

Screening of anti-bacterial activity of *Solanum trilobatum* Linn. seed extract against dental pathogens

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ABSTRACT

In the present study, seed crude extracts of *Solanum trilobatum* was studied for its ability to inhibit the growth of dental (bacterial) pathogens (*Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus sanguinis* and *Lactobacillus acidophilus*). Collected seeds of *S. trilobatum* were properly washed and shade dried at room temperature, crushed and extracted in acetone, petroleum ether and chloroform. The anti-bacterial activity of extracts was examined by agar well diffusion method at 200 mg mL⁻¹ sample concentration. Phytochemical analysis was done for plant extracts. The results of anti-bacterial activity were found that acetone extract of *S. trilobatum* was most effective against all tested bacterial pathogens. Maximum anti-bacterial activity was observed against *S. salivarius* (23 mm) and lowest activity against *S. sanguinis* (9 mm). The phytochemical analysis revealed the presence of alkaloids, flavonoids, steroids, terpenoids, tannins and saponins which might be accountable for its anti-bacterial potentiality. The results validate the traditional uses of *S. trilobatum* in treatment of dental diseases.

Key words: Dental pathogens, *Solanum trilobatum*, Acetone, anti-bacterial activity, seed extract.

INTRODUCTION

Dental infections are one of the most common health problems in the human community worldwide [1] and are mainly of three types, viz.: dental plaques, dental caries and periodontal diseases [2]. Dental plaque is a dense mass of bacteria that adhere tight to tooth surface. Dental caries is a common oral bacterial pathology caused by a biofilm consisting of microorganisms present on the tooth surface which causes destruction of enamel, dentin or cement of teeth due to bacterial activities [3-4]. Periodontal diseases are bacterial infections that affect the supporting structure of the teeth. Gingivitis, an inflammatory condition of gum, is the most common form of periodontal disease results in tooth loss. A large number of residents i.e., *Streptococcus*, *Actinomyces*, *Lactobacillus* and *Porphyromonas* bacteria responsible for this type of disease in humans [5-7].

Solanum trilobatum Linn. is an important medicinal plant, 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 [8]. In Sanskrit it is known as 'Alarka', English : Climbing Brinjal, in Telugu 'Alarkapatramu', in Tamil 'Tuduvalai' and in Malayalam 'Tutuvalam'. It has been widely used as an expectorant and in the treatment of respiratory diseases including bronchial asthma [9] febrile infections, and tuberculosis [10]. The methanolic extract of *S. trilobatum* has been shown to possess antioxidant activity [11] and hepatoprotective activity [12]. Sobatum, the partially purified petroleum ether extract of *S. trilobatum* has been reported to be very effective in protecting UV induced damage

[13], radiation-induced toxicity [14] and inducing tumor reduction in mice [15]. Solasodine and sobatum isolated from *S. trilobatum* plant has been shown to possess anti-inflammatory activity [16]. Hence, this study is an assiduous attempt to find out the chemical constituents that could be useful for the development of antibacterial potential agents from *S. trilobatum* seed extracts against dental pathogens.

MATERIALS AND METHODS

Plant materials: Fresh seeds of were collected from the local village, agricultural area in Bhimavaram. The plant specimen are identified and authenticated in the department of Botany, D. N. R. College, Bhimavaram.

Preparation of Extracts:

The seeds were carefully washed under running tap water followed by sterile distilled water. These were air dried at room temperature (30°C) for two days and pulverized to a fine powder using a sterilized mixer grinder and stored in airtight bottles. Three different solvents namely acetone, petroleum ether and chloroform were used for extraction. A 100 g amount of seeds was separately soaked in 100ml of acetone, petroleum ether and chloroform for 72h and allowed to stand for 30min on a water bath with occasional shaking, and kept undisturbed for 24h. Each preparation was filtered through a sterilized WhatmanNo.1 filter paper and the filtered extract was concentrated under vacuum below 40 °C using rotaevaporator [17-18]. The dried extract thus obtained was exposed to UV rays for 24h and checked for sterility on nutrient agar plates and stored in labelled sterile bottles in a freezer at 4°C until further use [19].

Determination of extractive value: Estimation of extractive value was done according to the method of [20].

Phytochemical Screening: To detect various biologically active phytochemical constituents present in various solvent extracts the standard methods were followed [21-22].

Microorganisms tested: The strains of dental infection related bacteria used in this study were *Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus sanguinis* and *Lactobacillus acidophilus* respectively. All the bacterial strains were grown and maintained on nutrient agar slants at 4°C.

Inoculums preparation: Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from stock cultures to test tubes of Mueller-Hinton Broth for bacteria that were incubated without agitation for 24 h at 37°C.

Screening for antimicrobial activity: The acetone, petroleum ether and chloroform of *S. trilobatum* seeds extracts were used for the screening. Antimicrobial activity of various extracts was determined by the agar well diffusion method [23]. The plates were incubated at 37°C for 24 h 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

In the present study the extractive values from the *S. trilobatum* seed extract by using acetone, petroleum ether and chloroform were calculated. The result showed that the acetone extract has highest extractive value of 10.5 (%w/w) and the extractive values of petroleum ether and chloroform are 8.7 and 9.2 (%w/w) (Table 1). It may be due to the solubility of secondary metabolites in different organic solvents. The preliminary phytochemical analysis of the seed extract with acetone, petroleum ether and chloroform revealed the presence of alkaloids, flavonoids, steroids, saponins, Tannins, Terpenoids (Table 2). The alkaloids, steroids and terpenoids are absent in chloroform. Many plants are known to have beneficial therapeutic effects as noted in the traditional Indian system of medicine, *Ayurveda*. The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world. Much work has been done on ethnomedicinal plants in India. Interest in a large number of traditional natural products has increased. It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumoral and antimicrobial agents due to the presence of these secondary metabolites [24].

Table 1: Extractive values of different crude seed extracts of *S. trilobatum*

Solvents	Extractive values (%w/w)
Acetone	10.5
Petroleum ether	8.7
Chloroform	9.2

Table 2: Phytochemical screening of different crude seed extracts of *S. trilobatum*

Phytochemicals	Acetone	Petroleum ether	Chloroform
Alkaloids	++++	+++	-
Flavonoids	+++	++	++
Steroids	++	+	-
Terpenoids	+	++	-
Tannins	+++	++	+
Saponins	++	+	++

+ Present; - Absent; + Less; ++ Moderate; +++ High; ++++ Very high

The antibacterial activity of seed extracts of *S. trilobatum* was assayed under *in vitro* conditions by agar well diffusion method against the selected dental pathogens. The inhibition of microbial growth by various solvent extracts was summarized in Table 3. All the solvent extracts, acetone, petroleum ether and chloroform chosen for the extraction process showed significant antibacterial activity. Of all the solvent extracts acetone extract showed highest zones of inhibition on the selected dental pathogens. The highest zone of inhibition was recorded with *S. salivarius* (23 mm) in acetone extract and lowest zone was noticed against *S. sanguinis* (9 mm) in chloroform. The results are in agreement with the previous work done by [25-28]. This suggests that the antibacterial activity of these extracts was due to the presence of bioactive compounds (secondary metabolites) and the organic solvents are better medium of choice to solubilize different phytochemicals present in the plant parts ([26, 29]).

Table 3: Anti-bacterial activity of *S. trilobatum* against dental pathogens

Dental pathogens	Diameter of zones of inhibition (mm)		
	Acetone	Petroleum ether	Chloroform
<i>Staphylococcus aureus</i> ,	22	18	13
<i>Streptococcus mutans</i> ,	19	16	11
<i>Streptococcus salivarius</i> ,	23	20	17
<i>Streptococcus sanguinis</i>	18	14	9
<i>Lactobacillus acidophilus</i>	20	18	10

*Values are means of three replicates, Bore diameter is 6 mm

CONCLUSION

Since all the tested extracts of *S. trilobatum* were highly effective against two of the tested dental pathogens causing bacteria, purification and toxicological studies of the plant and *in vivo* trials should be carried out so that it can be used as a potential source for the development of a phytomedicine to act against dental caries causing bacteria. The antimicrobial activities can be enhanced if the phytoactive components are purified and adequate dosage determined for proper administration. As the global scenario is now changing towards the use of nontoxic plant products having traditional medicinal use, development of modern drugs from *S. trilobatum* should be emphasized for the control of dental pathogens.

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