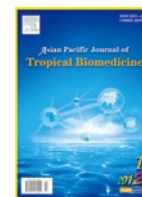




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Phytochemical and antibacterial studies of leaves of *Tridax procumbens* L.

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ABSTRACT

Objective: To investigate the phytochemical screening and antibacterial activity of pet. ether, chloroform and ethanolic extracts of *Tridax procumbens* (*T. procumbens*). **Methods:** The antibacterial activity of leaves of *T. procumbens* was evaluated by agar well diffusion method against three selected gram positive and gram negative bacterial species. **Results:** The presence of carbohydrates, proteins, tannins, steroids, alkaloids and purines in the different leaf extracts were established by phytochemical analysis. The chloroform extract was more effective against bacteria and the activity was comparable with that of standard drug ampicillin. **Conclusions:** The results in the present study suggest that *T. procumbens* leaves can be used for treating diseases caused by the tested organisms.

1. Introduction

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural resources. Traditional medicine is an important source of potentially useful new compounds for the development of chemotherapeutic agents [1]. Emergence of pathogenic microorganisms that are resistant/ multi resistant to major class of antibiotics has increased in recent years due to indiscriminate use of synthetic antimicrobial drugs [2]. In addition, high cost and adverse side effects are commonly associated with popular synthetic antibiotics (such as hypersensitivity, allergic reactions, immunosuppression, etc.) and are major burning global issues in treating infectious diseases [3]. Although pharmacological industries had produced considerable number of commercial antibiotics time to time but resistance in pathogens towards these drugs too has increased at high rate and multi drug resistant microorganisms have exacerbated the situation [4]. In the present scenario, there is an urgent and continuous need of

exploration and development of cheaper, effective new plant based drugs with better bioactive potential and least side effects. Hence, recent attention has been paid to biologically active extracts and compounds from plant species used in herbal medicines [5]. 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 [6]. 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.

Tridax procumbens Linn. (Family–Asteraceae; common name–Dhaman grass) (*T. procumbens*) is common herb found in India. It is denoted by different names; in English as Mexican Daisy, in ayurvedic as Jayanti, in siddha/tamil as Vettukkaaya–thalai and in folk as Akala kohadi. The whole plant was reported to treat various ailments, such as bronchial catarrh, dysentery, diarrhea, preventing hair loss, and to check hemorrhage from cuts [7,8]. Pharmacological studies have shown that *T. procumbens* possess properties like–anti inflammatory, hepatoprotective, wound healing, immunomodulatory, antimicrobial, antiseptic, hypotensive and bradycardiac effects [9–11]. Earlier workers [12] have reported that the presence of dexamethasone, luteoline,

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glucotureolin, β -sitosterol, flavone, glycoside and quercetin in this plant. This study aims to investigate the phytochemical and antibacterial effect of *T. procumbens*.

2. Materials and methods

2.1. Plant materials

The plants were collected from Gobichettipalayam, Erode district of Tamil Nadu by uprooting the whole plant, and then the leaves were removed carefully. The leaves were shade dried for one week. The authentication of the plant was done by the botanist of the college. A voucher specimen was deposited in herbarium of the college (ECP – 35).

2.2. Extraction of plant material

The dried leaves were reduced to coarse powder. 100 g of powder was successively extracted with three different solvents (pet. ether, chloroform and ethanol). The extraction process was carried out using soxhlet apparatus for 36 hours. The yield was found to be 0.483%, 0.734%, 1.234% w/w, respectively. The dried extracts were dissolved in dimethyl sulphoxide (DMSO) and subjected to antibacterial activity.

2.3. Phytochemical study for pet. ether, chloroform and ethanol extracts

The crude products obtained in soxhlet extraction technique were subjected to qualitative chemical evaluation of Carbohydrates & Glycoside, fixed oil and fat, proteins and free amino acids, saponins, phytosterols, tannins, flavanoids etc. [13,14].

2.4. Antibacterial assay

2.4.1. Testing organisms

The bacterial used for the test included gram-positive organisms were: *Bacillus subtilis* (*B. subtilis*), *Bacillus faecalis* (*B. faecalis*) and gram-negative organisms: *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*). They were procured from Microbial Type Culture Collection, IMTECH, Chandigarh, India.

2.4.2. Preparation of nutrient agar medium

A total of 1 000 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 between 7.8–8. 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 120 °C for 15 minutes.

2.4.3. Procedure

The well-diffusion assay was used to determine the antibacterial assay [15, 16]. 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 μ g/mL) were prepared from each extract. A solution of standard drug Ampicillin was prepared at the concentration 50 μ g/mL for *B. subtilis* and 200 μ g/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 μ g/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 33 °C for 18 hrs and the zone of inhibition was measured using a zone reader and the results are recorded.

3. Results

The phytochemical screening of *T. procumbens* pet. ether, chloroform and ethanol extract showed the presence of alkaloids, tannins, steroids, purines, carbohydrates, proteins (Table 1). The antibacterial activity of pet. ether, chloroform and ethanolic extracts were presented in table 2, 3 and 4, respectively. Comparatively chloroform extract was showing better (mild to moderate) activity against all the selected organisms. The activity of chloroform extract against *B. faecalis* and *E. coli* at the concentration 800 μ g/mL was comparable with that of standard drug ampicillin. The ethanolic extract showed moderate activity against *B. faecalis* and the extract was devoid of activity against all other selected organisms. The pet. ether extract also showed activity against *B. faecalis* similar to the activity of the standard.

Table 1
Pytochemical composition of various extracts of *T. procumbens*.

Phytoconstituents	Pet. ether	Chloroform	Ethanol
Carbohydrates	+	+	+
Glycosides	–	–	–
Proteins	+	+	+
Flavanoids	–	–	–
Tannins	–	+	–
Steroids	+	+	+
Fixed oil	–	–	–
Saponins	–	–	–
Alkaloids	+	+	+
Purines	+	+	+

4. Discussion

Table 2
Antibacterial activities of *T. procumbens* extracts .

Concentration (μ g/mL)	Zone of Inhibition (mm)											
	<i>B. faecalis</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>B. faecalis</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>B. faecalis</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
400	8	---	---	---	13	10	13	8	---	10	---	---
600	13	---	---	---	14	13	15	10	---	12	---	---
800	13	---	---	---	16	13	16	12	---	13	---	---
Std. ampicillin*200	15	22	10	16	18	24	18	13	10	24	8	16

Concentration of std. ampicillin for *B. subtilis* is 50 μ g/mL.

The therapeutic value of medicinal plants lies in the various chemical constituent's presents in it. The bioactivity of plant extracts is attributed to phytochemical constituents. In order to extract the important phytochemical categories such as alkaloids, glycosides, proteins, terpenoids, volatile oils, steroids, flavonoids, etc., effectively, we were employed this three different solvents with varying polarity. In the present study, the antibacterial activity of the three extracts were evaluated against both gram positive and gram negative organisms. In the antibacterial study, ampicillin at a concentration 50 μ g/mL was employed as reference standard for *B. subtilis* for the remaining three organisms the ampicillin at the concentration 200 μ g/mL was used as standard. As we found that *B. subtilis* is more susceptible towards ampicillin the concentration was reduced to 50 μ g/mL for evaluation. Among the three extracts, the chloroform 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[17]. Possibly this may be the mechanism of action of chloroform extract which also showed the presence of tannins by phytochemical analysis. The pet. ether and ethanolic extract also showed activity against *B. faecalis* this may be due to the presence of alkaloids. Alkaloids are commonly found to have antimicrobial properties[18]. It is concluded that the chloroform extract possess antibacterial activity against tested organisms. The zone of inhibition varied suggesting the varying degree of efficacy and different phyto-constituents of *T. procumbens* on target organism. The antibacterial activity of the 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.

Conflict of interest

We declare that we have no conflict of interest.

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