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EVALUATION OF ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF MEDICINAL PLANT *SOLANUM TRILOBATUM*.

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ABSTRACT

The ethanolic leaf extracts of *Solanum trilobatum* plant were used for antibacterial study by disc diffusion method. Different concentrations (10mg/ml, 20mg/ml, 30mg/ml) of the concentrated ethanolic leaf extract were tested for its antibacterial activity against bacterial strains such *Bacillusubstilis, Staphylococcus aureus, Bacillus cereus*, *E. Cali & Pseudomonas aeruginosa.* The bacterial cultures were grown in Muller hinton agar and Muller hinton broth. The zone of inhibition was comparatively more in the concentration of 30mg/ml. Phytochemical screening of the ethanolic leaf extract of the plant revealed the presence of tannins, saponins, flavonoids, carbohydrates and alkaloids. Thin Layer Chromatography (TLC) value of the leaf extract showed the highest Retention Factor (RF) value. The plant extracts showed better inhibitory activity against the tested organisms.

KEYWORDS :*Solanum trilobatum,* bacterial strains, ethanol extract, TLC, phytochemicals, retention time

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INTRODUCTION

Nature has a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, mainlybased on their use in traditional medicine[1]. It is reported that over 50% off all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the Pharmaceutical industry[2].Many infectious microorganism are resistant to synthetic drugs, hence alternative therapy is very much needed. *Solanum trilobatum*, a thorny creeper with bluish violet flower, more commonly available in southern India has been used in the treatment of various diseases[3, 4]. It has been widely used to treat respiratory disorder, cancer. It was reported that *S.trilobatum* possess antioxidant activity, hepatoprotective activity. *S.trilobatum* was reported effective in treating tumour reduction[4] and protect *peneaus modon* past larvae from bacterial attack[5]. New concepts have appeared with this trend, such as nutraceuticals nutritional therapy, phytonutrients and phytotheraphy. This functional or medicinal foods and phytonutrients or phytomedicines play positive role in maintaining well being, enhancing health and modulating immune function to prevent specific disease. The history of plants being used for medicinal purpose is probably as old as the history of mankind. According to world health organization, medicinal plants are the best source to obtain a variety of newer herbal drugs about 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties safety and efficacy. Infectious diseases are the leading cause of death worldwide. Bacterial and fungal pathogens have evolved numerous defense mechanisms against antimicrobial agents and resistant to hold and newly produced drugs in on the rise. The increasing failure of chemotherapeutics and antibiotic résistance exhibited by pathogenic microbial infections agents has led to the screening of several medicinal plants for their potential antimicrobial activity. The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health has been widely observed. Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs, chemotherapeutics from these plants as well as from traditionally used rural herbal remedies. The results of investigation performed in the 19 and 20th century and the advent streptomycin and other antibiotics provide the ground for experimentation of a vast of plants for antibiotic or antimicrobial activities that are useful to man [5, 6]. *Solanum trilobatum* Linn. (Solanaceae-herbs) is an important medicinal plant. It contains rich amount of calcium, iron, phosphorus, carbohydrates, fat, crude fibre and minerals in the leaves. It is used to cure asthma, arrest blood vomiting, to reduce blood glucose level and bilious matter phlegmatic rheumatism and several kinds of leprosy. It is also antibacterial, antifungal, antimitotic, antioxidant and antimtumours[7]. The present study was carried out to evaluate the preliminary phytochemical screening and antibacterial activity of medicinal herb *Solanum trilobatum*.

MATERIALS AND METHODS

Plant collection and identification

Fresh leafs of *S.trilobatum.* L were collected from the Herbal Garden from Madhavaram, Chennai, Tamil Nadu, India. The identity of the plant confirmed with the help of a Botanist. The plant materials were cleaned, shade dried and powdered. Fresh plant materials were washed with tap water, air dried, homogenized to a fine powder and stored in air-tight bottles. Qualitative and quantitative phytochemical screening*:* The solvent extracts were subjected to preliminary phytochemical screening to identify the presence of phytoconstituents such as alkaloids, flavonoids, saponins, tannins, phenols, glycosides and steroids according to [1]

Estimation of total free phenolics

Total phenolic constituents of plant extracts were estimated byFolin-Ciocalteau's method using Folin-Ciocalteu reagent. The estimation was done spectrometrically at 760 nm and the results were expressed as gallic acid equivalents (GAE) [8].

Estimation of total flavonoids

Aluminium chloride method was employed to quantify the total flavonoid content in the plant extracts. The results were expressed as quercetin equivalents (QE) [9].

Estimation of total alkaloids

Total alkaloid content of the plant extracts was determined according to [10]. Five gram of the sample was filtered and concentrated to one quarter of the original volume on a water bath after treatment with 200 mL of 10% acetic acid in ethanol. Concentrated NH4OH was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with diluteNH4OH, filtered and weighed.

Estimation of total saponins

Powdered sample (20 g) was treated with 100 mL of 20% aqueous ethanol, heated over a hot water bath for 4 h at about 55°C with continuous stirring. The mixture was filtered and the residue re-extracted. The combined extracts were reduced to 40 mL over a water bath at about 90°C and the concentrate was transferred into a separating funnel and 20 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60 mL of n-butanol was added to the combined extracts and washed twice with 10 mL of 5% aqueous NaCl. The remaining solution was heated in a water bath, dried in an oven to a constant weight and the saponin content was calculated as a percentage.

Thin layer chromatography

Preliminary identification of phytochemicals was made by thin layer chromatography (TLC) using silica gel plates (5gm of silica gel dissolved with 90ml of water). The extracts were eluted with chloroform : ethanol : water (30:20:4) and the chromatogram were developed by spraying with methanol : Sulphuric acid (1:1) and heating to 110ºC. Then RF value was calculated as the ratio of distance travelled by the solute to the distance travelled by the solvent [11].

Bacterial strains

Bacterial strains used in this study such as *Pseudomonas aeruginosa, Bacillus substilis, Escherichia coli, Staphylococcus aureus* and *Bacillus cereus*. All the strains were confirmed by cultural and biochemical characteristics. The bacterial cultures were grown in Muller Hinton Agar and Muller Hinton Broth.

Antibacterial activity

The antibacterial assay of Ethanolic extracts was performed by agar well diffusion method[12, 13]. Three types of concentrations were used, namely 10mg/ml, 20mg/ml and 30mg/ml of plant extract dissolved in Dimethylsulfoxide. For antibacterial assay all bacterial strains were grown in Muller Hinton Broth medium for 24 hours at 37ºc and plated on Muller Hinton Agar. Then 0.1 ml of each culture of bacteria was spread on agar plate surfaces. Sterile disc (Hi Media, 6mm in diameter) were placed on the agar medium to load 20 (l of different concentration (10 -30mg /ml) of ethanolic leaf extracts of *Solanum trilobatum* were tested. Inhibition diameters were measured after incubation for 24 hours at 37 (C. Blanks of solvent only (processed in the same way), were also tested for antibacterial activity. The diameter of inhibition zones was measured.

RESULTS AND DISCUSSION

Qualitative phytochemical analysis

The preliminary phytochemical screening of *Solanum trilobatum* revealed the presence of phenolics, carbohydrates, alkaloids, flavonoids, sopanins, tannins and steroids, in high amounts followed by glycosides, aminoacids and proteins. (Table 1)

Table 1
Phytochemical estimation.

Compound	Result
Alkaloids	+
Carbohydrates	+
Glycosides	+
Saponins	+
Proteins and amino acids	+
Phenolic compounds	+
Steroids	+
Flavonoids	+
Tannins	+

(+) Presence of all phytochemicals

Quantitative phytochemical analysis

The major phytochemicals present in the selected plant extracts were phenols, flavonoids, alkaloids and tannins were quantified. According to the results, suggested with the antioxidant activity of each extract tested. In general, the ethanolic extracts showed higher content of phenols and flavonoids, and likewise higher antioxidant activity. The results of total phenol content, alkaloids, saponins and flavonoids are given in (Table 2).

Table 2
Quantitative phytochemical estimation.

Bioactive compound	Result
Total phenols	259.63 µg GAE/g sample
Alkaloids	1.43 mg/g sample
Saponins	0.116 mg/g sample
Tanins	1.43mg/g dry weight
Flavonoids	64.3µg Quercetin equivalent/g

Thin layer chromatography

The chromatogram developed with 10% methanol in chloroform revealed the presence of five major compounds at Rf value of 0.33; 0.48; 0.56; 0.76; 0.86 as visualized under iodine vapour and UV illumination.

Antibacterial activity

The ethanol extract from *S.trilobatum* leaves showed antibacterial activity against tested bacterial strains in the order *Bacillus subtilis* (13mm), *Bacillus cereu* (11mm), *Pseudomonas aeruginosa* (9mm) *Staphylococcus aureus(* 8mm) *Escherichia coli* (*6mm)* The minimum inhibitory concentration was found in *Bacillus substilis* with the concentration 30mg/ml[15, 16]. The zone of inhibition was comparatively more in the concentration of 30mg/ml. The zone of inhibition was interpreted in millimeter and was tabulated in Table.3 (Figure1). From the studies, it was concluded that antibacterial activity of *Solanum trilobatum* extracts against the organism indicates the medicinal value and support the claim of traditional healer that it has been used to cure various diseases like asthma, liver disorder, cancer, cough and cold. The presence of antibacterial substances in the higher plants is well established[17]. Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contributions towards human health. Phytomedicine can be used for the treatment of diseases as is done in case of Unani and Ayurvedic. Traditional healers found more medicinal plants which are highly effective to treat various diseases and also it is necessary to prove scientifically in order to develop new drug molecules. Antimicrobial activity of *S. trilobatum* extract against a number of bacteria has been reported. Swapna Latha [18] they reported the plant extracts from leaves, flowers, stems and fruits of *S. trilobatum* revealed antimicrobial activity against Gram (+)ve bacteria and Gram (-) ve. These results reveal that the

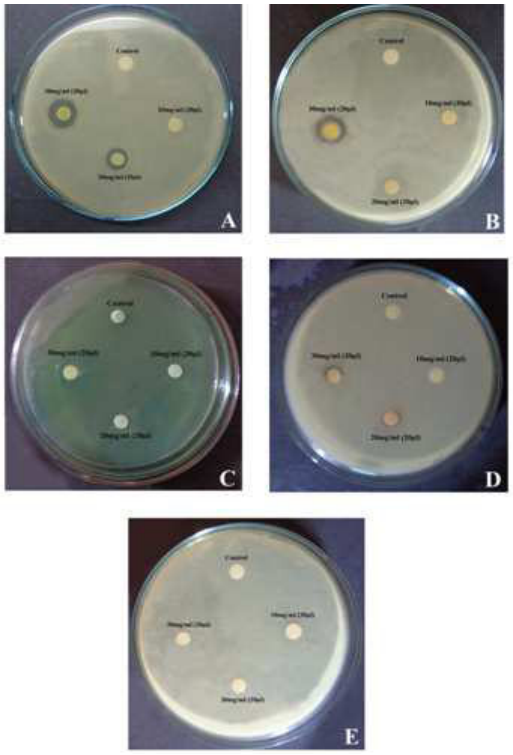
leaves of *S. trilobatum* could be a potential source of traditional medicine for infections caused by *Escherichia coli, Pseudomonas aeruginosa, Bacillus cereus* and *Bacillus subtilis, Staphylococcus aereus.* It is necessary to elucidate the exact bioactive compound which is responsible for the destined antibacterial action. However, further studies are needed to isolate its active principles.

Table 3
Antibacterial activity of Solanum trilobatum extracts

Inhibition Zone in diameter (mm)*			
Micro-organisms Tested	Concentrations of extract		
Ethanolic Leaf extract	10mg/ml	20mg/ml	**30mg/ml**
Bacillus subtilis	-	9	**13**
Bacillus cereus	-	-	**11**
Pseudomonas aeruginosa	-	-	**9**
Staphylococcus aureus	-	-	**8**
Escherichia coli	-	-	**6**

This strain was obtained from MTCC, Includes diameter of disc (6mm); Average three replicates

Figure 1
Antibacterial activity of ethanolic leaf extract of Solanum trilobatum



(A) Antibacterial activity of leaf extract of S. trilobatum on Bacillus subtilis.
(B) Antibacterial activity of leaf extract of S. trilobatum on Bacillus cereus
(C) Antibacterial activity of leaf extract of S. trilobatum on Pseudomonas aeruginosa
(D) Antibacterial activity of leaf extract of S. trilobatum on Staphylococcus
(E) Antibacterial activity of leaf extract of S. trilobatum on Escherichia coli

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