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Antibacterial Activity of Saponin Isolated from the Leaves of *Solanum trilobatum* Linn

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Abstract

This study was designed to test the antimicrobial potential of ethanol, acetone and ethyl acetate extracts of leaves, fruits and flowers of *Solanum trilobatum* and pure saponin fraction extracted from the leaves against selected bacterial strains, *Staphylococcus aureus*, *Escherichia* coli, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The antibacterial activity was tested by agar disc diffusion and agar well diffusion method. The plant parts tested were extracted with ethanol, acetone and ethyl acetate. Almost all the organic solvent extracts exhibited good inhibitory effect against tested bacterial pathogens. The most susceptible Gram-positive bacterial species was *S. aureus,* while the most susceptible Gram -negative bacteria was *P. aeruginosa.* Pure saponin fraction exhibited remarkable antibacterial activity when compared to crude extracts. The antibacterial activity of extracts was found to be comparatively higher than the standard antibiotics used in this study. These results provide evidence for the antagonistic activity of steroidal saponin against tested bacterial pathogens. Further, it could be developed as a bactericidal drug to be used as therapeutic agent against bacterial infections.

Key words: Medicinal plant, antibacterial activity, bactericidal drug, disc diffusion, agar well diffusion

INTRODUCTION

Emergence of more and more multidrug –resistant pathogens was reported to be one of the leading causes of death world-wide [1]. Many infectious microorganisms are resistant to synthetic drugs; hence an alternative therapy is very much needed. Medicinal plants have been used as an exemplary source for centuries as an alternative remedy for treating human diseases because they contain numerous active constituents of immense therapeutic value [2]. Even now, approximately 80% of the third world population is almost entirely dependent on traditional medicines for maintaining general health and combating many diseases [3].

Solanum trilobatum Linn (Family: Solanaceae), a thorny creeper with bluish white flower, widely distributed throughout India and has long been used in Siddha system of medicines to treat various diseases [4]. It has been widely used as an expectorant and in the treatment of respiratory diseases including bronchial asthma [5], febrile infections, and tuberculosis [6]. The methanolic extract of *S. trilobatum* has been shown to possess antioxidant activity [7] and hepatoprotective activity [8]. Sobatum, the partially purified petroleum ether extract of *S. trilobatum* has been reported to be very effective in protecting UV induced damage [9], radiation-induced toxicity [10] and inducing tumor reduction in mice [11]. Solasodine and sobatum isolated from *S. trilobatum* plant has been shown to possess antiinflamatory activity [12]. The methanolic extract has been reported to be very effective in protecting *Penaeus monodon* post larvae from bacterial attack [13] and the acetone extract has been shown to possess ovicidal activity against C*ulex quinquesfasciatus* and *Culex tritaeniorhnchux* [14]; and oviposition deterrent and skin repellent activity against *Anopheles stephensi* [15].

Various chemical constituents are reported to be isolated from *Solanum* species, which includes alkaloids, phenolics, flavonoides, steroidal saponins and their glycosides [16]. Alkaloides such as soladunalinidine and tomatidine were isolated from the leaf and stem of *Solanum* species. In the present study an attempt was made to screen and to isolate a lead chemical constituent that could be useful for the development of antibacterial agent to control common bacterial diseases.

MATERIALS and METHODS

Plant materials and preparation of extracts

S. trilobatum was collected from the VIT University medicinal garden and a voucher specimen was prepared and deposited in the herbarium section of the VIT University, Vellore, Tamil Nadu, India. Leaves, flowers and fruits of *S. trilobatum* were washed with distilled water, shade dried, powdered and stored in an air- tight container until further use. Organic solvent extracts were prepared by transferring 1g of the powder to sterile wide-mouthed screw-capped bottles. 10 ml of the solvent was added to the powdered samples which were allowed to soak for 24 hours at room temperature, after heating the extracts for one hour at 100°C, the mixture was then centrifuged at 2000 rpm for 10 minutes at 4°C. The supernatants were filtered through a sterile funnel containing sterile Whatmann filter paper no.1 and then filter sterilized using syringe filter containing 0.2µ cellulose acetate membrane (Sartorius).

Test organisms

The bacterial species, *Staphylococcus aureus* (ATCC 700699), *Escherichia coli* (ATCC 10412), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 2719), were used as test organisms and they are maintained on Mueller Hinton Agar solid media (MHA).

The powdered leaves sample of *S. trilobatum* was defatted by petroleum ether for 3 h at 40 °C. After filtering the petroleum ether, the sample was extracted with methanol for 3 h with mild heating. The methanol extract was concentrated and dissolved in methanol and acetone mixture (1:5 v/v) to precipitate the steroidal saponins [17]. The precipitate was dried under vacuum, which became a whitish amorphous powder named as crude saponin extract (CSE). The CSE was loaded on silica gel-60 (230-400 mesh, Merck) chromatography column and eluted with chloroform- methanol-water (70:30:10) [18]. The first fraction collected was evaporated under reduced temperature, the resultant residue was called pure saponin fraction (PSF).

Assay of antibacterial activity

Agar diffusion assay was carried out to evaluate the antimicrobial activity of the plant extract as described previously [19]. The plates were incubated at 37°C for 24 hours during which activity was evidenced by the presence of a zone of inhibition surrounding the well. Each test was repeated three times and the antibacterial activity was expressed as mean of diameter of inhibition zones (mm) produced by the extracts when compared to controls.

RESULTS and DISCUSSION

The antibacterial activity of aerial parts of *S. triolobatum* was assayed under in vitro conditions by agar disc diffusion and well diffusion method against four bacterial species. The inhibition of microbial growth by various solvent extracts was summarized in Table 1. All the solvents, ethanol, acetone, ethyl acetate used for the extraction of leaves, flower and fruits were shown significant antibacterial activity except acetone and ethyl acetate extracts of flower against *K. pneumoniae*. Gram –positive bacteria *S. aureus* was more susceptible to inhibition when compared to the Gram-negative bacterial species tested. This is in agreement with previous reports that plant extracts are more active against Gram-positive bacteria than Gram-negative bacteria [20, 21]. Amongst the Gram-negative bacteria *P. aeruginosa* was more susceptible to inhibition by the extracts when compared to other bacterial species tested. The inhibition of bacterial growth by *S. triolobatum* extracts was found to be significantly higher than that of standard antibiotics tested.

Earlier reports in our laboratory have shown that the aqueous extract of aerial parts of *S. triolobatum* was effective against *S. aureus*, *B. subtilis* and *K.pneumoniae* species [22]. The results of the present study indicates that the ethanol, acetone and ethyl acetate extracts of *S. triolobatum* aerial parts exhibited more consistent antimicrobial activity against tested bacterial strains. This suggests that the organic solvents are better medium of choice to solublize different phytochemicals present in different parts of plants.

Table 1. Antibacterial activity of with ethanol, acetone and ethyl acetate extracts of *Solanum trilobatum* aerial parts

Plant parts	Diameter of zone of inhibition (mm)*			
	S.aureus	***E. coli***	***P.aeruginosa***	***K.pneumoniae***
Penicillin disc (25 mg)	11[a]	-[b]	-	-
Streptomycin disc (25 mg)	7	8	-	7
Ampicillin disc (25 mg)	10	10	-	-
a. **Leaves**				
Ethanol extract (100mg/ml)	15	14	20	12
Acetone extract (100mg/ml)	14	16	17	13
Ethyl acetate extract (100mg/ml)	16	15	17	16
b. **Flower**				
Ethanol extract (100mg/ml)	12	14	18	15
Acetone extract (100mg/ml)	18	15	20	-
Ethyl acetate (100mg/ml)	16	14	15	-
c. **Fruit**				
Ethanol extract (100mg/ml)	20	20	15	19
Acetone extract (100mg/ml)	20	18	18	10
Ethyl acetate (100mg/ml)	20	14	14	15

Each value was mean of three experiments.

[a] Inhibition zones are including the disc and the well diameter.

[b] No zone of inhibition.

Table 2. Antimicrobial activity of pure saponin fraction of *Solanum trilobatum* leaves

Bacterial strains	Diameter of Zone of inhibition (mm)*			
	Standards Antibiotics			Pure saponin fraction (10 mg/ml)
	P	S	A	
E. coli	-[b]	8[a]	10	19
P. aeruginosa	-	-	-	20
K. pneumoniae	-	7	-	19
S. aureus	11	7	10	21

Each value was mean of three experiments.

[a] Inhibition zones are including the disc and the well diameter.

[b] No zone of inhibition.

P : Penicillin disc (25 mg).

S : Streptomycin disc (25 mg).

A : Ampicillin disc (25 mg).

Pure saponin isolated from the leaves of *S. trilobatum* has shown significant antibacterial activity against tested organisms (Table 2). *S. aureus* (21mm) followed by *P. aeruginosa*

(20mm). The inhibition of bacterial growth exhibited by the saponin fraction was significantly higher than the inhibition shown by the crude extracts. Our results support the fact that the antimicrobial activity of plant extracts depends on the phytochemicals present in it. It has been reported that the antibacterial activity depends on the total saponins and tannins content present in the plant extract [23]. Several reports are available in support of antimicrobial activity of saponins against bacterial and fungal pathogens [24, 25]. Sobatum, β-solamarine, solanine, solasonine, solasodine, glycoalkaloid and diosogenin and tomatidine are the phytochemical constituents isolated from *S trilobatum* and already been reported (26). The solasodine and sobatum isolated from *S. trilobatum* has been reported very effective in reducing carrageenan induced paw oedema in rats [12]. The pharmacological activity of any plant extract has been reported to be associated with phytochemical constituents and botanical properties [27]

It was reported in a clinical study, that oral administration of *S. trilobatum* for 3 days at a dose of 300 mg dry powder, thrice a day was found to be very effective in controlling mild and moderate bronchial asthma and moreover the bioactivity is equivalent to that of administration of 200 mg of deriphylline [5].

The results obtained in this study confirmed the antimicrobial potential of ethanol, acetone and ethyl acetate extracts of *S. trilobatum* aerial parts. Pure saponin of *S. trilobatum* leaves had higher inhibitory potential against tested bacterial pathogens. The inhibition exerted by *S. trilobatum* saponin against the oppturnistic pathogen, *S. aureus* is of considerable importance because it is considered to be one of the major causative agents for numerous hospital and community acquired infections. Similarly inhibition against *P. aeruginosa*, the oppturnistic pathogen of immunocompromised individuals responsible for causing blood, pulmonary and urinary tract infections is of considerable significance. Further exploration of its broad spectrum of activity against common human pathogens and structural elucidation of *S. trilobatum* steroidal saponin would prove its bioactive potential. The efficacy of pure saponin as a bactericidal agent needs to be studied further under *in vivo* conditions to make it useful for therapeutic applications against common bacterial infections.

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