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Drug Discovery

Studies on phytochemical screening, Antibacterial potential and Hemostatic activity of *Tridax procumbens*

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ABSTRACT

Nature always stands as a golden mark to exemplify the outstanding phenomenon of symbiosis. The plants are indispensable to man for his life. Nature has provided a complete store house of remedies to cure all ailments of mankind. The Phytochemical screening of acetone water and chloroform leaf extracts *T. procumbens* showed the presence secondary metabolites such as Alkaloids, Terpenoids, Tannins, Saponins, Flavanoids and Steroids, Amino acids, Phenol, Proteins and Glycosides. The Haemostatic activity of the acetone extract of the leaves of *T. procumbens* reduces the clotting time uniformly in the blood samples of all the subjects, it can be suggested that the same possesses hemostatic activity, thus affecting hemostasis. The Antibacterial activity of the acetone leaf extract of *T. procumbens* showed a varied inhibitory effects on the gram positive and gram negative strains.

Key words: *T. procumbens,* phytochemical, Haemostatic activity, antibacterial

1. INTRODUCTION

India is a country where very rich culture, folk medicine and nature go hand in hand. Since India is blessed by all kinds of environmental conditions like Himalayan to temperate to tropical, very rich flora is observed throughout the year. In nature many of the plants are present to which we call as Weeds, since their cultivation and economical status is not very high. But such weeds can be of great medicinal value. Traditional medicines or folk medicines are an important source of potentially useful new compounds for the development of chemotherapeutic agents. The essential values and uses of some plants have been worked out and published, but many of them remain unexplored to date.

Tridax procumbens (*T. procumbens*) is native of tropical America and naturalized in tropical Africa, Asia, Australia and India. Its widespread distribution and importance as a weed are due to its spreading stems and abundant seed production **(**Chauhan *et al.,* 2008**)**. The Yoruba people of Nigeria use the leaf of the plant for treating high blood pressure. In other West Africa sub-region and other tropical countries of the world, traditional medical practitioners and the native people use the leaves of the plant as remedy against conjunctivitis (Nia *et al*., 2003**)**. In Nigeria, the plant is known with many local names. The Ibo people call it "mbuli" while in south-western Nigeria it is called Igbalode or Muwagun (Olowokudejo, 1987). In Nigeria, *T. procumbens* is traditionally used in the treatment of fever, typhoid fever, cough, asthma, epilepsy and diarrhea (Mann *et al*., 2003).

Free radicals are produced in normal and/or pathological cell metabolism. Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, the uncontrolled production of oxygen derived free radicals is involved in the onset of many diseases such as cancer, rheumatoid arthritis, cirrhosis and arteriosclerosis as well as in degenerative processes associated with aging. Exogenous chemical and endogenous metabolic processes in the human body or in the food system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing biomolecules, resulting in cell death and tissue damage (Halliwell. 2004). Awareness of the potential benefits of antioxidant nutrients in health maintenance is growing. Antioxidants are means for the substances or group of the substances that delay or inhibit oxidative damage to a molecule.

2. MATERIALS AND METHOD

COLLECTION OF PLANT MATERIALS AND IDENTIFICATION

The leaves of *Tridax procumbens* were collected from Erode district, Tamilnadu, India. The collected leaves were identified by Dr.Balasubarmanium Department of Botany kongunadu Arts and Science College Coimbatore, The collected plant leaves *Tridax procumbens* (*T. procumbens*) were washed twice with tap water and rinsed with distilled water to remove or dust particles attached with leaves and the plant leaves subjected to dry in shade. Followed by this step, the dried plant leaves were then subjected to cold percolation method to obtain *T. procumbens* leaves powder

PREPARATION OF PLANT EXTRACT

About 10 g of air dried powder was taken in 100 mL of methanol. Plugged with cotton wool and then kept on a rotary shaker at 220 rpm for 24 h. Then the supernatant was collected and the solvent was evaporated to make the final volume one-fourth of the original volume and stored at 4 ^{o}C in air tight container.

A. QUALITATIVE ANALYSIS OF PHYTOCHEMICAL

The acetone, water and chloroform extract *T. procumbens* was screened for the presence of secondary metabolites using the procedures of (Harborne, 1984; Kokate *et al.,* 2011).

B. ANTI-BACTERIAL ASSAY

BACTERIAL CULTURE

Clinical isolates of microorganisms *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Klebsiella peneumoniae* were obtained from PSG Hospital, Coimbatore.

PREPARATION OF INOCULUMS

A loopful of strain was inoculated in 30 mL of nutrient broth bacteria composition (Dextrose, 4 g; Peptone, 1 g; Distilled water, 100 mL) in an Erlenmeyer flask and incubated on a rotary shaker at 37 ^{o}C for 24 h to activate the strain.

BIOASSAY (Perez *et al.,* 1990)

The antibacterial activity of the leaf extract was determined in accordance with the agar-well diffusion method. Nutrient agar plates were swabbed with a suspension of Staphylococcus *aureus* using sterile cotton swab. Wells of 6 mm were bored with a sterile cork borer in the swabbed plates and filled with the extract. Inoculated plates were incubated uninverted at 37 ^{o}C for 24 hrs. Controls were set up in parallel using the solvent that was used to reconstitute the extract. The plates were observed for zones of inhibition after 24 h. The results were compared with the standard antibiotic Neomycin (150 mg mL^{-1}).

C. HEMOSTATIC ACTIVITY OF THE LEAVES OF *T. PROCUMBENS*

As a guide to study the hemostatic activity of these leaves, clotting time (CT) of the various solvent extracts was determined *In vitro*. For the same, venous blood was collected in a clean and dry test tube without the addition of an anticoagulant and the time required for clotting was noted (normal clotting time is 5-12 min). Primarily, all the three extracts were screened for their effect on CT by testing on blood samples from the subjects. Venous blood was collected and stop-watch was started as soon as the blood entered the syringe. A set of 4 test tubes were filled, each with blood up to 1 mL mark, the first test tube being for normal CT. In the next 3 test tubes of the same set, 0.5 mL of extracts - ethanol extract (I), acetone extract (II) ,chloroform extract (III) and fresh leaf juice (IV) - were added. All these test tubes were then placed in water-bath at 37°C. Each of the test tubes was removed after 3 min and tilted at an angle of 45°C to see whether clotting had taken place. The test tubes in which clotting had not started were returned to the water-bath and examined at 30-s intervals to see if clotting had occurred. The watch was immediately stopped when there was clotting in a particular test tube and the time was noted in minutes. Likewise, CT was recorded for the remaining samples Out of the three extracts, extract (I) reduced CT while extracts (II) and (III) increased CT considerably than normal. This procedure was carried out *In vitro*, by drawing blood from 10 human volunteers; 2 males and 2 females, to minimize subject variation (Godkar and Praful, 1994).

4. RESULTS AND DISCUSSION

The phytochemical analyses of acetone and chloroform leaf extract of *T. procumbens* were analyzed for the compounds such as alkaloids, steroid, flavonoids, saponin, phenol, and tannins. The preliminary phytochemical analysis revealed the presence of six compounds i.e. alkaloids, cardiac glycosides, flavanoids, saponins, steroids and tannins and absence of glycosides, amino acid and Terpenoids (Table-1).

Various tests have been performed to find out the phytochemical constituents (Pimporn and Srikanjana, 2011). The results have shown that each and every phytochemical has the ability to get extracted with different solvents. This might differ according to the polarity of the solvent. It is denoted elsewhere in this paper that the leaves and the pod are being consumed as food and also acts as a supplement meal and as a vegetable. The usage of the plant parts is generally done with water.

With aqueous extract has shown the presence of phenol, alkaloids, tannins, terpenoids and acetone leaf extract has shown that it has extracted most of the compounds and this is confirming that acetone is being used as a solvent in ayurveda centers for extracting bioactive compounds (Malviya and Sharma, 2013). Every solvent has its own polarity and the polarity of the solvent is the major characteristic of them to be used as a base for extraction. Our experiment has shown clearly that methanol can be used as a active extracting solvent as the evaporation of acetone is soon thus we suggest that acetone can be used as an active extracting solvent (Biju John and Reddy, 2013). The different extracts of the leaves have clearly indicated that all the major phytchemicals are present in the extracts and so this plant leaves can be used perfectly as a major plant for extraction of bioactive compounds. Many

more experiments can be conducted in this plant where the plant can be grown at different conditions and checked assayed for the phytochemicals.

Table 1 screening of phytocompound in *T. procumbens*

Phytochemicals	W	A	C
Alkaloids	+	+	+
Flavonoids	_	+	+
Glycosides	-	-	-
Steroids	_	+	+
Tannins	_	+	-
Terpenoids	+	-	-
Protein	-	-	-
Amino acids	-	-	+
Phenols	+	+	+
Saponin	-	+	+

W-water, A- acetone, C-Chloroform

A. HEMOSTATIC ACTIVITY OF *T. PROCUMBENS*

The effect of various extracts of the plant on CT was observed (Table.2) and it was found that chloroform extract of plant leaves showed CT that exceeded the normal CT and so this was rejected from further studies. After testing on blood samples of 3 subjects, it was observed that CT was reduced only by acetone extract. CT less than that for control with acetone by approximately 1min in blood samples obtained from all subjects. Increase in normal clotting time thus signifies these deficiencies in coagulation. As the acetone extract of the leaves of *T. procumbens* reduces the clotting time uniformly in the blood samples of all the subjects, it can be suggested that the same possesses hemostatic activity, thus affecting hemostasis.

Table 2 Effect of various extract of *T. procumbens* on clotting time

Subject	Normal CT (min)	E CT(min)	A CT (min)	Fresh leaves juice (min)
1.	7	3.13	2.18	5.52
2.	8.02	5.09	1.30	8.00
3.	7.07	5.55	3.33	6.52
MEAN	22.09	14.07	7.21	20.04

Studied the effect of various extracts of the plant on CT was observed and it was found that fresh leaf juice and petroleum ether extract of the plant leaves showed CT that exceeded the normal CT, and so these were rejected from further studies. After testing on the blood samples of 10 subjects, it was observed that CT was reduced only by ethanolic extract. CT for (A) was less than that for control with ethanol (BA) by approximately 1min in blood samples obtained from all the subjects. Also, CT for (A) was less than that for normal CT by 2-3 min in blood samples of all the subjects. To relate between clotting time and hemostatic activity, the process of hemostasis was considered, which serially involves three processes: vasoconstriction, platelet plug formation and clot formation. In the last process, coagulation occurs in the blood which has come out of the blood vessel (extrinsic clotting) as well as within the occluded vessel by vasospasm (intrinsic clotting) and the plugs are formed due to extra vascular as well as intravascular clots, respectively (Shauhan and Johnson, 2008).

Clotting time determination is a routine laboratory test, which is carried out when there is coagulation factor deficiency; for example, deficiency of factor VIII, which causes haemophilia. Increase in normal clotting time thus signifies these deficiencies in coagulation. As the ethanolic extract of the leaves of *T. procumbens* reduces the clotting time uniformly in the blood samples of all the subjects, it can be suggested that the same possesses hemostatic activity, thus affecting hemostasis. In Present study the testing on blood samples of 3 subjects, it was observed that CT was reduced only by acetone extract. CT for was less than that for control with acetone by approximately 1min in blood samples obtained from all subjects. Increase in normal clotting time thus signifies these deficiencies in coagulation. As the acetone extract of the leaves of *T. procumbens* reduces the clotting time uniformly in the blood samples of all the subjects, it can be suggested that the same possesses hemostatic activity, thus affecting hemostasis.

B. ANTIBACTERIAL ACTIVITY

Infectious diseases are the number one among all causes of death, accounting approximately one-half all deaths throughout the world. About 50-75% of hospital deaths are reported due to infectious diseases (Gnanamani *et al*., 2003). These numbers are still increasing due to development of resistance in microorganisms to the existing first line drugs. Scientists from divergent fields are investigating plants with a new eye for their antimicrobial usefulness and as an alternative source to existing drugs. Plants with their wide variety of chemical constituents offer a promising source of new antimicrobial agents with general as well as specific activity (Evan's, 1996).

The present results revealed that the extract of *T. procumbens* was effective against both Gram-positive and Gram-negative bacteria. Presence of chemical compounds viz. alkaloids, tannins, flavonoid and saponins of *T. procumbens L.* may inhibit the bacterial growth. Traditionally, *T. procumbens* L. was employed using/mixing with aqueous for treating the antibacterial and other infections.

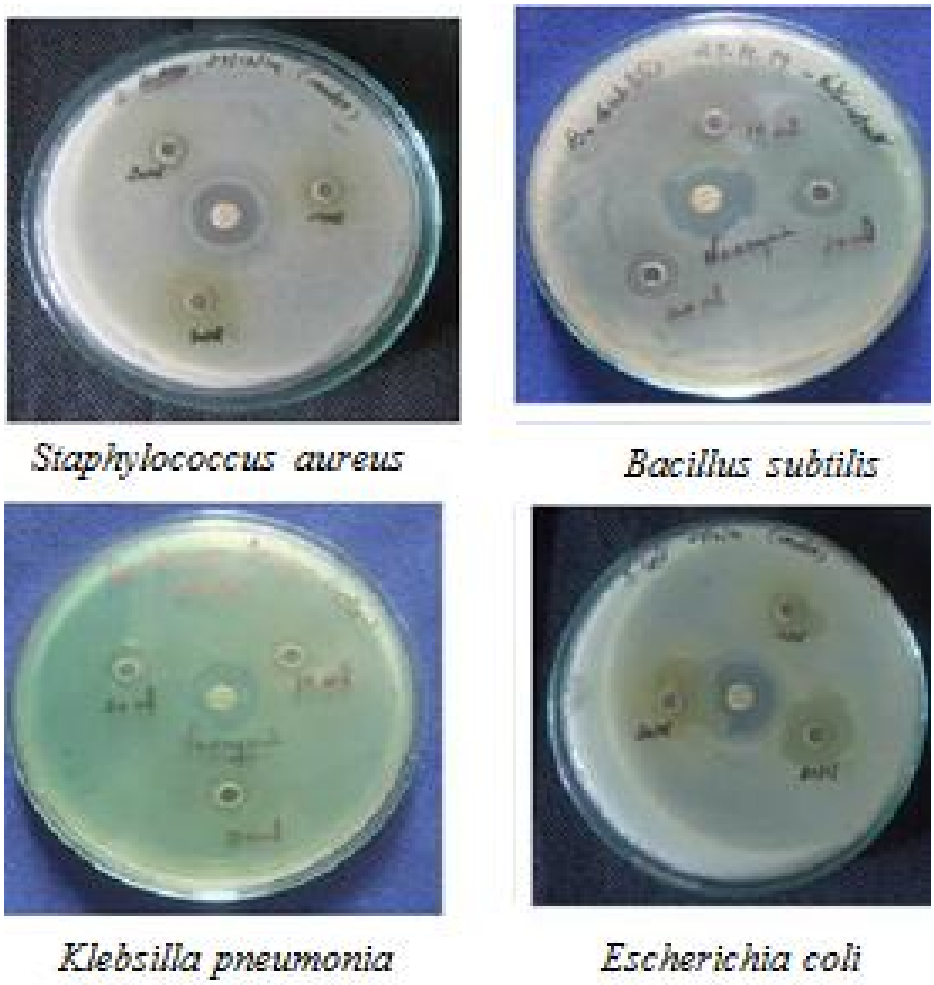

Plate 1 Antibacterial activity on various concentration of acetone leaf extract of *T. procumbens*

Table 3 Antibacterial activity on various concentration of acetone leaf extract of *T. procumbens*

Test organism	Control (Ampicillin)	Zone of inhibition (mm) / *T. procumbens* (mg/mL)		
		10	20	30
Gram positive strains				
Staphylococcus aureus	19±0.13	6.23±0.82	13.3±1.53	7.2±1.32
Bacillus subtilis	18±0.57	11±1.1	13.3±1.53	12±0.50
Gram negative strains				
Klebsilla pneumonia	16.7±0.8	12.3±1.53	10.80±0.6	14.24±0.04
Escherichia coli	14±0.03	9.20±1.50	12.6±0.94	11.50±0.8

Present study shows the antibacterial activity against the acetone leaf extract of *T. procumbens* against pathogenic bacteria's such as *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Klebsiella peneumoniae* showed varied results. The 25mg/mL concentration has shown maximum activity against *Bacillus subtilis* measuring 12±0.1and minimum against *Escherichia coli* 5±0.3. The *Klebsiella peneumoniae* and *Staphylococcus aureus* showed 12±0.1. The 50mg/mL concentration showed maximum activity against *Staphylococcus aureus* (18±0.35) and minimum activity against *Escherichia coli* (7±0.1). The other strains showed 15±0.63, 13±1.2 and 10±0.4 against *Klebsiella peneumoniae*and *Bacillus subtilis* respectively. The 75mg/mL concentration measured maximum activity against *Bacillus subtilis* (15.20±0.76) and minimum inhibitory activity against *Escherichia coli* (8±1.5). The other strains such as *Staphylococcus aureus* and *Klebsiella peneumoniae* showed 14±1.1 and 12±0.50 respectively.

Stem of *T. 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* (Jain, 1986).

5. CONCLUSION

The present study confirms the presence valuable chemicals present in the plants further thorough studies may bring out the real potential of these widely used medicinal plants in the preparation of antibiotic, antioxidant and anticancer drugs.

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REFERENCE

1. Biju John, Reddy VRK. Total Phenolics and Flavonoids in Selected *Justicia* Species, Pharmacognosy and Phytochem.2013, 2(4): 72-73.
2. Evan's, WC. Trease and Evan's Pharmacognosy. 14th edition W.B Saunders 1996, Company Ltd, UK.
3. Gnanamani, A., Shanmuga, Priya, K., Radhakrishnan, N. and Mary, Babu. Antibacterial activity of two plants extracts on eight burn pathogens. J. Ethnopharmacol. 2003. 86 (1); 59-61.
4. Godkar B. Praful. 1994. Text book of medical laboratory technology, Bhalain publishing house, Mumbai.
5. Halliwell, B. Biochemistry of oxidative stress. *Biochemical Society Transactions*, 2007, 35: 1147-1150.
6. Jain, N.C. The deer. In: Shalma O.W., Jam, N.C., Carol, E.J. Editors Schalm's Veterinary Haematology. 5th ed. Philadelphia. Lea and Febiger. 1986, 321-322.
7. Malviya R, Sharma. Agasthya (Sesbania grandiflora Linn.): Ayurvedic approach. UPJ, 2013, 02(04) :1-5.
8. Mann A, Abdulkadir NU and Muhammad G. Medicinal and Economic Plants of Nupe Land. *Juber Evans Books and Publication,* 2003, 78.
9. Nia R, Paper DH, Essien EE, Oladimeji OH, Iyadi KC and Franz G. Investiagation into in-vitro radical scavaging and in-vivo anti-inflammatory potential of *Tridax procumbens*. *Nig journal of physio sci.* 2003, 18(1-2):39-43.
10. Olowokudejo JD. Medicinal plants used as Vermifuges in Nigeria and Their Conservation. *J. Economic and Taxonomic Bot.,* 1987 9:459-466.
11. Pimporn A, Srikanjana K. An antibacterial activity of Sesbania grandiflora extracts Drug Discoveries & Therapeutics. 2011, 5(1):12-17.
12. Shauhan BS Johnson DE. Germination ecology of two troublesome Asteraceae species of rainfed rice: Sam weeds (*Chromolaena odorata*) and coat buttons (*Tridax procumbens*). *Weed Sci.,* 2008, 56(4): 567-573.