



Different extractions and their impact on bioactive potentials of leaf extracts of *Solanum trilobatum* against oral pathogens

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ABSTRACT

Aim: The enormous medicinal value of *Solanum trilobatum* was utilised in indigenous medicine. Diverse portions of *S. trilobatum*, like leaves, flowers, stems and fruits were utilised for the treatment purposes. The diverse secondary phytochemicals like phenolics, flavonoids, alkaloids, terpenoids, glycosides, etc. are rich in the *S. trilobatum* attributes numerous health beneficial activities like antimicrobial, anticancer, antioxidant and antidiabetic properties. Here, we evaluated the influence of the extraction methods on the bioactive properties of *S. trilobatum*. **Materials and methods:** The leaf tissues of *S. trilobatum* were extracted with aqueous (water) and ethanol, which were further examined for the extraction yield and antibacterial, antioxidant and *in vitro* biocompatibility studies. **Results:** Results showed an enhanced extraction yield in ethanolic extract compared to aqueous, which also corresponds with enhanced antioxidant and antibacterial activity. Water and ethanolic extracts of *S. trilobatum* revealed antimicrobial activities against the dental pathogens like *Staphylococcus aureus*, *Streptococcus mutans*, *Escherichia coli* and *Pseudomonas aeruginosa*. *S. trilobatum* extract has no adverse toxicity on hemolytic and zebrafish embryo toxicity analyses. **Conclusion:** The ethanolic extracts of *S. trilobatum* shown to have improved biological performance compared to the water extract. **Clinical significance:** Therefore, the utilisation of *S. trilobatum* extracts could be easy in controlling oral and clinical-related pathogens in clinical employment. In addition, an exhaustive mechanism of action of *S. trilobatum* extract in pathogenic growth repression at a molecular level is essential to utilise its application in disease control.

Keywords: *S. trilobatum*, antibacterial, cytotoxicity, antioxidant, well-being, extraction.

INTRODUCTION

Solanum trilobatum Linn. (Solanaceae) is a well-known shrub known as 'Tuduvelai' that is found in a number of Asian nations, comprising India, Sri Lanka, Malaysia, Indonesia, and Singapore.1 (Nadkarni, 1976). In Ayurvedic and Siddha medicine, the roots and leaves are used to treat a variety of respiratory tract conditions, such as acute and chronic bronchitis, sinusitis, asthma, tonsillitis, common colds, coughs, and pulmonary illness (Govindan et al., 2004, Kiritikar & Basu, 1999; Sastri, 1972; Pandurangan et al., 2009). The leaf tissues are primarily consumed for most of the conditions, like spermatorrhoea, ear infections, TB, and dyspepsia (Mohan et al., 1998).



Numerous studies have been conducted on *S. trilobatum's* antioxidant (Sini & Devi, 2004), antidiabetic (Ahmed et al., 2016), antimicrobial (Nagarajan et al., 2009), anticancer (Mohanan et al., 1998; Shahjahan et al., 2005; Venkatesan et al., 2008), anti-inflammatory (Emmanuel et al., 2006; Pandurangan et al., 2008), antinociceptive (Pandurangan et al., 2010), and mosquitocidal properties (Premalatha et al., 2013). *S. trilobatum* has long been used for its alleged medicinal features in natural medicine. The employment of various extraction methods and solvents greatly affects the bioactive extractions and their biological functions. The effect of different solvents during extraction varies depending on the plant and the type of phytochemical needed to be extracted. Hot aqueous extract is shown to contain a high amount of flavonoids in calamondin (*Citrus mitis* Blanco) (Lou et al., 2014), whereas in *Moringa oleifera*, ethanolic extract is said to contain the highest flavonoid content compared to other solvent extracts like methanolic and aqueous extracts (Nobossé et al., 2018). The nanomaterials synthesised using plant extracts showed better biological activities. The selenium nanoparticles synthesised using *S. trilobatum* plant extracts exhibit potent antioxidant activities (Ashritha et al. 2020). The green-synthesised silver nanoparticles using the aqueous extracts of *S. trilobatum* also showed good antibacterial efficacy (Sakthiraj, 2024).

In order to ascertain the bioactive potential of *S. trilobatum*, we have utilised two commonly employed solvents, like aqueous and ethanol, for phytochemical extraction. The extraction yield, antibacterial, and antioxidant potentials of the resulting extracts were assessed. Additionally, the plant extracts' biocompatibility and cytotoxicity were evaluated using hemolytic and zebrafish embryo cytotoxicity tests, which may assist in identifying their potential use in clinical trials and drug development.

MATERIALS AND METHODS

Plant sample extractions

Liquid nitrogen was used to crush *S. trilobatum* leaf samples into a fine powder after they were obtained and rinsed with sterile distilled water. One gram of plant leaf powder was mixed with 10 mL of distilled water (aqueous) and ethanol before agitating at 120 rpm for 2 h. The extracts were further centrifuged at 2000 rpm to separate the contents using Whatman Grade 1 filter paper. After that, the filtrates were gathered and stored at -20°C until needed again.

Percentage Recovery

The plant extract yield percentage was analysed in the aqueous and ethanolic extracts of *S. trilobatum* leaf samples.

Using the following formula, the yield percentage was evaluated: extraction yield (%) = (weight of extract (g)/weight of plant sample (g)) x 100.

DPPH (2,2-Diphenyl-1-Picrylhydrazyl) activity



The DPPH assay, as stated by Brand-Williams et al. (1995), was used to determine the *S. trilobatum* leaf samples' scavenging potential. The scavenging activity (%) is equal to $((A_0 - A_s)/A_0)$ times 100.

The absorbance of the *S. trilobatum* leaf extract is A_s , and the optical density (OD) value of the control is A_0 .

Assessment of *In Vitro* Toxicity

In vitro toxicity of *S. trilobatum* leaf samples was determined using zebrafish eggs. Zebrafish eggs were exposed to *S. trilobatum* leaf samples at different concentrations for a predetermined amount of time, and the results were compared to untreated embryos. In accordance with the OECD-203 criteria, the toxicity of *S. trilobatum* leaf samples in Hank's solution was evaluated against the selected zebrafish embryos at different times (24, 48, 72, 96, and 120 hours). Subsequently, the eggs were moved to a different well so that the head, tail, and eyes could develop. This process was observed under a 40X microscope.

Antibacterial susceptibility examination

The Kirby-Bauer disc diffusion assay is used to test the antibacterial efficacy of the plant extract against common oral bacterial pathogens, including Gram (+)ve (*Streptococcus mutans* and *Staphylococcus aureus*) and Gram (-)ve (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria (Bauer et al., 1966). The cultures were spread out on Muller-Hinton agar plates for the susceptibility test. For Gram (+)ve and Gram (-)ve bacterial cells, amoxicillin and tetracycline were used as standard drugs respectively. The corresponding standard antibiotic, ethanolic, and aqueous plant extract were added to the plates featuring 5-mm wells. The results were listed as inhibitory zones in millimeters, and all plates were kept for 24 h at 37°C.

Hemolytic Activity Test

The hemolytic characteristic of *S. trilobatum* leaf samples was evaluated using the hemolysis test. To separate the erythrocytes from the plasma, 10 mL of collected blood was placed in vacutainers with EDTA and spun at 1500 rpm for 15 min at 25 °C. Then, it was washed three times with 10 mL of phosphate-buffered saline (PBS, pH 7.4). After shielding them from light, the erythrocyte suspensions were combined with diluted plant extracts in 2 mL microfuge tubes at concentrations ranging from 100 µg/mL to 1000 µg/mL at 37 °C for approximately half an hour (Eppendorf SE, Hamburg, Germany). The half-maximal inhibitory concentration (IC₅₀) rates were calculated as the sample concentration required to hemolyze 50% of human RBCs. The hemolysis activity was assessed by measuring the ODs at 540 nm. The proportion of hemolysis percentage was measured with the given formula: $(OD_{\text{treatment}} - OD_{\text{negative control}}) / (OD_{\text{positive control}} - OD_{\text{negative control}}) \times 100$.

Statistical analysis

The values were expressed as the standard deviation plus the means of the triplicate samples (n = 3). Following that, the data was assessed using IBM SPSS Statistics for Windows, Version 21



(released 2012; IBM Corp., Armonk, New York, United States), a statistical package program, and Duncan's multiple range test ($p < 0.05$).

RESULTS

Extraction yield

The extraction yield percentage was analysed in the aqueous and ethanolic extracts of *S. trilobatum* leaf samples. The results implied that ethanolic extract (25.87%) had more extraction yield compared to the aqueous extract (21.26%) (Fig. 1).

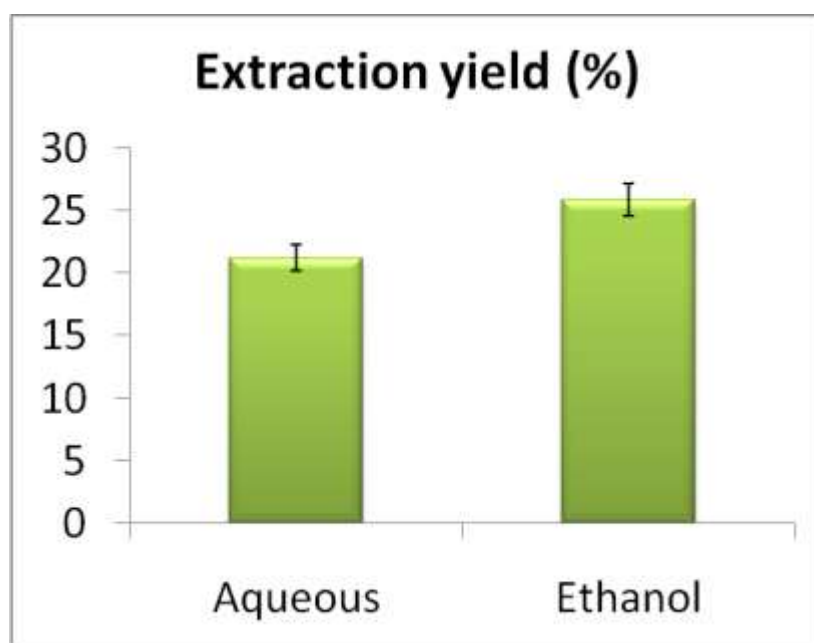


Fig. 1. Extraction yield percentage in the aqueous and ethanolic leaf extracts of *S. trilobatum*

Antioxidant capacity of *S. trilobatum* extracts

DPPH radical scavenging analysis was employed to examine the antioxidant efficiency of different extracts of *S. trilobatum*. Ascorbic acid was used as a standard. The concentration-dependent antioxidant efficiency was noticed in all the treatments (Fig. 2). Antioxidant status was found to be superior in the ethanolic extract of *S. trilobatum* than in the aqueous extract. Further, the radical scavenging activity of the *S. trilobatum* ethanolic extract (6.391%) was similar to the standard ascorbic acid (6.452%) (Fig. 2).

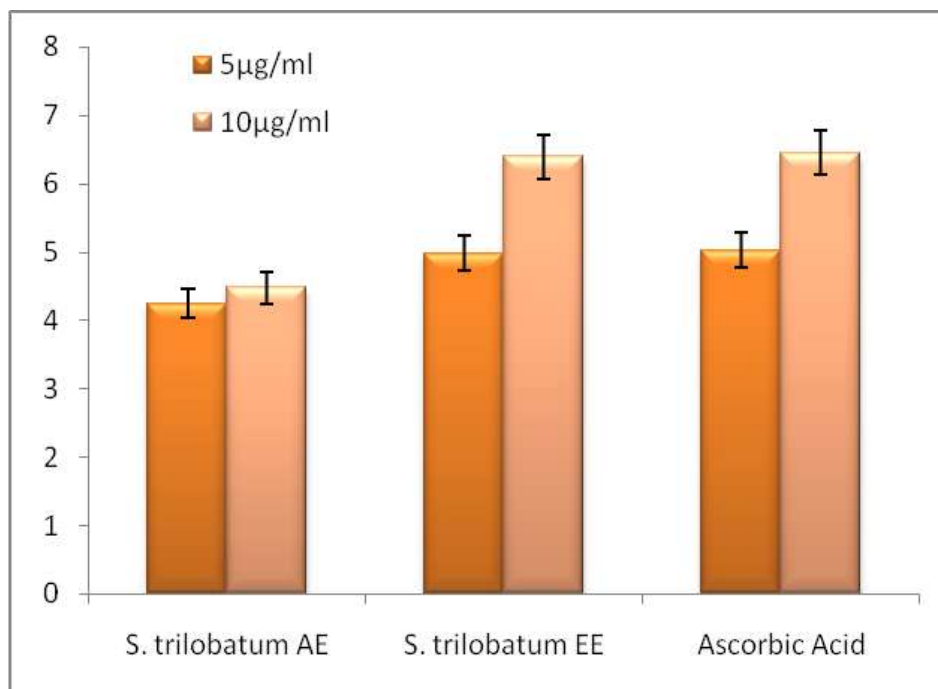


Fig. 2. Antioxidant potential of the aqueous and ethanolic leaf extracts of *S. trilobatum* using the DPPH assay

Antibacterial efficacy of *S. trilobatum* extracts

The *S. trilobatum* was shown to possess differential extraction yield percentages and increased antioxidant activities, and hence, the antimicrobial properties of the diverse extracts of *S. trilobatum* were studied against the dental biofilm-producing pathogens, namely *S. aureus*, *S. mutans*, *P. aeruginosa*, and *E. coli*. The ethanolic extracts of *S. trilobatum* were comparatively more potent against gram-positive and gram-negative pathogens than positive control drugs and aqueous extracts. Ethanolic extract of *S. trilobatum* was more effective against *S. aureus* (17 mm) and *E. coli* (18 mm) (Fig. 3).

| S.No | Bacterial sps (G +ve) | Amoxicillin | Aqueous Extract | Ethanol extract |
|------|-------------------------------|--------------------------|-----------------|-----------------|
| | | Zone of inhibition in mm | | |
| 1 | <i>Staphyloccous aureus</i> | 10 | 10 | 17 |
| 2 | <i>Streptococcus mutans</i> | 10 | 13 | 16 |
| S.No | Bacterial sps (G-ve) | Tetracycline | Aqueous Extract | Ethanol extract |
| 1 | <i>Pseudomonas aeruginosa</i> | 13 | 12 | 16 |



| | | | | |
|---|-------------------------|----|----|----|
| 2 | <i>Escherichia coli</i> | 11 | 12 | 18 |
|---|-------------------------|----|----|----|

Table 1: Antibacterial efficacy of the different *S. trilobatum* leaf extracts against gram-positive and gram-negative bacterial species.

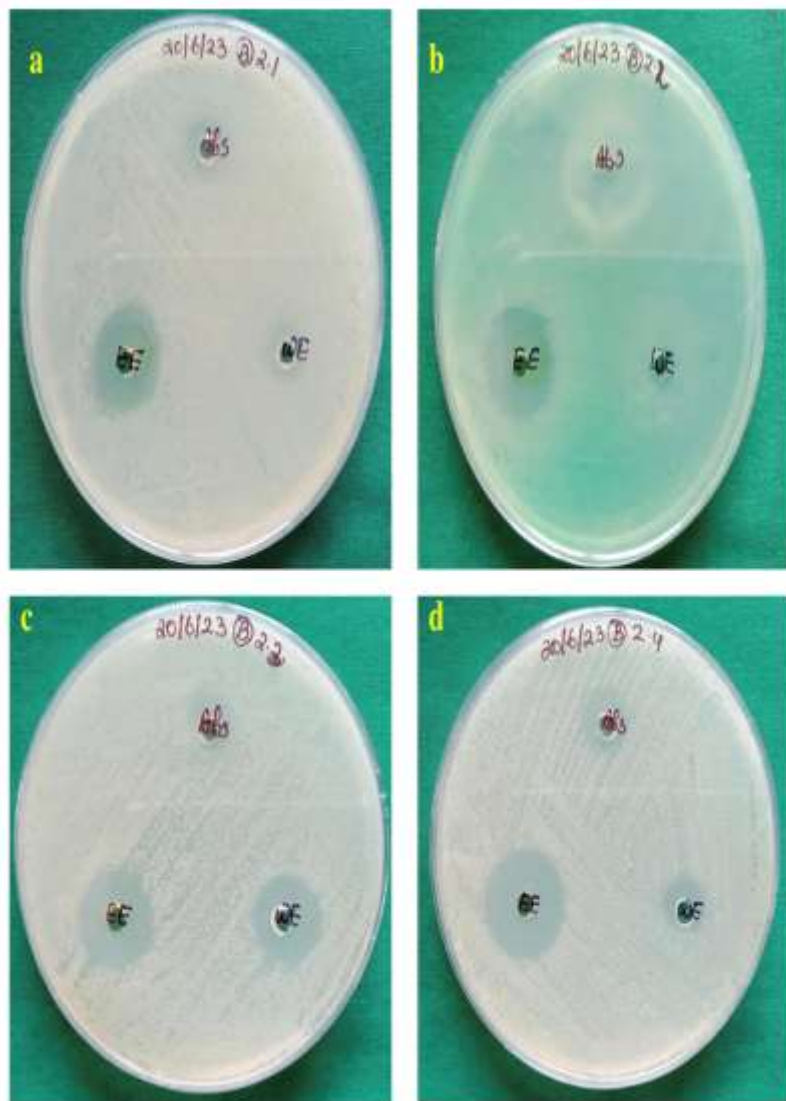


Fig. 3. Antibacterial activity of the different *S. trilobatum* leaf extracts against gram-positive (a. *S. aureus*, c. *S. mutans*) and gram-negative (b. *P. aeruginosa*, d. *E. coli*) bacterial species.

Impact of ethanolic extract of *S. trilobatum* on zebrafish embryos



Ethanollic extract revealed higher extraction yield and antioxidant and antimicrobial activity, and to check their influence on toxicity in biological systems, we utilised the zebrafish embryo toxicity assay. The toxic properties of the ethanollic extract of *S. trilobatum* were studied using a zebrafish embryo toxicity assay at different time intervals from 24 hrs to 120 hrs. There were no obvious toxic actions of ethanollic extract observed, which is similar to the control administered with distilled water. The lethal rate of embryos applied with ethanollic *S. trilobatum* extract was slightly increased depending on the time intervals, which is also similar to control embryos (Table 2).

| Time in hrs | Control (Mortality %) | Sample (Mortality %) |
|-------------|-----------------------|----------------------|
| 24h | 0.45 | 0.54 |
| 48h | 0.65 | 0.67 |
| 72h | 0.68 | 0.78 |
| 96 h | 0.97 | 1.24 |
| 120 h | 1.03 | 1.34 |

Table 2: Impact of ethanollic extract of *S. trilobatum* against the zebrafish embryo's mortality. The numbers indicate the % of dead embryos after being administered with samples.

Hemolytic Activity

Similarly, the impact of ethanollic *S. trilobatum* extract on red blood cells was studied by a hemolytic activity test. Higher hemolytic activity was observed in distilled water, whereas negligible hemolytic activity was found in the ethanollic extract of *S. trilobatum* (0.76%). The results of hemolytic activity stated that *S. trilobatum* extract did not show hemolytic activity, which suggests that *S. trilobatum* extract is not having any adverse effect and is considered safe (Table 2).

| Samples | OD values | SE |
|----------------------|-----------|-------|
| Positive control | 1.39 | 1.71 |
| <i>S. trilobatum</i> | 0.05 | 1.691 |

Table. 3. The impact of *S. trilobatum* extract on RBCs using a Hemolytic assay.

DISCUSSION

Natural sources, especially plants, have the potential to be sources of natural drugs which could contribute various health benefits. Solanaceae, sometimes called Alarka in Sanskrit, is the family



to which *Solanum trilobatum* belongs. Numerous studies provide evidence of this plant's varied characteristics. *S. trilobatum* protects mice from radiation-induced toxicity and damage (Mohan and Devi, 1998). It also has hepatoprotective and antioxidant properties (Shahjahan et al., 2004; Shahjahan et al., 2005). It was discovered that *S. trilobatum* had anti-inflammatory and antidiabetic and antibacterial properties (Emmanuel et al., 2006; Ahmed et al., 2016; Swapna Latha and Kannabiran, 2024).

The exploration of *Solanum trilobatum's* bio-potential against microbial and oxidative stress issues through the lens of differential extraction techniques opens avenues for understanding and utilising the therapeutic properties of this traditional medicinal plant (Swapna Latha and Kannabiran, 2024).

The extraction of phyto-constituents can be increased with diverse extraction approaches like steam distillation, maceration, and use of different solvents from *S. trilobatum*. The choice of extraction approach can greatly affect the nature and level of extracted phytochemicals, impacting bioactive potential of the plant (Sun et al. 2025). *S. trilobatum's* historical use in traditional medicine for combating microbial infections is substantiated by contemporary scientific research. Megala et al. (2025) examined the bioactives and antimicrobial features of various solvent extracts of diverse tissues of *Solanum trilobatum*. Their results indicated that among the various solvent extractions, methanolic extracts were found to have more antimicrobial activities. Moreover, silver nanoparticles synthesised using the extracts of *Plectranthus amboinicus* and *S. trilobatum* exhibited enhanced antibacterial activities against several human pathogens (Mohanaparameswari et al. 2024). Similarly, in our study we found that the ethanolic extract possesses improved antimicrobial properties compared to the water extracts of *S. trilobatum*. The bioactive potentials of the plants directly correspond to the phytochemicals present in the plants. It was also shown that the ethanolic extracts of *S. trilobatum* comprise the numerous bioactive constituents (Senthilkumar, 2018). In line with this study, we observed that the enhanced extraction yield, antioxidant and antibacterial activities in the ethanolic extracts of *S. trilobatum* might be due to the availability of diverse bioactive compounds. A previous study conducted by Hema and Sathya Savithri (2022) demonstrated that the *S. trilobatum* extracts possess phyto-compounds like flavonoids, alkaloids, terpenoids and phenolic compounds which are also responsible for their significant DPPH radical scavenging efficiency and improved antibacterial functions. The silver nanoparticles synthesised using *S. trilobatum* extracts are attributed to the positive effects of the membrane stability on human RBCs (Manimegalai et al. 2021). Previous studies showed that the *Solanum trilobatum*-derived compound sobatum did not produce toxic activities or mortality in the mice (Mohan and Devi, 1998). Similarly, in our study the *S. trilobatum* extracts did not show any toxic impacts on the hemolytic assays. The superior antibacterial and antioxidant properties of the *S. trilobatum* leaf extracts could suggest that it could be used as a candidate for integrative natural medicine in addressing the clinical issues.

CONCLUSION

The diverse parts of Solanum trilobatum were routinely employed in natural remedies for different treatment applications. The biological activities of the plants directly correspond to the bioactive substances and are greatly influenced by various extraction methods and the solvents used for the extractions. Here, we noticed that ethanolic extracts of *S. trilobatum* exhibited enhanced extraction yield and antioxidant status compared to the aqueous extracts. The improved extraction yield and



antioxidant status in ethanolic extracts also correspond to the increased antibacterial activities. The adverse impacts of *S.trilobatum* extracts was studied using hemolysis and zebrafish embryos, indicating that they did not show any toxic actions, which suggests that they could be used as an alternative to synthetic drugs. However, a comprehensive biochemical and molecular analysis should be performed to assess their chemopreventive features.

REFERENCES