

Analysis of Phytochemistry and Antimicrobial activity of *Tridax procumbens* Linn

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Abstract

The aim of the present study was to investigate the phytochemistry and bioactive nature of *Tridax procumbens*. The ethanol, benzene and petroleum ether extracts of dried roots of this plant were investigated for phytochemistry and antibacterial analysis. The antibacterial activity was evaluated against different bacterial strains viz. *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Shigella flexneri* by detecting zone of inhibition. The zone of inhibition was compared with standard discs of Gentamicin. From these extracts, two of them (ethanolic and pet-ether extracts) revealed significant results as compared to benzene extract. The qualitative analysis revealed the presence of flavonoids, pholabatannins, resins, tannins, phenols, lipids and fats and carbohydrates.

Keywords: *Tridax procumbens*; Phytochemistry; Antibacterial; Zone of inhibition; Gentamicin

Introduction

India is a country rich in indigenous herbal resources which grow on their and varied topography and under changing agro climatic conditions permitting the growth of almost 20,000 plant species, of which about 2,500 are of medicinal value [1]. *Tridax procumbens* is a species of flowering plant belonging to family asteraceae. It is best known as widespread weed and pest plant. It is native to the tropical Americas but it has been introduced to tropical, subtropical and mild temperate regions worldwide. It is listed as a noxious weed in the United States and has a pest status. The extracts of *Tridax procumbens* have been reported to have various pharmacological effects, anti-inflammatory [2], hypoglycemic [3], antimicrobial [4], antioxidant [5], immunomodulatory [6], anti-diabetic [7], anti-hyperglycemic [8] and antifungal [9]. The plant bears daisy like yellow-centered white or yellow flowers with three toothed ray florets. The leaves are toothed and generally arrowhead-shaped. Its fruit is hard achene covered with stiff hairs and having a feathery, plume like white pappus at one end. Calyx is represented by scales or reduced to pappus. The plant is invasive in part because it produces so many of these achenes, upto 1500 per plant and each achene can catch the wind in its pappus and be carried to some distance. This weed can be found in fields, meadows, crop lands, disturbed areas, lawns and roadsides in areas with tropical or semitropical climate.

Tridax procumbens is employed as indigenous medicine for a variety of ailments. It has been extensively used in Indian traditional medicine for wound healing, as anticoagulant, antifungal and insect repellent, in diarrhea and dysentery. Leaf extracts are used to treat infectious skin diseases in folk medicines. It is also dispensed as 'Bhringraj' which is well known Ayurveda medicine for liver disorders. Antioxidant properties have been demonstrated, also hair growth promoting activity have been analyzed. Phytochemically limited studies reporting compounds from the roots of the plant are available. Hence the present work has been designed as to involve the preliminary investigation on the phytochemistry and antibacterial activity of *Tridax procumbens*.

Materials and Methods

Chemicals required

The solvents like ethanol (95%), petroleum ether, benzene, ethyl acetate were purchased from Merck (India). The chemicals like HCl,

Ferric Chloride, magnesium chloride and α -naphthol were taken from Sigma-Aldrich (India) and were used without further purification.

Plant source

The roots of *Tridax procumbens* were collected from the local area of Bundelkhand region (Bundelkhand University Campus) and were identified by Dr. Gaurav Nigam; Taxonomist, (ID NO. Bot. Ast. 09) Department of Botany; Bundelkhand University Jhansi (U.P).

Preparation of plant extracts (Soxhlet extraction): The shade-dried plant material was coarsely powdered and extracted with 95% ethanol using Soxhlet apparatus for 45 h. The solvent was changed after every 15 h. The extract was concentrated under reduced pressure and a dark brown solid (12 g) was obtained. This ethanolic extract was then fractionated with petroleum ether, benzene and ethyl acetate on a steam bath for 8 h. The petroleum ether extract (3.31 g), benzene extract (1.5 g) and ethyl acetate extract (1 g) were obtained.

Qualitative phytochemical analysis of plant extracts: The root extracts were analyzed for the Flavonoids, Pholabatannins, Resins, Lipids and Fats, Tannins, Phenols, Carbohydrates as follows [10-13].

Flavonoids (Shino or Pew test): 0.5 g of the extract was dissolved in 2 mL of ethanol and treated with few drops of conc. HCl and 0.5 g of magnesium. The pink colour was observed.

Pholabatannins: 0.5 g of the extract was dissolved in 2 mL of ethanol in a test tube, few drops of aqueous solution HCl (1 g/dL) was added and allowed. The red precipitate was observed.

Resins: 0.5 g of the extract was dissolved in 2 mL of ethanol in a test tube and treated with 2 mL of distilled water and observed for turbidity.

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Lipids and fats: A small quantity of extract was rubbed on a filter paper and observed for a permanent translucent strain.

Tannins: 0.5 g of the extract was dissolved in 2 mL of ethanol and added with 3 mL of hot distilled water and then filtered. Few drops of FeCl₃ (0.1 g/dL) were added and allowed to stand for some time and observed for brownish green or blue black colour.

Phenols: 0.5 g of the extract was dissolved in 2 ml of ethanol and added with water. To this 2 ml of FeCl₃ was added and observed for the formation of green or blue colour.

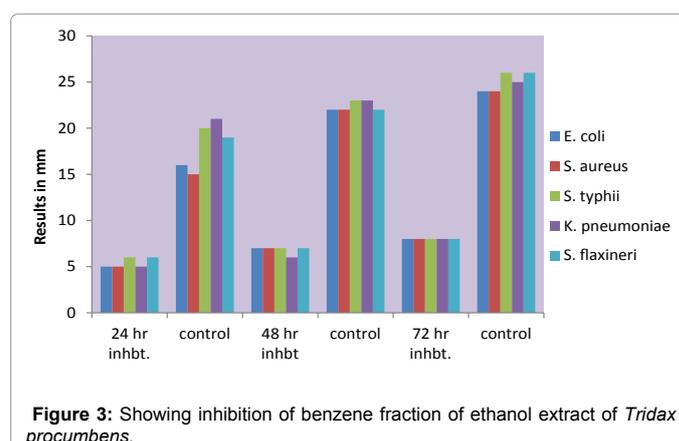
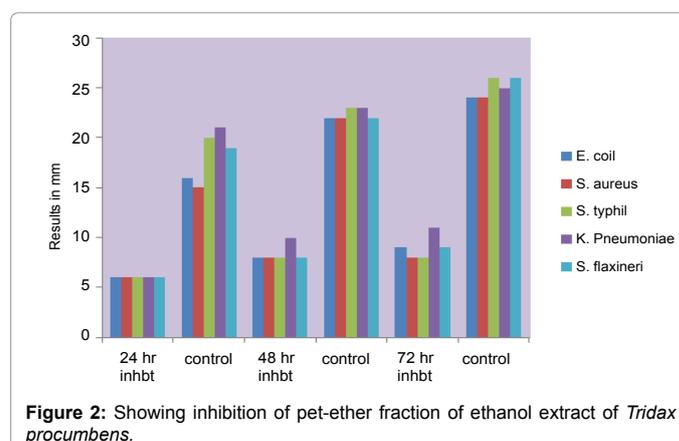
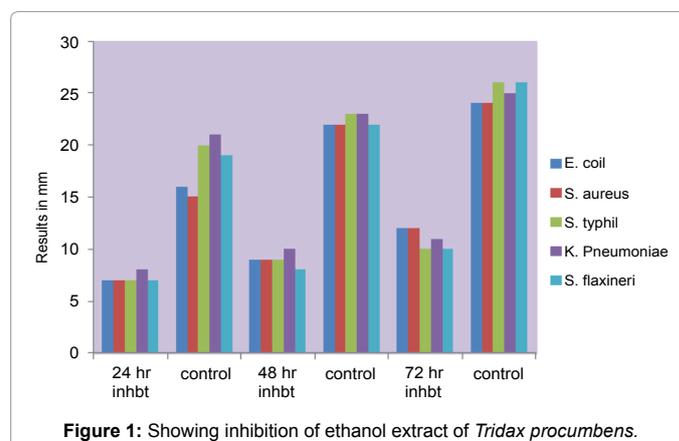
Carbohydrates (Molish test): The extract (0.5 g) was dissolved in 2 mL of ethanol and added with 1 mL of distilled water and filtered. To this solution, 2-3 drops of α-naphthol were added followed by 1 mL of H₂SO₄. The formation of violet coloured ring was observed at the interface of two layers.

Antibacterial activity

The antibacterial activity was carried out by known protocol [14]. During the activity, nutrient agar plates were prepared and stirred well till the extract was completely dissolved. Medium was then autoclaved (15 psi for 30 min) and was poured on petridish plates in a laminar flow and solidified. These plates were incubated at 36°C for 24 h to check any sort of contamination. The discs were prepared from Whatmann No.1 filter paper and were sterilized. After sterilization, the moisture discs were dried. Gentamicin was taken as positive control. One set of dilution (400 µg/ml) of plant extracts were prepared in a suitable solvent. Three sterile filter paper discs were soaked in three solvent extracts and placed on the surface of flooded plates, marked at the back of the petri dishes. The petri dishes were incubated at 36°C for 24 h.

Results and Discussion

The use of plants, both the wild and domesticated species has been recorded since ancient times in almost all major civilizations. Ayurveda has been known to be practiced in the Indian subcontinent since long. The specimen under consideration in this particular experiment has also come to notice due to its already predominant use as home strung recipe for infections. Hence the present study was carried out on *Tridax procumbens* Linn. Revealed the presence of bioactive constituents of medicinal value. The phytochemical compounds of the plant were qualitatively analysed and showed promising results for all the major phytochemicals. The qualitative analysis revealed the presence of flavonoids, pholabatannins, resins, tannins, phenols and carbohydrates (Table 1). The presence of these phytochemicals has also been investigated as per literature survey [9,10,15]. Antibacterial activity (assessed in terms of zone of inhibition) of the plant extracts, tested against selected microorganisms was recorded. In the present study three extracts from *Tridax procumbens* were tested for their bioactivity, among which two of them (ethanolic and pet-ether extracts) revealed significant results as compared to benzene extract. The ethanolic extract showed largest zone of inhibition ranging from 7-12 mm (Table 2; Figure 1), pet-ether extract showed 6-11 mm (Table 3; Figure 2) while as benzene extract showed 5-8 mm (Table 4; Figure 3), revealing its great medicinal potential for treatment of microbial induced ailments. The ethanolic extract of this plant showed highest zone of inhibition against *Escherichia coli* and *Staphylococcus aureus* ranging from 7-12 mm, the pet-ether extract of this plant showed highest zone of inhibition against *Klebsiella pneumoniae* while as the benzene fraction showed the same zone of inhibition against the tested bacteria. The results were promising in relevance that pet- ether and benzene extracts were fractions of ethanolic extract.



S No	Name of the test	Ethanolic extract
1	Flavonoids	+
2	Pholabatannins	+
3	Resins	+
4	Lipids and fats	+
5	Tannins	+
6	Phenols	+
7	Carbohydrates	+

(+) = Presence

Table 1: Showing the qualitative phytochemical analysis of root extract of *Tridax procumbens*.

Cultures	24 hours of inhibition		48 hours of inhibition		72 hours of inhibition	
	(400 µg/ml)	Gentamicin (Control)	(400 µg/ml)	Gentamicin (Control)	(400 µg/ml)	Gentamicin (Control)
<i>Escherichia coli</i>	7	16	9	22	12	24
<i>Staphylococcus aureus</i>	7	15	9	22	12	24
<i>Salmonella typhi</i>	7	20	9	23	10	26
<i>Klebsiella pneumoniae</i>	8	21	10	23	11	25
<i>Shigella flexneri</i>	7	19	8	22	10	26

Table 2: Showing zone of inhibition (mm) of ethanolic extract of *Tridax procumbens*.

Cultures	24 hours of inhibition		48 hours of inhibition		72 hours of inhibition	
	(400 µg/ml)	Gentamicin (Control)	(400 µg/ml)	Gentamicin (Control)	(400 µg/ml)	Gentamicin (Control)
<i>Escherichia coli</i>	6	16	8	22	9	24
<i>Staphylococcus aureus</i>	6	15	8	22	8	24
<i>Salmonella typhi</i>	6	20	8	23	8	26
<i>Klebsiella pneumoniae</i>	6	21	10	23	11	25
<i>Shigella flexneri</i>	6	19	8	22	9	26

Table 3: Zone of inhibition (mm) of pet-ether fraction of ethanolic extract of *Tridax procumbens*.

Cultures	24 hours of inhibition		48 hours of inhibition		72 hours of inhibition	
	(400 µg/ml)	Gentamicin (Control)	(400 µg/ml)	Gentamicin (Control)	(400 µg/ml)	Gentamicin (Control)
<i>Escherichia coli</i>	5	16	7	22	8	24
<i>Staphylococcus aureus</i>	5	15	7	22	8	24
<i>Salmonella typhi</i>	6	20	7	23	8	26
<i>Klebsiella pneumoniae</i>	5	21	6	23	8	25
<i>Shigella flexneri</i>	6	19	7	22	8	26

Table 4: Zone of inhibition (mm) of benzene fraction of ethanolic extract of *Tridax procumbens*.

The possible mechanisms for its antibacterial activity may be due to enzyme inhibition, substrate deprivation, complex with cell wall, membrane disruption, metal-ion complexation, intercalates into cell wall and DNA of parasites. Resistance of microorganisms to many antibiotics has resulted in morbidity and mortality from treatment failure and increased health care costs. Though many antibiotics are available but multidrug resistance has encouraged search for new, safe and effective bio active agents of herbal origin. Most of the secondary metabolites were identified in the polar extracts. The concentration of polar metabolites is higher than the non-polar metabolites. Flavonoids are known to be synthesized by plants in response to microbial infection; hence it should not be surprising that they have been found to be effective *in-vitro* antibacterial substances against a wide array of infectious agents [16]. Tannins (commonly referred as tannic acid) are also known potential antimicrobial agents. They are water soluble polyphenols present in many plants. Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein, the growth of many fungi, yeasts, bacteria and viruses were inhibited by this group of compounds. They show physiological effects like antisecretolytic, antiphlogistic, anti-microbial and antiparasitic effects [15]. Phytotherapeutically, tannin containing plants are used to treat nonspecific diarrhea, inflammation of mouth, throat and slightly injured skins [17]. Since from ancient times, the leaf juice of *Tridax procumbens* was shown to depress wound contraction in experimented animals. In addition to the possible antibacterial behavior, it was suggested that involvement of complex interaction between epidermal and dermal cells, the extra cellular matrix, controlled angiogenesis

and plasma derived proteins coordinated by an array of cytokines and growth factors [18]. *Tridax procumbens* antagonized antiepitelization and tensile strength depressing effect of dexamethasone (a known healing suppressant agent) without affecting anticontraction and antigranulation action of dexamethasone. Aqueous extract was also effective in increasing lysyl oxidase but to a lesser degree than whole plant extract. Further it has been shown that extract of leaves of this plant also promotes wound healing in normal and immune compromised (steroid treated) rats. The plant increase not only lysyl oxidase but also, protein and nucleic acid content in the granulation tissue, probably as a result of increase in glycosaminoglycan content [19].

Conclusions

Tridax procumbens Linn. is widely distributed weed. Each and every part of it is useful having pharmacological activity. The plant product over synthetic compound is the need in treatment of diseases, as it does not have any deleterious effect in higher animals including man. The work done showed that the different extracts of plant have potential antibacterial activity. The qualitative analysis revealed the presence of the biomolecules such as flavonoids, Pholabatannins, resins, lipids and fats, phenolic compounds, saponins, steroids, tannins and terpenoids. The studies on plant *Tridax procumbens* also desire development of novel therapeutic agents from the various types of compounds by means of modification and derivatization to design more potent and selective therapeutic agents. Therefore, there is huge room for research in direction of more pharmacological activities of plant and to elucidate the mechanism of action of same in future.

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