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Antibacterial Activity of different parts of *Tridax procumbens* against Human Pathogens

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KEYWORDS

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A B S T R A C T

Treatment of infections continues to be problematic in modern time because of the severe side effects of some of the chemically prepared drugs and the growing resistance to antimicrobial agents. An extract of the leaves of *Tridax procumbens Linn* possesses antiseptic, antipyretic, anticoagulant, antifungal and insect repellent; in bronchial catarrh, diarrhoea and dysentery. Crude extracts of different parts of the plant were prepared from the plant *Tridax procumbens Linn* a tropically distributed medicinal plant in India. Antimicrobial activity of extracts of the plants was investigated by agar disc well-diffusion method against bacterial pathogens gram positive organisms *Bacillus cereus*, *Staphylococcus aureus* and gram-negative organisms: *Escherichia coli*, *Proteus mirabilis* and *Klebsiella pneumonia* ampicillin as standard. The plant extracts from stem and whole plant showed higher inhibitory activity against the tested human pathogens, Phytochemical screening of the plant revealed the presence of tannins, flavonoids,saponins and alkaloids. In conclusion, this study scientifically validated the use of plant in traditional medicine.

Introduction

Tridax procumbens a common weed found in India and many countries all over the world, growing primarily during raining season, is also one such plant. *Tridax procumbens* plant has been considered as a gregarious weed, distributed throughout the tropics and sub tropics. It has been extensively used in Indian traditional medicine as anticoagulant, antifungal and insect repellent; in bronchial catarrh, diarrhoea and dysentery. The leaf juice possesses antiseptic, insecticidal and antiparasitic properties. It is also used to check hemorrhage from cuts, bruises and wounds. An aqueous extract of the plant produced reflex tachycardia and showed a transient hypotensive effect on the normal blood pressure of dogs; it had also a marked depressant action on the respiration (Ali Rawinder and Ramachamdram, 2001).

Tridax procumbens is known for several potential therapeutic activities like antiviral, anti oxidant antibiotic efficacies, wound

healing activity, insecticidal and anti-inflammatory activity. Some reports from tribal areas in India state that the leaf juice can be used to cure fresh wounds, to stop bleeding and as a hair tonic. *Tridax procumbens* is traditionally used in the treatment of fever, typhoid fever, cough, asthma, epilepsy and diarrhoea (Nino *et al.*, 2006).

The scientific literatures on this medicinal herb are enormous and have reported to confer activity against gastritis, heart burn, antioxidant, anti diabetic, antimicrobial, antiseptic, insecticidal and antiparasitic (Syed Baker and Sreedharamurthy, 2013*). Tridax procumbens* has effect against blood pressure, bronchial catarrh, malaria, dysentery, diarrhoea, stomach ache, headache, wound healing, it also prevents hair fall and check hemorrhage from cuts and bruises. Its flowers and leaves possess antiseptic, insecticidal and parasiticidal properties, shows various pharmacological activities like Immunomodulatory, Anti-diabetic, Anti hepatotoxic and Anti-oxidant, Anti-inflammatory, Analgesic, and marked depressant action on respiration (Jain Ankita and Amita Jain, 2012).

The most important of the bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds (Ali Rawinder and Ramachamdram, 2001). The occurrence of fumaric acid is reported. The β –sitosterol and tannins were reported.

The water soluble novel polysaccharides were reported (Raju and Davidson, 1994). Luteolin and glucoluteolin from the flowers were isolated from *Tridax procumbens* (Ravikumar et al 2005). 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).

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. The phytochemical screening revealed the presence of alkaloids, carotenoids, flavonoids (catechins and flavones), fumaric acid, fl-sitosterol, saponins and tannins (Verma *et al*., 1988).

The extracts of *Tridax procumbens* have been reported to have various pharmacological effects. Flavones, sterols, tannins, glycoside, luteolin, glucoluteolin, saturated and unsaturated fatty acids, campesterol, stigmasterol, amyrin, sitosterol, quercetin, polysaccharide and monosaccharide have been isolated from the plant. These extracts are found to have antibacterial properties (Shirish Pingale, 2013).

There are number of reports on different parts of the plant and whole plant of *Tridax* has been reported for its antimicrobial activity on various species of bacteria. Antibacterial activity is mostly investigated by disc diffusion (Kirby-Bauer method) and agar-well diffusion method (Krishnavignesh *et al*., 2012).

The objective of the present study was to prepare crude ethanolic, methanolic and chloroform extracts of leaves, stem and flower of *Tridax procumbens*. Perform the phytochemical screening of ethanolic, methanolic and chloroform extracts of leaves, stem and flower for *Tridax procumbens*. To extracts were studied for its activity against both Gram positive and Gram negative bacteria by agar well diffusion method and their Minimum

Inhibitory Concentration of the different extracts was tested against sensitive organisms.

Materials and Methods

Plant materials

The plants were collected from Stella Maris College campus, Chennai, Tamil Nadu by uprooting the whole plant, and then different parts of the plant was separated and shade dried for one – two weeks.

Preparation of plant extracts of *Tridax procumbens*

The collected plant samples were washed with tap water and then with distilled water. The leaves, stem and flowers were separated and dried under shade and dried for two weeks. The dried plant parts were powdered separately, using a mechanical grinder. The powdered sample was stored in air-tight containers. Then, each powdered plant part was subjected to Soxhelet apparatus extraction using the solvents Ethanol, Methanol and Chloroform. 20 g of powdered sample and 300 ml of solvent was used. The extracts were collected and concentrated using Rotary Vacuum Evaporator. The crude semi-solid extracts were collected and stored in small vials. The extract was stored at 4°C until further use for various evaluations. The dried extracts were dissolved in dimethyl sulphoxide (DMSO) and subjected to antibacterial activity.

Phytochemical screening test of *Tridax procumbens*

These extracts were used to conduct the phytochemical evaluation, by dissolving a small quantity in the respective solvents. The phytochemical screening of the plant extracts was carried out to detect the presence or absence of certain bioactive compounds. The crude products obtained in soxhlet extraction technique were subjected to qualitative chemical evaluation of Carbohydrates and Glycoside, proteins and free amino acids, saponins, phytosterols, tannins, flavanoids etc (Hegde *et al*., 2010)

Antibacterial assay

Organisms used in the study

The bacterial culture used for the test included gram-positive organisms were: *Bacillus cereus* (NCIM 2106), *Staphylococcus aureus* (NCIM 2127), and gram-negative organisms: *Escherichia coli* (NCIM 2068), *Proteus mirabilis* (ATCC 29906) and *Klebsiella pneumoniae* (NCIM 2883)

Preparation of nutrient agar medium for Agar well diffusion method

A total of 1000 mL of nutrient agar medium is prepared by dissolving all the ingredients except agar by dissolving in distilled water with the aid of heat and filtered through cotton fiber. The pH was adjusted between7.8-8. The agar was dissolved in the solution by stirring on a water bath and transferred in test tubes (5 mL in each), plugged with cotton and sterilized in an autoclave at 121° C for 15 minutes.

Procedure

The well-diffusion assay was used to determine the antibacterial assay (Laouer et al 2009, Nair and Chanda 2005). The glass petri-dishes were cleaned and sterilized. Previously liquefied medium was inoculated with requisite quantity of suspension of microorganism and the suspension to the medium at a temperature between 40° C to

50°C and immediately poured the inoculated medium into petri-dishes to give a depth of 3 to 4 mm. The media was allowed to solidify at room temperature. A sterile borer was used to prepare four cups in the agar media. Stock solution for the three crude extracts was prepared with dimethyl sulphoxide and various concentrations (200, 600 and 800 mg/mL) were prepared from each extract. A solution of standard drug Ampicillin was prepared at the concentration 50 mg/mL for *B.cereus* and 200 mg/mL for the rest of organisms were prepared. To each plate, one bore was filled with 0.1 mL of ampicillin solution as reference standard and marked accordingly. To the other bore, 0.1 mL of the extract solution 200, 600 and 800 mg/mL were added respectively in clockwise manner. Micropipette was used to measure 0.1 mL of standard and test solutions. Petri dishes were then incubated at 37 ° C for 24 hrs and the zone of inhibition was measured using a zone reader and the results are recorded.

Results and Discussion

The phytochemical screening of *T. procumbens* with different parts of the plant and the whole plant with ethanol, methanol and chloroform extract showed the presence of alkaloids, tannins, saponin, coumorin, purines, carbohydrates, proteins (Table 1). The antibacterial activity of ethanol, methanol and chloroform extracts of different parts of the plant and the whole plant against human pathogenic organisms, both gram positive and gram negative organisms are presented in Table 2 and 3 respectively. From Table 2 it is shown that Ethanolic extract of different parts of the plant showed better antibacterial activity against two of the pathogenic strains, *E.coli, and B.cereus* than with *S.aureus*. While the bacterial strains *Kelbsiella sp.* and *Proteus sp.* didn't show much effect with the plant extarct. The alcoholic extracts (400 ug/mL) of the leaves and whole plant showed a better antibacterial activity when compared to other parts of the plant. The therapeutic value of medicinal plants lies mainly on the various phytochemical constituent's presents in their extract and the biological activity is attributed based on the components. Table 3 represents the antibacterial activity of the three alcoholic extract prepared from different parts of the plant. In the antibacterial study, gentamycin at a concentration 100 ug/mL was employed as reference standard for all the bacterial strains. It is shown that Ethanolic extract at 800 mg/L of different parts of the plant showed better antibacterial activity against three of the pathogenic strains, *E.coli, S.aureus and B.cereus* than the bacterial strains *Kelbsiella sp.* and *Proteus sp.*.

In microdilution, minimum inhibitory concentration (MIC) values of ethanolic, extracts of stem and whole plant of *Tridax procumbens* against five bacteria tested were shown in Table-4. The results of the tests for minimum inhibitory concentration with the stem extract revealed that the *Escherichia coli* and *Bacillus cereus* between (17.6-15.8 mg/mL). While *Klebsiella pneumoniae, Staphylococcus aureus, Proteus sp.* showed a lesser range from (10-12.5 mg/mL). The results of the tests for minimum inhibitory concentration with the whole extract revealed that the *Escherichia coli* and *Bacillus cereus* between (17.9-15.3 mg/mL). While *Klebsiella pneumoniae, Staphylococcus aureus, Proteus sp.* showed a lesser range from (14.2-8 mg/mL). This is means that higher doses of the antibacterial agent will be needed in the treatment of infections caused by *Klebsiella pneumoniae, Staphylococcus aureus, Proteus sp* provided they are not toxic to the tissues.

A number of compounds were reported to be present in *Tridax procumbens*. Tannin, Saponin, Alkaloid and Coumarin are

detected in the present study. It has been proved that the phytochemical components play an important role in the antimicrobial activity of medicinal herbs. Phytotherapeutically, tannin-containing plants are used to treat nonspecific diarrhoea, inflammations of mouth, throat and slightly injured skins (Naveen Prasad *et al.,* 2008). In the present study, the ethanolic and methanolic extracts were effective against *E. coli* and *B. cereus*. Among the ethanolic extracts, the stem extract and whole plant extract were more active than the leaf extract and flower extract against all the five bacterial strains.

It is found that among the inhibited bacteria, most are susceptible only to the ethanolic extracts, indicating that many of the active principles are soluble only in organic solvents like ethanol. This in accordance with the explanation that nearly all of the identified components from plants which are active against microorganisms are aromatic or saturated organic compounds and they are often obtained through ethanol or methanol extraction (Taddel, 2007). In the present study, the activity of the ethanol, methanol and chloroform extracts showed inhibition against *Escherichia coli* and *Bacillus cereus*.

The inhibition zones produced against the various micro organisms ranged from 8 mm to 21 for 400 ug/L and 8 mm -25 mm for 800 ug/L in my study. It has been shown that the inhibition diameters in case of commercial antibiotics are bigger than the ones produced by the crude extracts of *Tridax procumbens*, because of the purity and concentration of the commercial antibiotics. In case of crude extracts, neither the purity nor the concentration of the active components is known (Cowan, 1999).

From the present study, the minimum inhibitory concentration (MIC) values of ethanolic extracts of stem of *Tridax procumbens* against *E. coli* was 800 µg/ml. The results of minimum inhibitory concentration also revealed that ethanolic extract of stem (800 µg/ml) was more effective than the ethanolic extract of flower (600 µg/ml), against *B. cereus*.

In the present study in broth dilution technique, the ethanolic extracts of *Tridax procumbens* L. successfully control the *E.coli* and *Bacillus cereus* and *Staphylococcus aureus*. Minimum Inhibitory Concentration value also revealed that almost all tested bacterial strains were sensitive to the ethanolic extracts of our study plant. From the earlier studies it is also revealed that the organic solvent extract is better than aqueous extracts (Nair et al 2005). *Proteus sp* was the most resistent bacteria amongst all the bacterial strains investigated in the present work. The highest MIC values of whole plant extracts on *E.coli* in the inhibition showed that either the plant extracts are less effective on some Gram-negative bacteria and gram negative bacteria or that the organism has the potential of developing antibiotic resistance, while low MIC values for other bacteria is an indication of the efficiently of the plant extraction. Now the present study revealed that the ethanolic and methanolic extracts were effective control the bacterial growth than the chloroform extract. This probably indicates that there are bioactive ingredients that are able to inhibit the growth of these common pathogens. The present results revealed that the extract of *Tridax procumbens* L. was effective against both Gram-positive and Gram-negative bacteria used in the study. Presence of chemical compounds viz. alkaloids, tannins, flavanoids and saponins of *Tridax procumbens* L. may inhibit the bacterial growth.

Table.1 Phytochemical Screening of various Extracts of Tridax procumbens

EXTRACTS		Phytoconstituents						
		Tannin	**Saponin**	**Alkaloid**	**Coumarin**	**Carbohydrate**	**Protein**	**Purines**
Ethanol	Leaf	+	+	+	+	+	+	+
	Stem	-	-	-	-	+	+	+
	Flower	+	+	-	-	+	-	+
	Whole plant	+	+	+	-	+	+	+
Methanol	Leaf	+	+	+	+	+	+	+
	Stem	+	+	-	-	+	+	+
	Flower	-	-	-	-	+	-	+
	Whole plant	+	+	+	-	+	+	+
Chloroform	Leaf	+	+	+	+	+	+	+
	Stem	-	-	-	-	+	+	+
	Flower	-	-	-	-	+	-	+
	Whole plant	+	+	+	-	+	+	+

'+' indicates Presence; '-' indicates Absence

Table.2 Antimicrobial activity of ethanol, methanol and chloroform extracts (400 ug/mL) of *Tridax procumbens* against test organisms

Components (400 µg/mL)	**Diameter of Zone of Inhibition (mm) Ethanolic (E), Methonolic (M) and Chloroform (C) extract**															
	Organisms															
	E.coli			***B. cereus***			***S. aureus***			***K. pneumonia***			***Proteus sp.***			**NC**
	E	M	C	E	M	C	E	M	C	E	M	C	E	M	C	-
Leaf	18	10	15	13	13	14	10	8	14	7	6	4	5	6	-	-
Stem	17	10	11	12	12	10	8	4	5	5	6	4	4	8	-	-
Flower	12	15	16	11	15	10	10	8	5	7	5	4	8	9	-	-
Whole plant	21	15	17	19	14	12	10	12	8	9	4	5	4	10	-	-
PC	12	12	14	15	14	15	13	10	14	12	15	16	11	12	13	-

'PC' indicates Positive Control – Gentamycin; 'NC' indicates Negative Control – Chloroform

Table.3 Antimicrobial activity of ethanol, methanol and chloroform extracts (800 ug/mL) of Tridax procumbens against test organisms

Components (800 µg/mL)	**Diameter of Zone of Inhibition (mm) Ethanolic extract**															
	Organisms															
	E.coli			***B. cereus***			***S. aureus***			***K. pneumonia***			***Proteus sp.***			**NC**
	E	M	C	E	M	C	E	M	C	E	M	C	E	M	C	-
Leaf	18	14	19	12	15	17	12	10	10	6	8	6	-	-	-	-
Stem	19	12	14	15	13	12	8	10	10	6	4	-	-	-	-	-
Flower	14	15	17	15	14	11	10	8	8	8	5	-	-	4	-	-
Whole plant	25	14	15	21	11	10	12	5	4	10	12	8	8	4	-	-
PC	13	12	18	13	15	17	13	10	10	12	14	13	12	13	11	-

Table.4 Minimum inhibitory concentration (MIC) of the whole plant extracts of Tridax procumbens L. Organisms stem extracts and Whole plant

Organisms	**Stem extracts (Ethanolic extract mg/mL)**	**Whole plant (Ethanolic extract mg/mL)**
E.coli	15.8	17.9
B. cereus	17.6	15.3
S. aureus	12.5	14.2
K. pneumonia	11.2	15
Proteus sp.	10	8

Traditionally, *Tridax procumbens* L. was employed using with aqueous for treating the antibacterial and other infections. Naturally, the biological active compounds whose activity can be enhanced in the presence of ethanol could have been produced number of active compound responsible for antibacterial activity.

The present study provides information about the different parts of plant extract of *Tridax procumbens* L. and supports the usage of this plant for curing many bacterial diseases by traditional healers.

Conclusion

The results of this present study have proven that the stem extract and whole plant extraction of *Tridax procumbens* L. have great potential as antibacterial agents in the treatment of infectious organisms. Further, detailed investigation of the active compounds of the plant for the exact mechanism of action will contribute greatly to the development new pharmaceuticals.

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