

PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF EXTRACT OF *SOLANUM TRILOBATUM* L.

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ABSTRACT

Solanum trilobatum L. is one of the oldest known therapeutic herb belonging to the family Solanaceae, widely used in cough, chronic bronchitis and the leaves are cooked as vegetable. The leaf broth used as medicine for several diseases. The present investigation was undertaken to evaluate the phytochemicals screening analysis for the eleven solvent used such as methanol, ethanol, acetone, ethyl acetate, hexane, chloroform, petroleum ether, n-butanol, n-propyl alcohol, diethyl ether and aqueous broths. The extraction capacity of methanol, ethanol and acetone is higher than the other eight solvent used. The preliminary phytochemical studies showed the presence of alkaloids, saponins, tannins, flavonoids, steroids, glycosides, phenolic compounds carboxylic acid and quinine. The antibacterial activity of crude extract of *S. trilobatum* was studied against gram-negative

bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* and gram-positive bacteria such as *Bacillus subtilis*, *Staphylococcus aureus*.

KEYWORDS: *Solanum trilobatum*, Phytochemical analysis, Antibacterial Activity.

INTRODUCTION

Screening of medicinal plants for antimicrobial agents has gained much more importance because lately World Health Organization (WHO) is fanatically interested in the development and utilization of medicinal plant resources in the traditional system of medicine in the developing countries so as to extend the health care to maximum number of population in

these countries (Dagawal and Ghorpade, 2011). The exploitation of plants by human beings for the treatment of diseases has been in practice for a very long time. Herbal drugs, mostly secondary metabolites constitute a major part in all the traditional system of medicines (Sharma et al., 2013). These secondary metabolites showed various biological activities and act in plant defense mechanisms. Higher plants, as sources of medicinal compounds continue to play a dominant role in maintenance of human health since antiquities (Jain et al., 2014). Earlier studies reported that the plant based drugs as good sources of antibiotics, anti-inflammatory and antioxidant agents (Mathur et al., 2011; Ahad et al., 2011; Afzal and Parveen, 2011). The methanolic extract of *S. trilobatum* has been shown to possess antioxidant activity, hepatic protective activity (Shahjahan et al., 2005) anti-inflammatory activity and anti-ulcerogenic activity (Amir et al., 2004) skin repellent activity against *Anopheles stephens* (Rajkumar and Jebanesan, 2005). The secondary metabolites are chemical substances used by plants for defense system and serve as the bioactive principles for various drugs in modern chemotherapy (Cragg and Newman, 2005).

MATERIALS AND METHODS

Plant materials

The fresh and healthy leaves of *S. trilobatum* were collected from the location of Rajapalayam, Virudhunagar District. The plant materials were identified in the Department of Botany, Ayya Nadar Janaki Ammal College, Sivakasi.

Microorganism used

Gram-negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* and gram-positive bacteria such as *Bacillus subtilis*, *Staphylococcus aureus* were obtained from the Department of Microbiology, Ayya Nadar Janaki Ammal College, Sivakasi.

Preparation of plant extract

Leaf extracts were prepared according to the method described by (Ahmad and Beg, 1998) with minor modification. The leaf extract thoroughly washed with distilled water and then dried under shade condition. The dried leaf was powdered and stored in air sealed plastic container at room temperature until the time of extraction. The leaf powders were subjected to extraction using organic solvents. Five gram of dried leaf powdered was soaked in 10ml of solvent for 72 hours, with stirring every 24 hours. At the end of extraction period, it was centrifuged and supernatant was filtered through Whatman No.1 paper. This extraction was

repeated three times. Filtrates were pooled and evaporated to air dry and stored at 20°C for further use.

Phytochemical Screening

The methanol, ethanol, acetone, ethyl acetate, hexane, chloroform, petroleum ether, n-butanol, n-propyl alcohol, aqueous and diethyl ether extracts were screened for phytochemical for the presence of its constituents.

Phytochemical analysis

Preliminary phytochemical screening was performed as per standardized procedure authors (Harborne, 1973; Patnaik et al., 2012). Alkaloids test carried out using 0.6g of Bismuth sulphate dissolved in 20ml of water and 6g of Potassium iodide was dissolved in 50ml of water. This solution were mixed and allowed to stand for some time. The supernatant was decanted from potassium iodide and make up to 100ml. Flavonoids test carried out by 1ml of stock alcoholic solution with few drops of neutral FeCl₃ and 5ml of extract with 1ml of alcohol subjected to the Ferric chloride test. Phenolic compounds test carried out using 1ml of extract with 5ml of alcohol and few drops of neutral FeCl₃. Tannin test carried out using 1ml of extract and minimum amount of H₂O. Filtered and to the filtrate add few drops of FeCl₃ solution. Saponins test carried out using 1ml of extract with 20ml of distilled water agitated vigorously for 15 minutes. Steroids test carried out using 1ml of extract with 1ml of methanolic extract of drug and 1ml of chloroform, 2-3ml of acetic anhydride and 1-2 drops of conc. H₂SO₄ were added. Quinine test carried out using 1ml of extract with few drops of alcoholic KOH was added. Glycosides test carried out using 1g powder with dissolved in 2-3 ml of distilled water and 2-3 drops of 1per cent solution of alcoholic -naphthol added side of test tube (Tensingh Baliah and Astalakshmi, 2014).

Antibacterial activity

The antibacterial test was carried out against gram positive and gram negative bacteria. The antibacterial activity of leaf extracts was tested against bacteria by disc diffusion method (Berghe and Vlietinak, 1991) and 100µl, 150µl and 200µl concentration of leaf extract loaded disc were placed on the surface of the agar medium by pressing with sterile forceps in an aseptic condition. The inoculated and treated plates were incubated at 37°C for 24 hours. After the incubation, the diameter of zone was measured. Standard antibiotic streptomycin (50mg/ml) used as control. The respective control was also run simultaneously using different solvents to compare the effect of plant extracts. After overnight incubation, the

diameter of each zone of inhibition was measured. In all measurements, the zones of inhibition are measured from the edges of the last visible colony-forming growth. The results were recorded in millimeters (mm) and interpretation of susceptibility was obtained by comparing the results to the standard zone sizes.

RESULTS

Phytochemical Screening: The result indicated that the leaf extract of *S. trilobatum* contained different types of phytochemicals such as alkaloids, flavonoids, saponin, phenolic compounds, steroids, carboxylic acid, tannin and glycoside. Alkaloids reacted with Dragendroffs reagents to produce reddish brown precipitate, indicate the presence of alkaloids in the leaf extracts. Ferric chloride reacted with leaf extract and formed blue colour precipitate. The above result indicated the presence of tannins in the extract of *S. trilobatum*. Glycosides reacted with α -naphthol and sulphuric acid to form brick red colour. Steroids reacted with chloroform, acetic anhydride and concentrated sulphuric acid to produce rosy red colour, indicated the presence of steroids. Saponin reacted with mercuric chloride to produce white precipitate as positive result. Flavonoids reacted with ferric chloride to form blackish red colour confirm the presence of flavonoids. Quinine reacted with alcoholic NaOH to colour change from red to blue. Phenolic compounds reacted with neutral FeCl_3 to colour change as positive result (Table 1).

In general, eleven solvent extracts of *S. trilobatum* were able to produce inhibition zones on all the five strains used indicating bactericidal activity. The acetone, hexane, petroleum ether, chloroform, n-butanol, diethyl ether and water extracts of *S. trilobatum* L showed least antibacterial activity when compared to other solvent extracts. Only the 150 μL and 200 μL concentration had effect on test organisms while at 100 μL the extract had no effect or least effect on bacterial strains (Fig 1,2 and 3). Hexane extract of *S. trilobatum* L showed no activity against *P. aeruginosa*. Likewise, petroleum ether extract showed no activity against *E. coli*. The extracts of acetone, methanol and n-propyl alcohol exhibited the maximum zone of inhibition (7-10mm) and other extracts had minimum zone of inhibition (3-7mm) (Figure 1 – 11). All the solvent extracts of *S. trilobatum* L. were able to produce inhibition zones on all the five bacterial strains.

Table 1. Phytochemical analysis in *S. trilobatum* with different solvents

S. No	Extract	Phytochemicals								
		A	F	S	S	Q	PC	T	G	CA

1.	Acetone	+	+	+	+	-	+	-	-	+
2.	Ethyl acetate	+	+	+	+	-	-	+	+	-
3.	Hexane	+	+	+	+	-	+	+	+	-
4.	Ethanol	+	+	-	+	-	+	+	+	-
5.	petroleum ether	+	+	+	+	-	+	+	-	-
6.	Chloroform	-	+	-	-	-	+	-	-	-
7.	Diethyl ether	+	+	-	-	+	+	+	-	-
8.	n-propyl alcohol	+	+	-	-	-	+	+	-	-
9.	n-butanol	+	+	-	+	-	+	-	-	-
10.	Methanol	+	+	+	+	-	+	+	-	-
11.	Water	+	+	-	-	-	+	-	-	-

A – Alkaloid,

C - Phenolic compound, F – Flavonoid, T - Tannin

S – Steroid, G – Glycoside S – Saponin, CA - Carboxylic acid and

Q – Quinine. (– indicates absence of compounds)

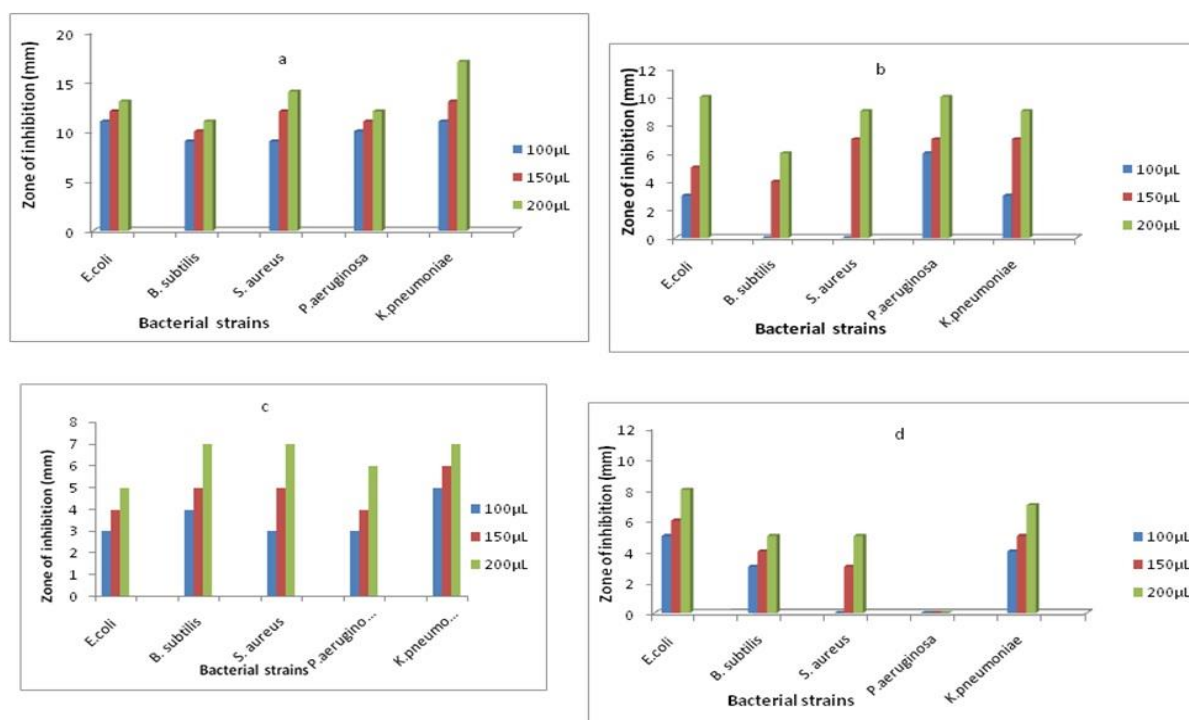


Fig 1: Antibacterial activity of methanol extract (a), Acetone extract (b), Ethyl acetate extract (c), Hexane extract(d) of *Solanum trilobatum* L

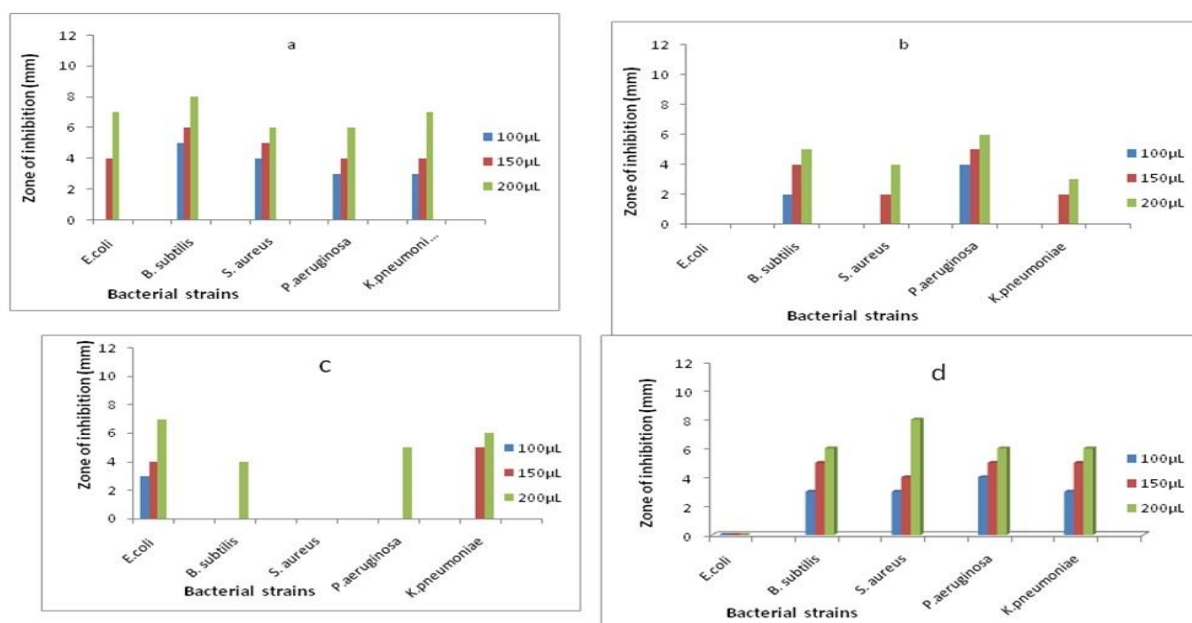


Fig 2: Antibacterial activity of ethanol extract (a), petroleum ether extract (b), chloroform extract (c), diethyl ether extract (d) of *Solanum trilobatum* L

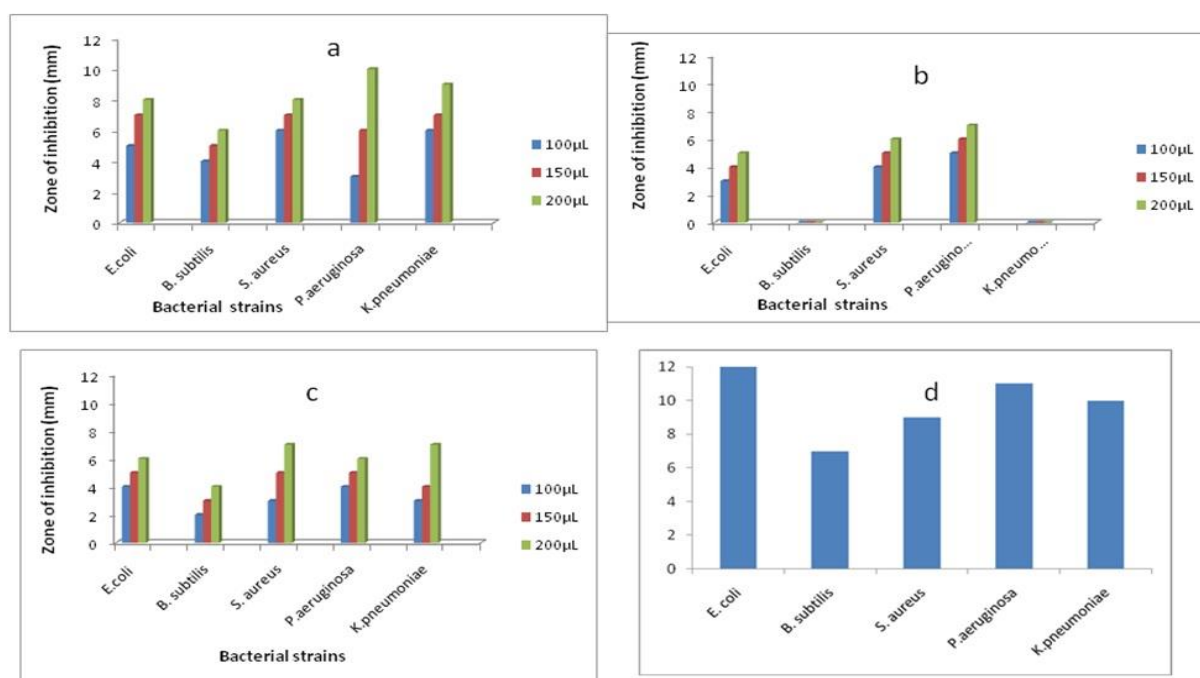


Fig 3: Antibacterial activity of n-propanol extract (a), n-butanol extract (b), water extract (c), Standard control (Streptomycin) (d) of *Solanum trilobatum* L

DISCUSSION

Dichrostachys cinerea contained tannins, alkaloids and glycosides. Amongst the gram-positive and gram-negative bacteria, gram-positive bacterial strains were more susceptible to the extracts when compared to gram negative bacteria. This may be attributed to the fact that these two groups differ by its cell wall component and its thickness. The ability of tannin

compounds to cause the bacterial colonies to disintegrate probably results from their interference with the bacterial cell wall; thereby inhibiting the microbial growth (Eresto et al., 2004). The major compound observed, piperitone, is a terpene ketone found in most of *Mentha* species well known to possess carminative properties. However, in recent investigation piperitone (4.2 %) along with other components showed antibacterial activity against *Bacillus dipsauri*, *Corynebacterium cystitidis* and *Corynebacterium flavescens* (Ozturk and Erclsi, 2006). Methanolic extracts of the root and stem bark of *B. crassifolia* was found to inhibit the growth of *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella flexneri*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Micrococcus luteus*. The methanolic extract of the leaves of *B. crassifolia* demonstrated weak activity against *Trypanosoma cruzi* and *in vitro* spasmogenic effects on rat fungus. Among these, epicatechin was the most active with MIC values of 64 µg/mL for *P. gingivalis*, 128 µg/mL for *Streptococcus mutans* and 260 µg/mL for *S. aureus*. The remaining compounds did not demonstrate comparable level of antimicrobial activity against all test bacteria when compared with epicatechin (Panda et al., 2004).

CONCLUSION

The present study revealed that the *S. trilobatum* was more effective in antibacterial activity of the eleven solvent used such as methanol, ethanol and acetone seems to be the best solvent when compare to other solvents. The extraction capacity of methanol, ethanol and acetone is higher than the other eight solvent used. This was understandable from the size of inhibition zone formed.

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