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Assessment of the Antibacterial Activity of *Spilanthes acmella* Against Bacteria Associated with Dental Caries and Periodontal Disease: An In-vitro Microbiological Study

S. Shivananda[1], Vidya G. Doddawad[2]*, Lipsa Bhuyan[3], Akhil Shetty[4] and V.H. Pushpa[5]

[1]Department of oral and Maxillofacial Surgery, JSS Dental College and Hospital (A Constituent College of JSS Academy of Higher Education & Research), Mysore, Karnataka, India.
[2]Department of Oral Pathology and Microbiology, JSS Dental College and Hospital, (A Constituent College of JSS Academy of Higher Education & Research), Mysore, Karnataka, India.
[3]Department of Oral and Maxillofacial Pathology, Kalinga Institute of Dental Sciences, KIIT Deemed to be University, Patia, Bhubaneswar, Odisha.
[4]Department of Orthodontics and Dentofacial Orthopedics, AB Shetty Memorial Institute of Dental Sciences (ABSMIDS), Nitte (Deemed to be University), Mangalore, Karnataka, India.
[5]Department of Pharmacology, JSS Medical College, JSS Academy of Higher Education and Research Mysuru, Karnataka, India.

***Correspondence:** drvidyagd@gmail.com

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Abstract

Dental caries and periodontal disease are two of the most common oral diseases caused by bacterial infections. Traditional medicine in India has a long history of using plant extracts for dental care. *Spilanthes acmella* (*S. acmella*), also known as the "Toothache Plant," is a medicinal plant that has been traditionally used for its medicinal properties but has not been extensively studied for its applicability and use in dentistry. This study aims to investigate the antimicrobial action of *S. acmella* ethanol extract on *Streptococcus mutans* (*S. mutans*), and *Lactobacillus fermentum* (*L. fermentum*), which causes dental caries, and *Porphyromonas gingivalis* (*P. gingivalis*), *Capnocytophaga gingivalis* (*C. gingivalis*), which causes periodontal infection. The ethanol extract of *S. acmella* in various dilutions of 10mg/ml, 20mg/ml, 40mg/ml, 80mg/ml, and 100mg/ml was tested for its antibacterial activity against the bacteria as mentioned above using the agar well diffusion method. Erythromycin 0.125mg/ml was used as a positive control, whereas distilled water was used as a negative control. The Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) were determined by the broth dilution method. The results of this study have shown that the ethanol extract of *S. acmella* demonstrated concentration-dependent inhibition of bacterial growth (13-16mm diameter), with the highest concentration of 100mg/ml showing the strongest effect. The findings of this study support the use of the *S. acmella* plant extract in the treatment of dental caries and periodontal infection and suggest that it may be a viable alternative to traditional antimicrobial agents.

Keywords: Herbal, Medicine, Traditional, Antibacterial Agent, Dental Caries, Periodontitis

INTRODUCTION

The oral cavity has around 500 different types of bacteria, making it one of the most diverse and complex microbial flora in the human body.[1] Dental caries and periodontal disease are two of the most common diseases of the oral cavity. The most common microorganisms causing dental caries are *S. mutans* and *L. fermentum*. Similarly, periodontal infections are caused by *T. denticola* and *P. gingivalis*. The use of an antimicrobial agent to control the bacteria in tooth plaque that cause caries and periodontitis is essential for dental disease prevention.[2]

India has a long history of using traditional medicine in the field of dentistry. In the modern period, the use of traditional remedies is increasing in newer areas. Plant extracts continue to be a novel source of structurally significant chemicals that lead to the creation of medicines.[3] Plant extract products are safe and their use as a medication is a viable option for the treatment of patients.[4] The increased recognition of plant products as non-narcotic, without side effects, and conveniently available at low rates is driving the demand for medicinal plants in both developing and developed countries.[5] *S. acmella* is renowned for its diverse medicinal attributes, encompassing anti-inflammatory, analgesic, and antimicrobial properties. Extensive research has revealed that the plant harbors a plethora of phytochemicals, such as flavonoids, terpenoids, and alkamides, which are accountable for their therapeutic effects.[6]

S. acmella is an important medicinal plant commonly known as the 'Akarkara plant' with a rich source of therapeutic constituents. It is referred to as the "Toothache Plant".[6-8] The whole plant is claimed to possess medicinal properties. However, little literature exists on its applicability and use in dentistry.[9-11] There are no studies on the efficacy of *S. acmella* on micro-organisms causing dental caries and periodontal infection. For instance, they could address the lack of investigations into the potential synergistic effects of *S. acmella* in combination with traditional dental treatments, or the scarce understanding of its efficacy against specific strains of oral pathogens commonly associated with dental diseases. Additionally, exploring the specific mechanisms of action that make *S. acmella* effective against oral infections could add depth to their research. As a result, the purpose of this research is to investigate the antimicrobial action of *S. acmella* essential oil on *S. mutans*, *L. fermentum*, which causes dental caries, and *P. gingivalis, C. gingivalis,* which causes periodontal infection.

MATERIALS AND METHODS

The present study is an In-vitro microbiological assay that was conducted in the Department of Microbiology, School of Life Sciences, JSS Academy of Higher Education and Research, Mysore. The ethical clearance (JSSDCH IEC no: 86/2021) was obtained priorly from the local ethical committee.

Collection and processing of plant material

The researchers have identified different species within the genus such as *S. oleracea, S. paniculata*, and *S. radicans* based on morphological characteristics. The plant material was collected and then separated into flowers, leaves, and stems.[11]

S. acmella plant leaves were randomly collected from at least 50 healthy plants from Mysuru district, Karnataka, India, and were provided by the Department of Pharmacognosy, JSS College of Pharmacy, Mysuru (Feb to May 2022). The voucher specimen is preserved in our laboratory for future reference. The dried leaves material was then ground into a coarse powder and extracted with different solvents such as 70% v/v of ethanol using a Soxhlet apparatus. The liquid extracts were then filtered and evaporated under reduced pressure at 40°C using a rotary evaporator to get a soft mass for about 24 hours. This extract of *S. acmella* was used to create different dilutions at a ratio of 3:1 (10mg/ml, 20mg/ml, 40mg/ml, 80mg/ml, and 100mg/ml) for further testing.

The type of bacteria used

In this study, specific strains of bacteria were used to assess the antibacterial activity of *S. acmella* plant extracts. Two strains of aerobic bacteria, *Streptococcus mutans* (ATCC 25175) and *Lactobacillus fermentum* (ATCC 14932), which are known to cause dental cavities, were used. Additionally, two strains of anaerobic bacteria, *Porphyromonas gingivalis* (ATCC 33277) and *Capnocytophaga gingivalis* (ATCC 33624), which are associated with periodontal infection, were also used. These strains were obtained from the American Type Culture Collection (ATCC), which is well-established and commonly used as reference strains in research.

Evaluation of antibacterial activity

The well diffusion procedure and Minimum Inhibitory Concentration (MIC) methods were used to determine the antibiotic susceptibility of the test substance. A standardized inoculum was prepared from bacterial culture (Using 0.25 McFarland standards) and spread onto Petri plates (i.e. Muller Hinton Agar supplemented with 5% sheep blood). Wells were made in the Agar plate using a cork borer and different concentrations of the test substance were added to each well (0.1ml). All the plates were kept in a refrigerator at 2 to 8°C for 2 hours for effective diffusion of test compounds and standards. The plates were then incubated at 37°C for 24-48 hrs. The diameter of the zone of inhibition or the MIC was measured and recorded. The *S. acmella* extract was prepared with 5 different dilutions (10mg/ml, 20mg/ml, 40mg/ml, 80mg/ml, and 100mg/ml) and Erythromycin (Colpaldas Visram and Co. Ltd, India; 0.125mg/ml) was used as a positive control, while distilled water was used as a negative control. The experiments were performed three times, and the mean diameter of the zone of inhibition was measured and recorded.[11]

Statistical analysis

The study data were analyzed using SPSS software version 24.0, with each result being the average of three measurements of inhibition zones in millimeters. The student's t-test was used to determine if there was a statistically significant difference between the means. Standard errors of the mean were represented by horizontal bars. Differences between the test groups and the control group were considered significant when $P < 0.05$ (95% confidence level).

RESULTS

The results of this study have shown that the ethanol extract of *S. acmella* possesses significant antibacterial activity against *S. mutans, L. fermentum*, *P. gingivalis,* and *C. gingivalis*, which are commonly associated with dental caries and periodontal infections. The extract demonstrated a dose-dependent inhibition of bacterial growth, with the highest concentration of 100mg/ml showing the strongest effect.

Table. Showing the effects of *Spilanthes acmella* extracts at various dilutions as zones of inhibition in mm on bacteria such as *Streptococcus mutans* (SM), *Lactobacillus* (LB), *Treponema denticola* (TD), and *Porphyromonas gingivalis* (PG)

Test material	Dilution of the Drug	Zone of inhibition (diameter in mm)			
		SM	LB	TD	PG
Spilanthes acmella (Ethanol extract)	10mg/ml	07	06	08	07
	20mg/ml	10	10	12	10
	60mg/ml	12	11	14	13
	100 mg/ml	14	13	16	15
Positive control Erythromycin	0.125mg/ml	22	20	24	24
Negative Control distilled water	-	00	00	00	00

The ethanol extract of *S. acmella* at a dilution of 10mg/ml demonstrated inhibition zones of 7mm, 6mm, 8mm, and 7mm against *S. mutans, L. fermentum*, *P. gingivalis*, and *C. gingivalis* respectively. A higher concentration of 20mg/ml and an increased concentration of 60mg/ml showed inhibition zones of 10mm, 10mm, 12mm, and 10mm and 12mm, 11mm, 14mm, and 13mm respectively. The highest dilution of 100mg/ml exhibited the greatest inhibition zone of 14mm, 13mm, 16mm, and 15mm. Erythromycin was used as the positive control drug.

In this research study, the Kirby-Bauer method was employed to assess the antimicrobial susceptibility of various bacterial strains. Antibiotic-impregnated discs were placed on agar plates containing bacterial cultures. The formation of clear zones around the discs indicated bacterial inhibition. Measurements of these zones were compared to established standards, revealing the susceptibility of bacteria to specific antibiotics. The standard values for the erythromycin breakpoints disk diffusion method (Kirby-Bauer method) were Sensitive (S): ≥ 23 mm zone diameter, Intermediate

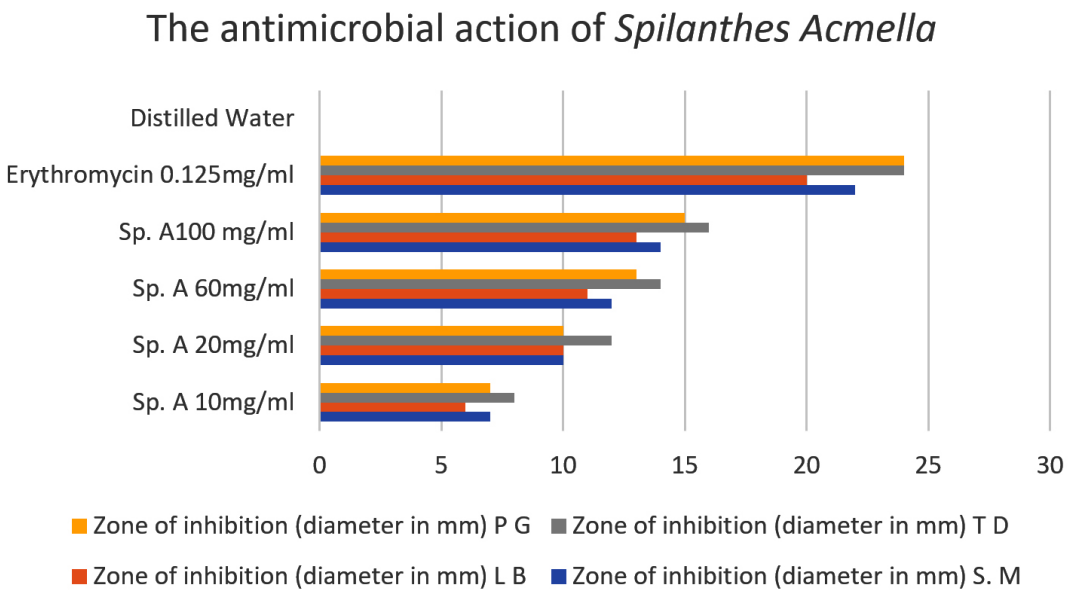

Figure. Showing the effects of *Spilanthes acmella* extracts at various dilutions as zones of inhibition in mm on bacteria such as *Streptococcus mutans* (SM), *Lactobacillus* (LB), *Treponema denticola* (TD), and *Porphyromonas gingivalis* (PG)

(I): 15-22 mm zone diameter, Resistant (R): ≤ 14 mm with a zone diameter. A concentration of 0.125mg/ml showed the largest inhibition zone of 22mm, 20mm, 24mm, and 24mm, respectively, against the same bacterial species. It is important to note that the negative control (distilled water) showed no zone of inhibition on any of the bacteria studied as shown in Table and Figure.

There is a significant impact of antibacterial activity against the mentioned bacterial species as the concentration of *S. acemella* ethanol extract increases. Therefore, the results indicate that the ethanol extract of *S. acmella* has the potential as an antimicrobial agent for the treatment of oral conditions caused by these bacteria.

DISCUSSION

The plant *Spilanthes acmella* (*S. acmella*) has been traditionally used in Ayurvedic and folk medicine for the treatment of various ailments, including oral health problems.[2,3] However, there have not been many studies investigating the antibacterial activity of *S. acmella* against the specific bacteria associated with dental caries and periodontal disease. This may be because of a lack of interest in natural remedies, insufficient funding for research on alternative medicine, limited knowledge about the medicinal properties of *S. acmella*,[12-15] or limited research without speculating on the reasons behind it.

Our study explores the potential of *S. acmella* extract as a natural alternative to traditional antimicrobial agents in the treatment of these bacterial infections. Additionally, there may not have been many studies done in the past on this topic, making it an area of interest for further research.

Our study used the agar well diffusion method to test the *S. acmella* extract, erythromycin, and distilled water against *S. mutans, L. fermentum*, *P. gingivalis*, and *C. gingivalis*. The results of this study have demonstrated that the ethanol extracts of *S. acmella* possess antibacterial activity against the bacterial species *S. mutans, L. fermentum*, *P. gingivalis,* and *C. gingivalis*, which are commonly associated with dental caries and periodontal infections. The extract showed a dose-dependent inhibition of bacterial growth, with the highest concentration of 100mg/ml showing the strongest effect. This suggests that *S. acmella* has the potential as an antimicrobial agent for the treatment of these oral conditions. Similar to our study, there is previous research on the antimicrobial activity of *S. acmella* by Onoriode O et al. found that the ethanol extract of the plant possessed strong antibacterial activity against *S. mutans, L. fermentum*, with an inhibition zone of 28mm-29mm and 24mm.[16] However, our research investigates the antibacterial activity of *S. amella* extract against two additional species, namely *P. gingivalis* and *C. gingivalis*, both of which are associated with periodontal problems.

The use of plant extracts as an alternative to traditional antimicrobial agents has been gaining popularity in recent years due to the increasing resistance of bacteria to conventional antibiotics.[17] *S. acmella* is known to have a wide range of medicinal properties, including anti-inflammatory, analgesic, and antimicrobial effects. Studies have shown that the plant contains a variety of phytochemicals, including flavonoids, terpenoids, and alkamides, which are responsible for its medicinal properties.[17-19]

Limitation of the study

One limitation of this study is that it only tested the antimicrobial activity of *S. acmella* ethanol extract against a small number of specific bacteria associated with dental caries and periodontal disease, and it is not clear if the extract would be effective against other types of bacteria or pathogens. Additionally, the study only used an in-vitro testing method, so further in-vivo studies are needed to confirm the efficacy of *S. acmella* in treating these conditions in a living organism. The studies are limited to the laboratory setting and more clinical studies are needed to confirm the results.

Further elaboration on the uncharted territories of *S. acmella*'s dental applications would not only justify the need for the current study but also pique the interest of the scientific community and potentially spur further research in this promising area.

CONCLUSION

This study has shown that the ethanol extract of *S. acmella* possesses strong antibacterial

activity against the bacteria commonly associated with dental caries and periodontal infections. This supports the traditional use of the plant in the treatment of these oral conditions and suggests that it may be a viable alternative to traditional antimicrobial agents. However, further studies are needed to fully understand the mechanism of action, and safety and to evaluate the extract's potential as a therapeutic agent *in vivo*.

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None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

SS and VGD designed, organized, conducted research, collected data and performed data analysis. SS, VGD, LB, AS and VHP wrote and revised the manuscript. All authors read and approved the final manuscript for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was approved by the Institutional Ethics Committee, JSS Dental College and Hospital, Mysore, India, with ethical clearance number JSSDCH IEC no: 86/2021.

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