Research Paper

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Antimicrobial activity of different solvent extracts of *Tridax procumbens* (Asteraceae) from leaf and stem against Human pathogens

Zoology

KEYWORDS : *Tridax procumbens*, solvent extracts, Antimicrobial activity and Thin layer chromatography

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ABSTRACT

To evaluate the antimicrobial activity of solvent extracts from leaf and stem of Tridax procumbens. The Ethyl acetate, Acetone, Hexane and Methanol extracts of Tridax procumbens were evaluated against Gram-positive Bacillus cereus (B. cereus), Staphylococcus aureus (S. aureus) and Gram-negative bacteria Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa), Klebsiella pneumoniae (K. pneumonia) by using agar well diffusion method. The results revealed that among five pathogenic bacteria. Bacillus subtilis and Staphylococcus aureus belongs Gram-positive bacteria showed higher susceptible for leaf and Stem extracts.

Introduction

Anti-microbial agents are undeniably one of the most important therapeutic discoveries of the 20th century. However, with the 'antibiotic era' barely five decades old, mankind is now faced with the global problem of emerging resistance in virtually all pathogens (Peterson and Dalhoff 2004). The herbal medicines serve the health needs of about 80% of the world's population, especially for millions of people in the vast rural areas of developing countries; more than 65% of the global population uses medicinal plants as a primary health care modality (WHO 2001). In view of this, the searches for new anti-microbial agents from medicinal plants are even more urgent in the countries like India where infectious diseases of bacterial origin are not only rampant, but the causative agents are also developing an increasing resistance against many of the commonly used antibiotics (Abebe *et al*., 2003). The results of the present study would be useful in promoting research aiming at the development of new agent for bacteria control based on plant source. This study was undertaken to assess the antimicrobial activity of *Tridax procumbens* leaf and stem extracts of against Gram-positive and Gram-negative bacteria.

Materials and methods

Plant Collection and identification

The leaf and stem of Tridax procumbens were collected from Auxilium College campus, Katpadi Vellore District, Tamil Nadu, (South India). The plant identified by identification was made by Ms. S. Isabella Rosaline, Department of Botany, Auxilium College, Katpadi, Vellore District, Tamil Nadu, and (South India).

Preparation of Extract

Fresh leaves and stem of *Tridax procumbens* was washed thoroughly with tap water and shade dried for 7 days. Coarse powder of leaf and stem was obtained by crushing the leaves in an electronic blender. The extraction procedure was carried out with 300 grams of coarse powder of the leaves of *Tridax procumbens* using 1200ml Hexane, Acetone, Ethyl acetate and Methanol in a Soxhlet extractor for 48 hours. A solvent extract was prepared similarly by using only the powder. The extracts were stored in desiccators for preliminary phytochemical analysis and further testing of antimicrobial activity.

Agar Well diffusion method

The antimicrobial activity was tested against solvent Methanol, Acetone, Hexane and Ethyl acetate extracts of *Tridax procumbens*. The inoculation of microorganisms was prepared from bacterial culture (Parihar and Bohar 2006). The inoculums suspension was spread uniformly over the agar plates using spreader, for uniform distribution of bacteria. Subsequently using a sterile borer, well of 0.5cm diameter was made in the inoculated media in addition to 0.2ml of each extract was a specially filled into the well. Later the plates were placed at room temperature for an hour to allow diffusion of extract into the agar. Then the plates were incubated for 24 hour at 37°C for room temperature. The result was recorded by measuring the diameter of inhibition zone at the end of 24-72 hour. Zone of inhibition surrounding the disc was measured using a transparent ruler and the diameter was recorder in mm.

Thin Layer Chromatography (TLC)

A sample mixture was dissolved in solvent (Ethyl acetate) and spotted at one end of the silica gel TLC (MERK) plate. The plate was kept in the tank beaker containing the mobile phase (Hexane: Methanol: acetic acid 4:3:0.5) in such a way that the end near the sample application should touch the mobile phase. The chromatogram was allowed to run about 30 min. The plate was dried at 30-40°C at hot air oven/ RT. The compound was viewed under UV Transilluminator. The R_F value was calculated as explained below

$$R_f = \frac{\text{Distance moved by the solute (b)}}{\text{Distance moved by the solvent (a)}}$$

Phytochemical analysis of *Tridax procumbens*

The extracts prepared were analyzed for the presence of alkaloids, tannins, flavonoids, anthraquinone, cardiac glycosides and reducing sugars based on the protocols available in the literature (Adetuyi *et al*., 2001).

Statistical analysis

Analysis was performed using Microsoft Excel 2007. The one way ANOVA test was used to determine any statistically significant difference in the MIC of the extracts and the antibiotics. P-values <0.05 were considered significant.

Result

The different solvent extract of *Tridax procumbens* showed effective antimicrobial activity against the test pathogens. The antimicrobial activity of solvent extracts (Methanol, Acetone, Hexane, and Ethyl acetate) of plant parts of *Tridax procumbens* (leaf and stem) against human pathogenic bacteria both Gram-positive and Gram-negative bacteria are presented in (Table-1and 2). *Bacillus subtilis and Staphylococcus aureus*

belongs Gram-positive bacteria showed higher susceptible for leaf and Stem extracts. In Gram-negative bacteria *Pseudomonas aeruginosa* showed maximum inhibition where as Gram-positive bacteria showed least susceptible for leaf and Stem. Among the four solvent extracts the methanol and acetone extracts showed highly significant activity against both Gram-positive and Gram-negative bacteria. Other solvent extracts Hexane, and Ethyl acetate showed less significant activity when compared with Ciprofloxacin. Among tested bacteria *Staphylococcus aureus* and *Bacillus subtilis* were sensitive to methanol and acetone extracts of leaf and Stem, while moderately sensitive to Hexane, and Ethyl acetate respectively. The results were expressed as mean ± standard deviation (n=3). The leaf extract showed maximum antimicrobial activity against *Pseudomonas aeruginosa* (16.0±0.5) and lowest activity against *E.coli* (5.0±1.5) compared with Ciprofloxacin. The Stem extract showed maximum antimicrobial activity against *Bacillus subtilis* (15.6±0.4) and lowest activity against *Klebsiella pneumoniae* (5.0±0.6) compared with Ciprofloxacin.

TLC analysis also suggests the presence of different kinds of phytochemicals in leaf extract. Table 3 reports the R_F values for various extracts. TLC of plant extract in Methanol and Acetone reports seven spots for various phytochemicals. The reported spots are separated with enough space and having various R_F values showing the presence of atleast seven phytochemicals in Methanol and Acetone solvent extracts. Extracts in Hexane and Ethyl acetate report only five spot. It is prominent and uniquely identified.

Discussion

The present results showed that the leaf extracts are more effective than the stem. Different solvents have been reported to have the capacity to extract different phytoconstituents depending on their solubility or polarity in the solvent acetone and methanol extracts in this study might have had higher solubility for more phytoconstituents, consequently the highest antimicrobial activity and four extracts (Acetone, Methanol, Hexane, and Ethyl acetate) were more active against the Gram-positive bacterial strains (*Staphylococcus aureus* and *Bacillus subtilis)* than the Gram-negative bacterial strains (Matu and Van Staden 2003).

Out of these three the maximum zone of inhibition (compared with ciprofloaxcin) was observed in the leaf methanolic extract of *Tridax procumbens* against *Pseudomonas aeruginosa* (16.0±0.5) Bacillus *subtilis (*15.4±0.5), *Klebsiella pneumonia* (15.5±0.3) and stem methanolic extract of *Tridax procumbens against Bacillus subtilis (*15.6±0.4) *Pseudomonas aeruginosa* (15.3±0.3) *Escherichia coli* (14.0±0.4) respectively. While work done by (Anjana Sharma 2009) using aqueous extract of plant *Terminalia chebula* and *Zinziber officinale* showed the zone of inhibition against *Proteus* (1mm, 0mm*), E.coli* (9mm, 0mm*), Pseudomonas aeruginosa* (5mm, 4mm) and *Klebsiella pneumoniae* (5mm, 6mm) respectively, thus our studied plant is showing more effective results and can be used to prepare drug against disease caused by ESBL producing bacteria.

TLC profiling of all four extracts gives an impressive result that directing towards the presence of number of phytochemicals. Various phytochemicals gives different R_F values in different solvent system. This variation in R_F values of the phytochemicals provides a very important clue in understanding of their polarity and also helps in selection of appropriate solvent system for separation of pure compounds by column chromatography. Mixture of solvents with variable polarity in different ratio can be used for separation of pure compound from plant extract. The selection of appropriate solvent system for a particular plant extracts can only be achieved by analyzing the R_F values of compounds in different solvent system. Different R_F values of the compound also reflect an idea about their polarity.

Conclusion

The present study is conducted to evaluate the phytochemical and antimicrobial activities of *Tridax procumbens*. This study has confirmed the antibacterial potentials of ferns, thus supporting their application as a biocontrol herbal remedy. With these, there is need for the preparation of different formulations towards ensuring acceptable dosing to field trials. From this study, we came to the conclusion that the plant is endowed with many potent phytochemicals like flavonoids, tannins, quercitin, chlorophyll B, carotenoids, and many others. Therefore, we have to exploit the potent possibilities of this plant which possess high therapeutic value and many other uses. The results obtained in the present investigation show the presence of phytochemicals which take part in defense mechanism of the plant.

Table: 1.

Antimicrobial activity of solvent extracts of *Tridax procumbens* leaf (agar well diffusion method) Zone of Inhibition diameter (mm)

S. No.	Test organisms	Methanol extract	Acetone extract	Hexane extract	Ethyl acetate extract	Control (Ciprofloxacin 10mcg)
1.	Bacillus subtilis	15.4±0.5	9.0±0.7	7.0±1.0	8.5±0.3	14.0±0.7
2.	Staphylococcus aureus	14.0±0.4	12.5±0.8	9.0±0.5	10.3±0.5	15.3±0.4
3.	Klebsiella pneumonia	15.5±0.3	14.0±0.7	10.4±0.6	12.5±0.5	15.6±0.5
4.	Pseudomonas Aeruginosa	16.0±0.5	15.3±1.2	10.5±0.5	14.0± 0.4	16.5±0.4
5.	Escherichia coli	14.3±0.8	10.0±0.6	5.0±1.5	8.4± 0.5	14.7±0.5

S.D: standard deviation.

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Table: 2. Antimicrobial activity of solvent extracts of *Tridax procumbens* stem (agar well diffusion method) Zone of Inhibition diameter (mm)

S.No.	Test organisms	Methanol extract	Acetone extract	Hexane extract	Ethyl acetate extract	Control (Ciprofloxacin 10mcg)
1.	Bacillus subtilis	15.6±0.4	14.0±0.6	7.2±1.2	12.5±0.5	15.0±0.4
2.	Staphylococcus aureus	12.0±0.5	10.5±0.4	6.0±0.7	8.0±0.6	13.3**±0.5**
3.	Klebsiella pneumonia	13.0±0.5	12.5±0.3	5.0±0.6	7.0±0.8	12.8**±0.6**
4.	Pseudomonas Aeruginosa	15.3±0.3	12.0±0.5	5.5±0.5	10.0± 0.4	15.5±0.4
5.	Escherichia coli	14.0±0.4	10.5±0.3	9.0±0.7	7.4± 0.5	15.5±0.5

S.D: standard deviation

Table: 3. Phytochemical analysis of different solvent leaf extracts of *Tridax procumbens*

S.No	Solvents	Phytoconstituents	**R_f value**
1.	Methanol	Alkaloids	0.45
		Flavonoids	0.52
		Quercitin	0.64
		Tannins	0.68
		Chlorophyll B	0.72
2.	Acetone	Alkaloids	0.49
		Flavonoids	0.52
		Quercitin1`	0.63
		Tannins	0.69
		Carotenoids	0.72
		Chlorophyll B	0.78
3.	Ethyl acetate	Alkaloids	0.47
		Flavonoids	0.59
		Quercitin	0.65
		Tannins	0.77
		Chlorophyll B	0.82
4.	Hexane	Alkaloids	0.43
		Flavonoids	0.55
		Quercitin	0.61
		Tannins	0.68
		Chlorophyll B	0.74

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