibacterial and other infections.

Naturally, the biological active compounds whose activity can be enhanced in the presence of ethanol and methanol could have been produced number of active compound responsible for antibacterial activity. The present study provides the scientific information about the plant extract of *Tridax procumbens* L. and supports the usage of this plant for curing many bacterial diseases by traditional healers. Further studies are needed to isolate and characterize the bioactive principles to develop new antibacterial drugs.

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