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A comparative antioxidant, antimicrobial, and biocompatible potential of various extracts of *Solanum virginianum*

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Abstract
Oral health challenges, such as microbial resistance, biofilm formation, and oxidative stress, necessitate innovative therapeutic solutions in dental care. This study evaluates the extraction yield, antioxidant, antimicrobial, and biocompatibility potential of Solanum virginianum extracts using aqueous and ethanolic solvents. The extraction yield was noticed to be higher in the ethanolic solvent than in the water extraction. The antioxidant potential was assessed using the DPPH assay, where ethanolic extracts demonstrated superior radical scavenging activity than the aqueous extracts. Antimicrobial efficacy was evaluated against important oral pathogens, *Streptococcus mutans, Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa,* using agar well diffusion methods. The outcome of the antibacterial assay showed that ethanolic extracts revealed enhanced antibacterial activity against these pathogens compared with the water extract. The highest antibacterial activity was observed against *Pseudomonas aeruginosa* (18 mm), followed by *S. mutans, S. aureus,* and *E. coli.* Further, biocompatibility of the extracts was studied by analyzing the toxicity in zebrafish embryos and *in vitro* hemolytic assays, which also implies that they did not impose any toxicity, suggesting their safe usage in therapeutic applications. This study highlights *S. virginianum,* particularly ethanolic extracts, as a promising natural resource for oral care and clinical applications. Their robust antioxidant and antimicrobial properties, combined with excellent biocompatibility, may position them as viable alternatives to conventional and synthetic drugs. Future research should focus on clinical trials and formulation development to fully harness their therapeutic potential in therapeutic interventions.

Keywords: *Solanum Virginianum*, aqueous extract, ethanolic extract, anti-bacterial, anti-oxidant, toxicity

1. Introduction

Oral health is a critical component of overall well-being, yet it faces numerous challenges (L et al., 2024), including microbial resistance, oxidative stress, and biofilm formation. These issues complicate oral disease management, particularly in conditions requiring surgical interventions. For instance, microbial resistance to conventional antibiotics and oxidative stress-induced tissue damage during surgery pose significant challenges in recovery and infection control (Website, n.d.). Traditional antimicrobial agents, while effective, often have limitations such as side effects, resistance development, and inadequate efficacy against biofilm-forming oral pathogens like *Streptococcus mutans* (L et al., 2024). As a result, there is a growing interest in exploring natural alternatives, particularly plant-based compounds, that can offer enhanced efficacy with minimal adverse effects.

Solanum virginianum, a medicinal plant from the Solanaceae family, has gained attention for its diverse therapeutic properties. Previous studies have highlighted the antimicrobial, antioxidant, and anti-inflammatory potential of plants from this family, making them attractive candidates for addressing oral health issues (Aksoy et al., 2007). The rich phytochemical composition of *S. virginianum*, including alkaloids, flavonoids, tannins, and phenols, is known to contribute to its bioactivity (Abbas et al., 2014; Al-Huqail et al., 2024). These compounds are well-documented for their roles in neutralizing oxidative stress, a key factor in surgical recovery and oral tissue health (Forssten et al., 2010; Patel et al., 2019). Oxidative stress occurs when the balance between reactive oxygen species (ROS) and antioxidant defenses is disrupted, leading to tissue damage and delayed healing. Antioxidants play a crucial role in mitigating these effects by scavenging free radicals and reducing oxidative damage (Prashith Kekuda et al., 2017). For oral surgery patients, enhanced antioxidant protection can improve outcomes by supporting tissue repair and reducing inflammatory responses. (Pavithra et al., 2023; Shenoy et al., 2023; Thomas & Jain, 2023) Studies have demonstrated that plant-derived antioxidants, such as those in *S. virginianum*, offer strong free radical scavenging activity, particularly when extracted using methanol, which efficiently isolates phenolic and flavonoid compounds (Habeeb Rahuman et al., 2022).

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The management of microbial infections is another crucial component of oral health care. Pathogens such as *S. mutans*, *E. coli,* and *Staphylococcus aureus* are common culprits in oral infections, including dental caries, biofilm formation, and surgical site infections (Chung et al., 2025). Biofilm formation by these pathogens exacerbates their resistance to conventional treatments, necessitating the exploration of alternative antimicrobial agents. (Ramsundar et al., 2023; Rieshy et al., 2023; S. Singh et al., 2023) Recent findings suggest that *S. virginianum* extracts exhibit significant antimicrobial activity against microbial pathogens, particularly when extracted with methanol, which enhances the concentration of active phytochemicals (Rajaselvam et al., 2023; Selvam, 2022). Biocompatibility is another essential criterion for integrating natural compounds into clinical practice. Dental treatments require materials and agents that do not harm surrounding tissues or interfere with healing. Biocompatibility testing of *S. virginianum* extracts has shown minimal cytotoxicity and excellent compatibility with human oral fibroblast cells, making them suitable for therapeutic applications (An & Zhao, 2018)

This study aims to evaluate the comparative antioxidant, antimicrobial, and biocompatibility potential of aqueous and ethanolic extracts of *S. virginianum*. By leveraging the therapeutic properties of *S. virginianum*, this study may provide a foundation for the development of innovative, safe, and effective solutions to address the challenges of modern dental and clinical care. Future research directions include clinical trials and the development of therapeutic products to fully harness the potential of this promising medicinal plant.

2. Materials and Methods

Plant Material Collection and Extraction

Solanum virginianum leaves were collected, identified, and confirmed by a qualified botanist. Plant parts were air-dried and ground into powder with liquid nitrogen. Approximately 1g of S. virginianum leaf tissues were weighed and ground with 10 mL of distilled water (aqueous) and ethanol separately before being agitated for two hours at 120 rpm. The extracts were further centrifuged at 2000 rpm to separate the contents using Whatman Grade 1 filter paper (Cytiva, Marlborough, Massachusetts, USA). After that, the filtrates were gathered and stored at -20°C until needed again.

Extraction yield

The following formula was used to calculate the yield percentage of plant extract in aqueous and ethanolic extracts of S. virginianum leaf samples.
The extraction yield (%) = (weight of extract (g)/weight of plant sample (g)) x 100.

Antioxidant Activity

There are different antioxidant methods commonly used to evaluate the antioxidant potential of the plant extracts. Among these, DPPH-based free radical scavenging activity was routinely used to analyse the antioxidant activity of the plant extracts. DPPH was measured based on the % inhibition of DPPH radicals as previously described. The scavenging activity (%) is equal to ((Ao - As)/Ao) times 100.
The absorbance of the *S. virginianum* leaf extract is As, and the optical density (OD) value of the control is Ao.

Antimicrobial Activity

The Gram-positive and Gram-negative bacterial pathogens, like *Streptococcus mutans*, *E. coli*, and *Staphylococcus aureus,* were obtained from microbial type culture collection (IMTECH, Chandigarh, India) repositories. The Kirby-Bauer disc diffusion method is used to test the bacterial susceptibility towards plant extracts. Bacterial cultures were spread out on Muller-Hinton agar plates for the susceptibility test. Amoxicillin and tetracycline were used as standard drugs for Gram-positive and Gram-negative bacterial cells, respectively. The corresponding standard antibiotic, ethanolic, and aqueous plant extracts were added to the plates featuring 5-mm wells. The results were recorded as inhibitory zones in millimeters, and all plates were kept for 24 hrs at 37°C.

Haemolytic assay

The hemolytic nature of *S. virginianum* leaf samples was evaluated using the hemolysis test. To separate the erythrocytes from the plasma, ten milliliters of collected blood were placed in venipuncture tubes with ethylenediaminetetraacetic acid (EDTA) and centrifuged at 1500 rpm for 15 minutes at 25°C. Then, it was washed three times with 10 mL of phosphate-buffered saline (PBS), which has a pH of 7.4. After shielding them from light, the erythrocyte suspensions were combined with diluted plant extracts in two mL Eppendorf 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) values were calculated as the sample concentration required to hemolyze 50% of human RBCs. The hemolysis activity was determined by measuring the ODs at 540 nm. The proportion of hemolysis was calculated using the following formula: hemolysis percentage is calculated as follows: OD treatment - OD negative control - OD positive control - OD negative control x 100.

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Statistical analysis
The values were expressed as the standard deviation plus the means of the triple examinations of the 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 ($p < 0.05$).

3. Results

Extraction yield and Antioxidant Potential
The percentage of extraction yield was examined in the leaf of *Solanum virginianum* extracted with aqueous and ethanolic solvents, and the results showed that the yield was higher in the ethanolic extract (24.91%) compared to the aqueous extract (20.76%) (Figure 1). The comparative antioxidant potential of aqueous and ethanolic extracts of *S. virginianum* was examined using the DPPH assay, and the results indicate that the ethanolic extracts reveal higher radical scavenging activity than the water extract (Figure 2). Furthermore, the antioxidant potential of ethanolic extracts was found to be closely related to that of ascorbic acid. Higher concentrations (10 µg/mL) demonstrated increased antioxidant activity.

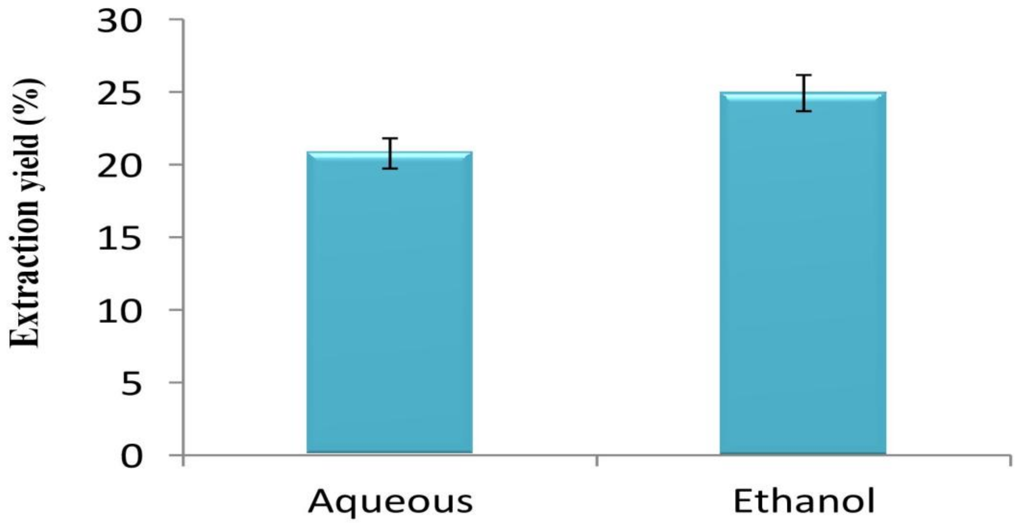

Figure 1. The percentage yield extraction of *Solanum virginianum* extracted with aqueous and ethanolic solvents.

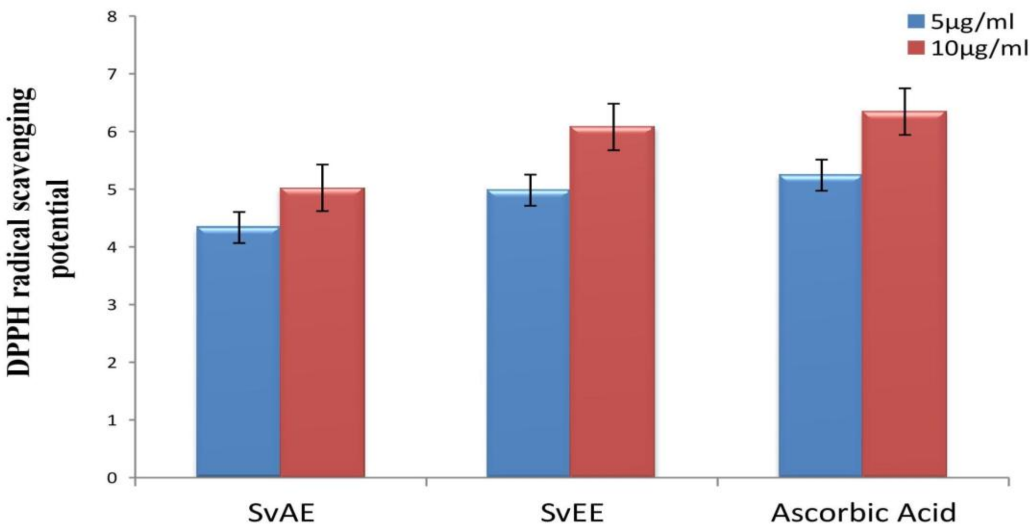

Figure 2. The DPPH antioxidant activity of the aqueous and ethanolic extracts of *Solanum virginianum* and the positive control, ascorbic acid, at 5 and 10 µg/mL concentrations.

Antimicrobial Activity
The antibacterial potentials of ethanolic and aqueous extracts of *Solanum virginianum* showed that the ethanolic extracts exhibited higher antimicrobial activity towards both gram-positive and gram-negative bacterial pathogens. Ethanolic extracts showed enhanced antibacterial efficacy compared to the aqueous extracts. Further, their antimicrobial efficacy is comparable to that of the standard antibiotics. These results illustrate that ethanolic extracts of *Solanum virginianum* could

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be suitable to use as antimicrobial drugs (Fig. 3 & Table 1).

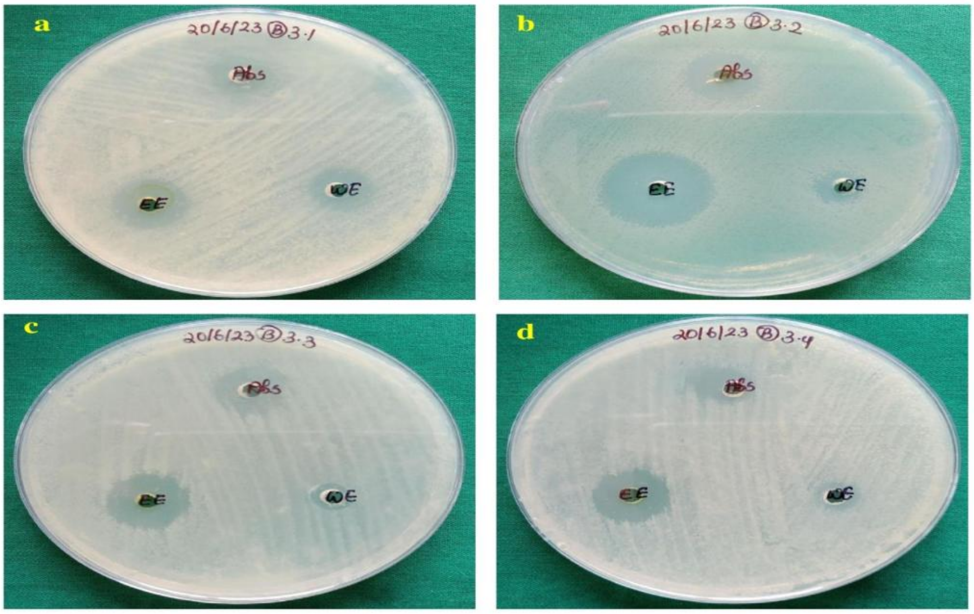


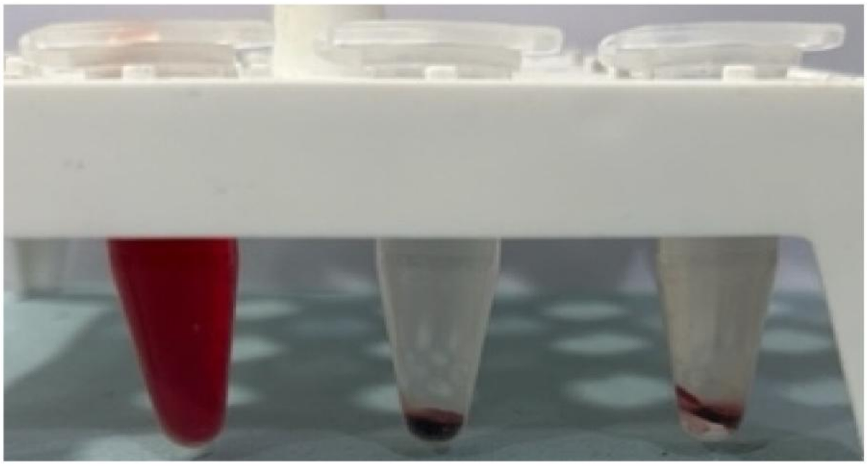
Fig. 3. Antimicrobial potential of the aqueous and ethanolic extracts of *S. virginianum* against a) *Staphylococcus aureus,* b. *Pseudomonas aeruginosa,* c. *Streptococcus mutans* and d. *Escherichia coli.*

S.No	Microorganisms (G+ve)	Amoxicillin	*S. virginianum* Aqueous Extract	*S. virginianum* Ethanol extract
1	*Staphylococcus aureus*	13	11	15
2	*Streptococcus mutans*	13	10	16
S.No	Microorganisms (G-ve)	Tetracycline	Aqueous Extract	Ethanol extract
1	*Pseudomonas aeruginosa*	13	11	18
2	*Escherichia coli*	11	10	14

Table 1. Antibacterial activity of aqueous and ethanolic extracts of *S. virginianum* against Gram-positive and Gram-negative bacterial pathogens.

Hemolytic assay

The biocompatibility of the *S. virginianum* extracts was determined using a hemolytic assay. The results implied that the plant extracts did not show any hemolytic activity, which suggests the safety and the biocompatibility of the *S. virginianum* extracts (Table 2).



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Samples	OD values	SE
Positive control	1.39	1.69
Solanum virginianum	0.052	1.631

Table 2. The table shows the RBC hemolytic pattern of the *S. virginianum* extracts and the positive control.

***In vitro* toxicity analysis using zebrafish embryos**

The adverse toxic effects of *S. virginianum* extracts were determined using a zebrafish embryo toxicity assay. The ethanolic extracts of *S. virginianum* leaf were studied at different time intervals (24 h to 120 h), and the results showed that no obvious or significant toxicity was noticed. The numbers indicate the % of dead embryos after treatment with samples (Table 3). The least toxic effects on zebrafish embryos suggest the safe utilization of *S. virginianum* in possible drug development.

Time in hours	Control (Mortality %)	Sample (Mortality %)
24 h	0.12	0.23
48 h	0.34	0.65
72 h	0.42	0.76
96 h	0.57	0.89
120 h	0.87	1.21

Table 3. Impact of ethanolic extract of *S. trilobatum* on the zebrafish embryo's mortality.

4. Discussion

The findings of this study highlight the promising potential of *S. virginianum* extracts, particularly ethanolic extracts, as effective agents for addressing oral health challenges. The superior antioxidant, antimicrobial, and biocompatibility profiles of these extracts make them viable candidates for modern dental applications.

The antioxidant properties of *S. virginianum* extracts were validated through the DPPH assay, which demonstrated the highest radical scavenging activity. This aligns with earlier findings that flavonoids and phenols play a critical role in neutralizing free radicals (Forssten et al., 2010; T. A. Singh et al., 2024). The significant difference between the IC50 values of ethanolic extracts and other solvents underscores the efficiency of ethanol in extracting potent antioxidants. These results are consistent with N. Abbas et al. (2025), which showed that solvent type impacts the antioxidant efficacy of plant extracts, with ethanol often yielding superior outcomes. (Doshi et al., 2023; Lampl et al., 2023; Pandiyan et al., 2023) The antimicrobial activity of the extracts against *S. mutans, E. coli,* and *S. aureus* further supports their application in oral and health care systems. The ethanolic extracts of *S. virginianum* exhibited the largest zones of inhibition, which is comparable with the standard drugs, signifying their potent antimicrobial properties. These findings corroborate the work of Selvam (2023), who observed similar efficacy of *S. virginianum* extracts against oral pathogens. The ability of ethanolic extracts to inhibit *S. mutans*, a primary causative agent of dental caries, emphasizes the need for effective alternatives to combat biofilm-forming pathogens in oral environments. (Janani et al., 2021; Kachhara et al., 2021; Subramanian et al., 2023) The comparative analysis underscores the superior performance of methanolic extracts across all parameters. The combined antioxidant and antimicrobial activities of these extracts are attributed to their rich phytochemical composition, particularly the high levels of flavonoids and phenols. (Gandhi et al., 2021; Katyal et al., 2023; Priyadharshini et al., 2023) These compounds have been extensively documented for their ability to disrupt microbial membranes and neutralize oxidative damage (Habeeb Rahuman et al., 2022; Jones et al., 2025). The aqueous and ethanolic extracts, while effective, demonstrated moderate activity, indicating that solvent choice is a critical factor in optimizing extract efficacy. This study also contributes to the growing body of evidence supporting the integration of herbal remedies into dental care. (Chokkattu et al., 2023; Dharman et al., 2023; Govindaraj & Shanmugam, 2023) Herbal extracts, like those from *S. virginianum*, offer a natural alternative to synthetic antimicrobials and antioxidants, with reduced risk of side effects. As highlighted by Kepp et al. (2024), the global burden of oral diseases necessitates the exploration of sustainable and accessible treatments. (Rajeshkumar & Lakshmi, 2021; Sivakumar et al., 2021) The current findings align with previous research suggesting that plants from the Solanaceae family exhibit significant bioactivity, as reported by H. Abbas (2014) and Modzelewski et al. (2025). The robust antioxidant, antimicrobial, and biocompatibility profiles of *S. virginianum* extracts, particularly methanolic, position them as promising candidates for addressing oral health challenges. These findings emphasize the need for further research, including clinical trials and the development of therapeutic formulations. As suggested by Cruz-Cunha & Mateus-Coelho (2020), the formulation of multi-herbal products incorporating *S. virginianum* extracts could enhance their therapeutic efficacy and broaden their applications in modern dentistry. Future studies should also investigate the long-term effects and stability of these extracts in various delivery systems to fully harness their potential (Stankiewicz et al., 2024).

5. Conclusion

This study highlights the immense potential of *Solanum virginianum* extracts, particularly ethanolic, as effective agents for oral and other health applications. Extraction yield was found to be higher in the ethanolic extracts of *S. virginianum.* The antioxidant activity was also higher in the ethanolic extracts than in the aqueous extracts. Further, the antioxidant activity was also comparable with that of the ethanolic extracts. The higher extraction yield and antioxidant activity in the ethanolic extracts also corroborate the enhanced antibacterial efficacy against crucial bacterial pathogens, including *Streptococcus mutans, E. coli,* and *Staphylococcus aureus,* underscoring their potential as natural alternatives to synthetic antimicrobials. Furthermore, these plant extracts were biocompatible in nature and did not show any adverse toxicity. These findings support the extracts' ability to mitigate oxidative stress, a critical factor in oral surgery recovery and overall oral health maintenance. This antimicrobial potential is particularly relevant for addressing microbial resistance and biofilm formation, which are significant challenges in oral healthcare. Future research should focus on clinical trials, exploring delivery mechanisms, and evaluating the long-term stability and efficacy of these extracts in therapeutic formulations. This could pave the way for their broader use in modern dentistry.

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