



ORIGINAL ARTICLE

Screening of wild plant species for antibacterial activity and phytochemical analysis of *Tragia involucrata* L.

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Abstract Eight wild plant species namely *Tragia involucrata* L., *Cleistanthus collinus* (Roxb.) Benth. Ex Hook.f., *Sphaeranthus indicus* L., *Vicoa indica* (L.) Dc., *Allmania nodiflora* (L.) R.Br. ex Wight., *Habenaria elliptica* Wight., *Eriocaulon thwaitesii* Koern. and *Evolvulus alsinoides* L. were used for phytochemical extraction with four different solvents. Antibacterial activity of these plants was studied against *Escherichia coli* NCIM 2065 using Kirby Bauer agar disc diffusion assay. Effective antibacterial activity was shown by *T. involucrata* acetone extract (27.3 mm), compared to standard medicinal drug amoxicillin (28.3 mm). Minimum inhibitory concentration (MIC) of *T. involucrata* extract was 15 mg/mL and hence, it could be pursued further for obtaining phytomedicine. Biochemical constituents of *T. involucrata* fresh leaf were: sugars (55 mg/g), starch (0.7182 mg/g), proteins (0.0166 mg/g) and lipids (170 mg/g). Alkaloids, tannins, phenolic compounds, flavonoids and steroids were also observed qualitatively.

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1. Introduction

Traditionally, wild plant parts are used as a source of herbal preparation for treatment of various ailments [1]. They are novel source of medicines as they have a reservoir of chemical agents with therapeutic properties [2] and plants are the cheapest and safer alternative sources of antimicrobials [3]. Plant extracts have both phytochemical and antimicrobial properties and can be of great significance in therapeutic treatments [4]. Therefore, screening and testing the efficacy of plants are undertaken to explore their antibacterial activity [5].

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In more than 80% of developed countries, plants have been used as traditional medicine as they are the good source of compound derivation. Therefore, plants are investigated for better understanding of their properties, safety and efficacy. Many plants have been used for their antimicrobial traits, which are chiefly due to the synthesis of secondary metabolites [6] and their inhibitory effect against the growth of human pathogens. Keeping this in view, efforts are underway to search for economic and safe phytochemicals for disease control. Despite the existence of potent antibiotic and antifungal agents, resistant microbial strains are continuously appearing, suggesting the need for a permanent search and the development of new drugs [7].

In this respect, *Escherichia coli* is a species of great genetic diversity and a resident of the large intestine in almost every individual [8]. It can cause serious health problems in human beings [9] such as abdominal pain, diarrhea and hemolytic uremic syndrome and it can produce shiga-like toxins; 2–7% of *E. coli* infections result in acute renal failure [10]. It can also have severe public health implication in many large disease outbreaks [11]. Especially, Enterohemorrhagic *E. coli* is one of the six groups of *E. coli* recognized as etiological agents of diarrhea. It produces cytotoxins referred as verocytotoxin (Shiga-like toxin) which is responsible for hemorrhagic colitis [12]. This organism was first identified as a cause of illness in 1982 and the infections have been reported with increasing frequency since then. It is now of public health importance as it is readily isolated from human and animal wastes that pollute the environment [13]. Therefore, present study is focused only on *E. coli* as it is a pathogen.

Moreover, many researchers have studied the antimicrobial activity of plant extracts on *E. coli* NCIM 2065 [14–19] and the National Collection of Industrial Microorganisms (NCIM) is the biggest microbial culture collection facility in India, which supplies *E. coli* NCIM 2065 for antimicrobial assay. Therefore, *E. coli* NCIM 2065 was chosen.

In the present study, we investigated the potential of eight wild Indian plant species (*Tragia involucrata* L., *Cleistanthus collinus* (Roxb.) Benth. Ex Hook.f., *Sphaeranthus indicus* L., *Vicoa indica* (L.) Dc., *Allmania nodiflora* (L.) R.Br. ex Wight., *Habenaria elliptica* Wight., *Eriocaulon thwaitesii* Koern., *Evolvulus alsinoides* L.) (Table 1) for antibacterial property against the gram-negative *E. coli* NCIM 2065.

2. Materials and methods

2.1. Collection of plant materials

Plants of the eight species were collected during December, 2010 from various regions of Pudukkottai district, Tamilnadu, India.

Plants were identified using the facility of Rapinat Herbarium, St. Joseph's College, Tiruchirappalli and the identified voucher specimens were deposited in the Research and PG department of Botany, H.H. The Rajah's college, Pudukkottai. Plants were thoroughly washed with water; leaves, stems, roots and flowers of each species were separated and kept between the filter papers in a dark room at room temperature to get rid of moisture until further analysis.

2.2. Preparation of extract

Dried plant materials were powdered with Waring blender, at room temperature and 2 g of each powder sample was soaked in 20 mL of different solvents (petroleum ether, chloroform, acetone and autoclavable distilled water) overnight. Later, the samples were filtered under vacuum using Whatmann No.1 filter paper and stored in airtight screw-capped bottles at 5 °C for further analysis.

2.3. Preparation of inoculum

The test organism *E. coli* NCIM 2065 was obtained from the National Collection of Industrial Microorganisms of National Chemical Laboratory, Pune. Stock culture was maintained at 5 °C on slants of nutrient agar. Active stock culture was inoculated in fresh tubes of Muller–Hinton broth medium (MHB) and the bacterium was incubated for 24 h at 37 °C. Ten fresh subculture Muller–Hinton agar slants were prepared and stored in refrigerator at 5 °C for future requirements.

2.4. Antibacterial susceptibility test

Spectrum of antibacterial activity was studied by using the technique described by Bauer et al. [20]. Amoxicillin sensitivity disc (30 mg; Hi-Media India Pvt. Ltd., Mumbai) was used as a positive control and respective solvents were taken as negative control. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. These tests were performed in triplicate.

2.5. Minimum inhibitory concentration

Minimum inhibition concentration (MIC) is defined as the lowest concentration of drug that prevents the growth of a particular pathogen [21]. Different dilution concentrations of the *T. involucrata* leaf crude extracts (5–50 mg/mL) were prepared and inoculated with the suspension of the overnight grown *E. coli* NCIM 2065 inoculum. After 24 h at 37 °C, MIC of each sample

Table 1 List of wild plant species used in the study.

S. no	Species	Family	Vernacular name	Plant materials screened
1	<i>Tragia involucrata</i> L.	<i>Euphorbiaceae</i>	Senthatti	Leaf, stem, root and flowers
2	<i>Cleistanthus collinus</i> (Roxb.) Benth. Ex Hook.f.	<i>Euphorbiaceae</i>	Oduvan thali	Leaf, stem, root and flowers
3	<i>Sphaeranthus indicus</i> L.	<i>Astreeaceae</i>	Kottaikaranthai	Leaf, stem, root and flowers
4	<i>Vicoa indica</i> (L.) Dc	<i>Astreeaceae</i>	Mukkuthi poo	Leaf, stem, root and flowers
5	<i>Allmania nodiflora</i> (L.) R.Br. ex wight.	<i>Amaranthaceae</i>	Vannikeerai	Leaf, stem, root and flowers
6	<i>Habenaria elliptica</i> Wight.	<i>Orchidaceae</i>	–	Leaf, stem, root and flowers
7	<i>Eriocaulon thwaitesii</i> Koern.	<i>Eriocaulaceae</i>	Ericalan	Leaf, stem, root and flowers
8	<i>Evolvulus alsinoides</i> L.	<i>Convolvulaceae</i>	Vishnukiranthi	Leaf, stem, root and flowers

was determined by measuring the optical density at 620 nm in UV-vis spectrophotometer-2402 (Shimadzu, Japan) [22].

2.6. Biochemical screening

Biochemical tests in the fresh leaf of *T. involucrata* were done using standard methods [23–26]. Secondary metabolites were qualitatively tested by the standard methods of Harborne [27] and Odebiyi and Sofowora [28].

3. Results

Plant parts extracts (128 extracts) of the eight species were investigated for antibacterial activity against *E. coli* NCIM 2065. Out of these, 35 extracts inhibited the growth of *E. coli* NCIM 2065. Among them, *T. involucrata* plant extract showed higher inhibition in all the solvent extracts except water and the maximum inhibition was observed in acetone leaf extract (27.3 ± 0.5 mm) and the minimum, in the petroleum ether leaf extract (6.3 ± 0.5 mm). *C. collinus* also showed inhibition in all the solvent extracts except water. Maximum inhibition was in the stem extract of petroleum ether (14.9 ± 0.3 mm) and the minimum was in the leaf extract of acetone (9 ± 0 mm). *V. indica* showed inhibition only in the solvent extracts of chloroform and acetone. Maximum inhibition was observed in the leaf extract of chloroform (11.6 ± 0.5 mm) and the minimum, in the flower extract of chloroform (9.6 ± 0.5 mm). *E. alsinoides* showed moderate activity in all the solvent extracts except water and the maximum inhibition was in the stem extract of chloroform (12 ± 0 mm) and the minimum was recorded in the leaf extract of the petroleum

ether (5.6 ± 0.5 mm), as per Table 2. *S. indicus* and *H. elliptica* inhibited only in the chloroform solvent. *A. nodiflora* (petroleum ether and chloroform) and *E. thwaitesii* (chloroform and acetone) showed only minimum inhibitory activity. In all the eight plant species, chloroform had a better solubility followed by acetone and petroleum ether. The amoxicillin antibiotic drug showed 28.3 ± 0.5 mm diameter zone of inhibition (Table 2).

T. involucrata leaf extract MIC was tested in 10 different concentrations from 5 mg/mL to 50 mg/mL and the MIC was 15 mg/mL (Fig. 1). Biochemical tests showed 55 mg/g of total sugar, 0.7182 mg/g of total starch, 0.0166 mg/g of total proteins and 170 mg/g of total lipids. Further, alkaloids, tannins, phenolic compounds, flavonoids, steroids, saponins and terpenoids were also present in *T. involucrata* leaf.

4. Discussion

Emergence of multi-drug resistance in human and animal pathogenic bacteria as well as undesirable side effects of certain antibiotics have triggered immense interest in the search of new antimicrobial drugs of plant origin [29]. Bioactive compounds from plants serve as a novel source for infectious disease management as an alternate to synthetic drugs and several phytochemicals have been derived from the plant materials like bark, stem, leaves, roots, fruits, seeds, fruit rind, flowers and whole plants [30]. In the present study, chloroform solvent extract had better solubility but the acetone extract showed maximum inhibition. Though chloroform can dissolve more compounds than acetone, the latter dissolves many hydrophilic and lipophilic compounds from plants and it is a very useful extractant for

Table 2 *In vitro* antibacterial activity of different plant solvent extracts against *E. coli* NCIM 2065.

Solvents	Diameter of inhibition zone (mm) ^a after 24 h								
	<i>T. involucrata</i>	<i>C. collinus</i>	<i>S. indicus</i>	<i>V. indica</i>	<i>A. nodiflora</i>	<i>H. elliptica</i>	<i>E. thwaitesii</i>	<i>E. alsinoides</i>	Standard (+ve) disc
Petroleum ether									
Leaf	6.3 ± 0.5	9.3 ± 0.5	–	–	–	–	–	5.6 ± 0.5	
Stem	–	14.9 ± 0.3	–	–	–	–	–	6.3 ± 0.6	
Root	–	–	–	–	8 ± 0	–	–	8.3 ± 0.5	
Flower	8.7 ± 0.15	–	–	–	–	–	–	–	
Chloroform									
Leaf	10.6 ± 0.5	–	–	11.6 ± 0.5	9 ± 0	10.3 ± 0.5	9.3 ± 0.5	11 ± 0	
Stem	11.5 ± 0.3	10.2 ± 0.1	–	10.1 ± 0.6	–	–	9.3 ± 0.5	12 ± 0	
Root	–	–	–	–	–	10.6 ± 0.5	–	10.1 ± 0	
Flower	9.3 ± 0.5	–	9.6 ± 0.5	9.6 ± 0.5	–	–	–	10.3 ± 0.5	
Acetone									
Leaf	27.3 ± 0.5	9 ± 0	–	11.3 ± 0.5	–	–	–	7.6 ± 0.5	
Stem	17.6 ± 0.5	13.5 ± 0.5	–	10.6 ± 0.5	–	–	–	7 ± 0	
Root	–	–	–	–	–	–	–	8.3 ± 0.5	
Flower	8.3 ± 0.5	–	–	–	–	–	8.6 ± 0.7	–	
Aqueous									
Leaf	–	–	–	–	–	–	–	–	
Stem	–	–	–	–	–	–	–	–	
Root	–	–	–	–	–	–	–	–	
Flower	–	–	–	–	–	–	–	–	
Amoxicillin									28.3 ± 0.6

Diameter of a sterile disc is 5 mm. Concentration of each plant extract was 20 mg/disc.

^aValues are mean \pm standard deviation of three determination; No inhibition (–).

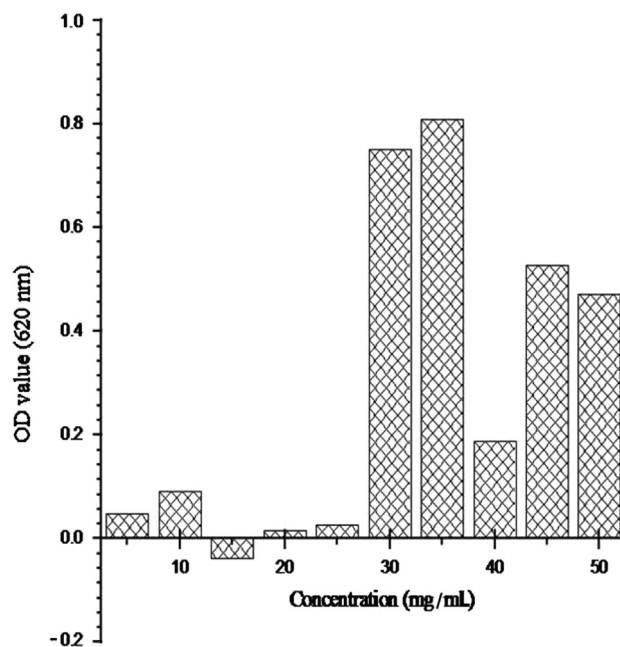


Fig. 1 MIC of *T. involucrata* leaf extract.

antimicrobial studies where more phenolic compounds are required to be extracted [31]. Vinoth et al. [32] reported predominant antibacterial activity in the organic solvent (acetone) as compared to water, which indicates that the active compounds responsible for the bactericidal activity are more soluble in the organic solvents. Similar to this, in the present study, acetone extract had the maximum inhibitory activity but the water extracts did not show antibacterial activity. Thus, the present study establishes that the organic solvents are having more powerful antibacterial activity than the water extracts, as opined by Maji et al. [33].

Chanda and Kaneria [34] reported that the bioactive compounds are normally accumulated as secondary metabolites in all plant cells but their concentration could vary in different plant parts. In this regard, leaf is one of the highest accumulatory plant parts and its compounds are generally preferred for therapeutic purpose [35]. Action mechanism of such compounds has not been unequivocally established, but they may interfere with peptidoglycon bacterial cell wall synthesis in the effected organisms [36] and in many other ways such as inhibiting protein synthesis, interfering with nucleic acid synthesis, breaking the peptide bonds, acting as chelating agents, inhibiting the metabolic pathway and preventing the utilization of available nutrients by the microorganisms. Some compounds may also cause the lysis of microbial cells. In the present study, acetone extract of *T. involucrata* leaf showed maximum inhibitory activity compared to that of others. This could be due to the fact that the leaf of *T. involucrata* might contain more number of secondary metabolites, responsible for the antimicrobial activity and inhibition of the growth of microbes. Plant components with phenolic structures are highly active against the microorganisms [37]. Several studies have also shown that alkaloids, saponins, tannins, flavonoids and phenolic compounds possess antimicrobial activities [38]. Especially, terpenoids from *Terminalia avicennioides* showed antibacterial activity against *E. coli* [39]. In *T. involucrata* also, terpenoides might be responsible for the inhibition of *E. coli* NCIM 2065 in addition to the phenolic compounds [40]. Similarly, Perumal Samy et al. [41] isolated nine bioactive compounds from *T. involucrata* leaf; among

these, vinyl hexylether and 2-methylnonane had the inhibitory activity against *E. coli*. Similar study was carried out by Panda et al. [42] in *T. involucrata* root extract and it was found that the compounds viz. TIR-01(10,13-dimethoxy-17-(6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1Hcyclopenta [a] phenanthrene) and TIR-05 (3-(2,4-dimethoxyphenyl)-6,7-dimethoxy-2,3-dihydrochro-men-4-one) possess antibacterial property against *E. coli*.

Potential of different plant species to yield newer antibacterial compounds was revealed by Shahid et al. [43]. In the present study, *C. collinus* showed reasonable inhibition activity in acetone, chloroform and petroleum ether. Similarly, various extracts of this plant had shown antimicrobial activity against *E. coli* [34,44,45]. Srinivasan et al. [46] reported that *V. indica* cold aqueous extracts alone showed a more potent inhibitory effect against *E. coli* as compared to hot and autoclaved waters. This lends support the present study where *V. indica* autoclaved water extracts also did not show any inhibitory effect.

S. indicus aqueous extract [47] and petroleum ether extract [48] were found to have antibacterial activity against *E. coli*. Whereas, Duraipandiyan et al. [49] found that *S. indicus* acetone and chloroform extracts did not show inhibitory effect against *E. coli*. In contrast, *S. indicus* chloroform extract has shown antibacterial activity in the present study. This variation could be due to the biological and environmental factors [50] and handling procedures. Previously, many researchers have reported on the antimicrobial property of the various solvent extracts of *E. alsinoide* [51–53]. Present study has also proved that *E. alsinoide* chloroform, acetone and petroleum ether extracts have antibacterial property against *E. coli* NCIM 2065.

Minimum inhibitory concentration is the lowest concentration of an antimicrobial that will inhibit the growth of microorganisms; 15 mg/mL of *T. involucrata* leaf extract was the MIC for *E. coli* NCIM 2065. Similarly, Kaur and Arora [54] have observed that the MIC of the acetone extract of seeds (*Anethum graveolens*, *Foeniculum vulgare* and *Trachyspermum ammi*) was 5–15 mg/mL. Dash et al. [55] reported that the MIC of Coriander and Fenugreek acetone extract was 16–128 µg/mL for *E. coli*.

It can be concluded that the wild plants investigated, have opened up a new perspective in pharmaceutical research and they can be used for the development of potential, novel antimicrobial agents for the treatment of microbial diseases. In this direction, *T. involucrata* can be pursued further as it is a promising source of antibacterial activity.

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