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ABSTRACT

Plants have been an important source of medicine for thousands of years. Even today, the World Health Organization estimates that up to 80 percent of people still rely mainly on traditional remedies such as herbs for their medicines. Its civilization is very ancient and the country as a whole has long been known for its rich resources of medical plants. Studying the plants used in folklore medicine promises to yield commendable results as investigating their antibacterial activity has led to a better understanding of the use of traditional medicines as potential drugs in addition to contemporary drugs. Many efforts have been done to discover new antimicrobial compounds from various kinds of sources such as soil, microorganisms, animals and plants. One of such resources is folk medicine and systematic screening of them may result in the discovery of novel effective compounds. *Tridax procumbens* Linn. (Family- Asteraceae; Common name-Dhaman grass) is a common herb in India. It is employed as an indigenous medicine for a variety of ailments including jaundice. The plant also has hepatoprotective activity and it is used in Ayurveda in various liver disorders. Phytochemical screening of the plant revealed the presence of tannins, flavonoids, saponins and alkaloids. Antimicrobial activity of *Tridax procumbens* young leaf extracts were investigated by agar disc well-diffusion method against selected human pathogens. The leaf extracts showed inhibitory activity against the tested organisms. The results lend credence to the folkloric use of this plant in treating microbial infection and shows that *Tridax procumbens* young leaves could be exploited for new potent antimicrobial agents.

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INTRODUCTION
Medicinal plants have played a significant role in ancient traditional systems of medication in many countries. Indigenous herbs are used as remedies against various diseases in the traditional system of medicine or in ethnomedical practices [1]. Plants continue to serve as possible sources for new drugs and chemicals derived from various parts of plants [2]. In the recent past there has been a tremendous increase in the use of plant based health products in developing as well as developed countries resulting in an exponential growth of herbal products globally. An upward trend has been observed in the research on herbals. Herbal medicines have a strong traditional or conceptual base and the potential to be useful as drugs in terms of safety and effectiveness leads for treating different diseases. World Health Organization has made an attempt to identify all medicinal plants used globally and listed more than 20,000 species [3]. According to the WHO more than 80% of the world’s population relies on traditional herbal medicine for their primary health care [4]. Recently much attention has directed towards extracts and biologically active compounds isolated from popular plant species. In the present era of drug development and discovery of newer drug molecules, many plant products are evaluated on the basis of their traditional uses.

*Tridax procumbens* Linn is a common herb found in India. The whole plant was reported to treat various ailments, such as bronchial catarrh, dysentery, diarrhea, preventing hair loss, and to check hemorrhage from cuts [5, 6]. Pharmacological studies have shown that *Tridax procumbens* possess properties like anti-inflammatory, hepatoprotective, wound healing, immunomodulatory, antimicrobial, antiseptic, hypotensive and bradycardiac effects [7-9]. The curative properties of medicinal plants are mainly due to the presence of various chemical substances of different compositions which occur as secondary metabolites [10].

The medicinal value of the plants lies in some active chemical substances called phytochemicals that produce a definite physiological action on the human body. Phytochemicals are divided into two groups, which are primary and secondary constituents according to their functions in plant metabolism. Primary constituents comprise common sugars, aminoacids, proteins and chlorophyll while secondary constituents consists of alkaloids, terpenoids, flavonoids, tannins, phenolic compounds [11]. It is necessary to focus and develop these compounds to be more effective drugs. Antimicrobials of plant origin have enormous therapeutic potential and have been used since time immemorial. They have been proved effective in the treatment of infectious diseases simultaneously mitigating many of the side effects which are often associated with synthetic antibiotics [12]. Positive response of plant based drugs (less/ no side effects) might lies in the structure of the natural products which reacts with toxins and/or pathogens in such a way that less harm is done to other important molecules or physiology of host. In view of its medicinal value, the present study is aimed to screen the pharmaceutically important bioactive substances from *Tridax procumbens* young leaves that greatly contribute the nutraceutical and drug research.

MATERIALS AND METHODS
Collection and identification of plant material
The specimen was collected from Coimbatore and authenticated by Botanical Survey of India, Coimbatore, India. A voucher specimen has been deposited in the laboratory for future reference (BSI/SRC/5/23/2012-13/Tech.371). The young leaves of *Tridax procumbens* were washed thoroughly 2-3 times with running tap water and once with sterile distilled water, air dried at room temperature on a sterile blotter. After complete drying, young leaves were powdered well using a mixer. Then the powdered material was weighed and kept in air tight container and stored in a refrigerator for future use. About 10g of this powdered sample was refluxed with petroleum ether, methanol and water in the ratio of 1:10 (w/v). The crude extracts were collected in amber coloured sample bottles and stored. All chemicals and reagents used including the solvents were of analytical grade.

Pharmacognostic Profile
Extractive values
Extract of the powdered leaves were prepared with different solvents for the study of extractive values.

Fluorescence Analysis
A small quantity of dried and finely powdered material was placed in a clean grease-free microscopic slide, treated with 1 - 2 drops of the freshly prepared reagent solution, mixed gently by tilting the slide and waited for 2 - 4 minutes. The slide was then viewed day light and ultraviolet radiations (365 nm). The colours observed on application of different reagents in different radiations were recorded.

Phytochemical Analysis
Phytochemical analysis was carried out in the petroleum ether, ethanol and aqueous extracts of the young leaves of *Tridax procumbens* using standard procedures [13-15].

Test for alkaloids
Dragendorff’s test
To 5 mL of the extract few drops of Dragendorff’s reagent was added for the formation of orange coloured precipitate.

Mayer’s test
To 5 mL of the extract few drops of Mayer’s reagent was added for the formation of cream coloured precipitate.
Wagner’s test
To 5 mL of the extract few drops of Wagner’s reagent was added for the formation of reddish brown coloured precipitate.

Hager’s test
To 3 mL of the extract few drops of Hager’s reagent was added for the formation of prominent yellow precipitate.

Test for flavonoids
To 3 mL of the extract few magnesium ribbons are dipped and conc. HCl was added over them and observed for the formation of magenta (brick red) colour indicating the presence of flavonoids.

Test for proteins
Biuret test
To 3 mL of the extract few drops of 10% sodium chloride and 1% copper sulphate was added for the formation of violet or purple color. On addition of alkali, it becomes dark violet.

Millon’s test
To 3 mL of the extract few drops of Millon’s reagent was added for the formation of red colour.

Test for carbohydrates
Molisch’s test
To a small amount of the extract few drops of Molisch’s reagent was added followed by the addition of conc. H₂SO₄ along the sides of the test tube. The mixture was then allowed to stand for 2 min and then diluted with 5 mL of distilled water. Formation of red or dull violet colour at the inter phase of two layers indicates the presence of carbohydrates.

Fehling’s test
The extract was treated with 5 ml of Fehling’s solution (A and B) and kept in boiling water bath. The formation of yellow or red color precipitate indicates the presence of reducing sugar.

Test for tannins
Liebermann-Burchard test
To a small amount of the extract few drops of chloroform, acetic anhydride and H₂SO₄ was added along the sides of the test tube to observe the formation of dark red or pink colour.

Test for glycosides
Baljet’s Test
To 5 mL of the extract few drops of sodium picrate was added to observe yellow to orange colour.

Keller-Killiani test
To 5 mL of the extract few drops of ferric chloride solution was added and mixed, then sulphuric acid containing ferric chloride solution was added, it forms two layer showed reddish brown while upper layer turns bluish green indicates the presence of glycosides.

Test for phenols
Ferric chloride test
A fraction of the extract was treated with 5% ferric chloride solution and observed for the formation of deep blue or black colour.

Test for saponins
Foam test
To a small amount of the extract few drops of distilled water was added and shaken vigorously until persistent foam was observed.

Test for terpenoids
Chloroform test
To 5 mL of the extract few drops of chloroform and conc. H₂SO₄ was added carefully along the sides of the test tube to form a layer and observed for the presence of reddish brown colour.
Antimicrobial activity of *Tridax procumbens* young leaves

Agar well diffusion method
The modified agar well diffusion method was employed [16, 17]. Muller-Hinton agar plates were inoculated by streaking the swab over the entire sterile agar surface. The agar well diffusion method was carried out to evaluate antibacterial activity. Test organism was spread on Muller-Hinton agar plates. The standard inoculums were evenly spread on the surface of the medium then wells of 6mm diameter were punched into the agar medium and filled with *Tridax procumbens* young leaves extracts of various concentrations. The plates were incubated for 24 hours at 37°C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism.

RESULTS AND DISCUSSION

Extractive values
Extractive values of the successive extracts of *Tridax procumbens* young leaves are given in Table 1.

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Extract values (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether</td>
<td>5.79</td>
</tr>
<tr>
<td>Ethanol</td>
<td>9.84</td>
</tr>
<tr>
<td>Water</td>
<td>7.28</td>
</tr>
</tbody>
</table>

Fluorescence analysis
The powdered sample of *Tridax procumbens* young leaves was subjected to fluorescence analysis, results are tabulated in Table 2.

<table>
<thead>
<tr>
<th>Plant sample</th>
<th>Day light</th>
<th>UV light (365nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powder</td>
<td>Green</td>
<td>Dark green</td>
</tr>
<tr>
<td>Powder+ NaOH</td>
<td>Dark green</td>
<td>Light green</td>
</tr>
<tr>
<td>Powder+Acetone</td>
<td>Pale green</td>
<td>Yellowish green</td>
</tr>
<tr>
<td>Powder+HCl</td>
<td>Light green</td>
<td>Green</td>
</tr>
<tr>
<td>Powder+HNO$_3$</td>
<td>Light green</td>
<td>Yellowish green</td>
</tr>
<tr>
<td>Powder+Acetic acid</td>
<td>Brown</td>
<td>Dark brown</td>
</tr>
<tr>
<td>Powder+CHCl$_3$</td>
<td>Yellowish green</td>
<td>Pale green</td>
</tr>
</tbody>
</table>

Phytochemical Analysis
Powdered *Tridax procumbens* young leaves were subjected to various qualitative tests for the identification of phytochemical constituents includes tests for alkaloids (Dragendroff’s test, Mayer’s test, Hager’s test, Wagner’s test), saponins, glycosides (Baljet’s test, Kellar-Killiani test), carbohydrates (Molisch’s test, Fehling’s test), proteins (Biuret test, Xanthoprotein test, Millon’s test), tests for tannins, flavonoids, steroids (Liebermann-burchard test), phenols, terpenoids were performed using specific reagents and results are tabulated in Table 3.

Phytochemical screening results of the powdered sample of *Tridax procumbens* young leaves extracted in water and ethanol showed the presence of all the constituents whereas the petroleum ether extract showed the presence of very few bioactive compounds. Chemical investigation on the different parts of the plant has resulted in the isolation of a large number of novel and interesting metabolites [18].

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Petroleum ether</th>
<th>Ethanol</th>
<th>Aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sterols</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

‘+’ present, ‘-’ absent
The antibacterial activity of *Tridax procumbens* young leaves extracts against the different pathogenic organisms are illustrated in Table 4. Among the extracts, the petroleum ether extract showed good activity against the gram positive and gram negative organisms. There are reports that plants rich in tannins have antibacterial potential due to their basic character that allows them to react with proteins to form stable water soluble compounds thereby killing the bacteria by directly damaging its cell membrane [19]. Possibly this may be the mechanism of action of chloroform extract which also showed the presence of tannins by phytochemical analysis. The petroleum ether and ethanolic extract also showed activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, this may be due to the presence of alkaloids. Alkaloids are commonly found to have antimicrobial properties [20]. The zone of inhibition varied suggesting the varying degree of efficacy and different phytoconstituents of *Tridax procumbens* on target organism.

<table>
<thead>
<tr>
<th>Pathogenic organism</th>
<th>Various extracts of <em>Tridax procumbens</em> young leaves and the Zone of Inhibition in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Petroleum ether</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>28 mm</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>12.2 mm</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>11.6 mm</td>
</tr>
<tr>
<td><em>K. pneumonia</em></td>
<td>13.1 mm</td>
</tr>
</tbody>
</table>

**CONCLUSION**

Plant derived substances have recently become great interest due to their multiple application. Medicinal plants are the richest bio-resource of drugs of traditional system of medicine, modern medicine pharmaceutical intermediates and chemical entities for synthetic drugs. Researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs. Results of phytochemical screening revealed the presence of alkaloids, flavonoids, proteins, carbohydrates, tannins, phenols, glycosides, saponins and terpenoids. The pharmacognostic profile, phytochemical screening and antimicrobial activity of the present study showed favorable effects for the standardization parameters of plant parts. In the present study, the preliminary phytochemical screening of the various extracts revealed the presence of major bioactive compounds which may retain a wide range of actions. The antibacterial activity of the young leaves may be due to the presence of various active principles in their leaves. Further studies are needed to isolate and characterize the bioactive principles to develop new antibacterial drugs. Thus, *Tridax procumbens* young leaves are quite promising as a multipurpose medicinal agent so further clinical trials should be performed to prove its efficacy.

**REFERENCES**


