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A Comparative Study on the Effect of *Spilanthes acmella* and Chlorhexidine (0.2%) Mouth Rinses on Salivary *Streptococcus mutans* Count in Children

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Authors' contributions

All the authors have contributed to the study design, data collection, analysis and drafting of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

In the wake of an increased interest in finding natural alternatives for chemical medicinal products, this study assesses and compares the effects and acceptance of *Spilanthes acmella,* a widely used plant in various traditional systems of medicine, with Chlorhexidine (0.2%) mouth rinse on the dental caries causing pathogen *Streptococcus mutans* colonizing the oral cavity of children. The study included 40 healthy children in the age group of 8-12 years based on inclusion and extrusion criteria. In the beginning of the study, non-stimulated whole salivary samples were tested for the baseline count of colony forming units (CFU) of *Streptococcus mutans* using Mitis Salivarius Agar supplemented with potassium tellurite. The same process was repeated 24 hours after 15 days of regular use of mouth rinses twice daily. Results showed that there was statistically highly significant decrease in CFU in the post rinse phase in both the groups. *S. acmella* showed greater acceptance

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by study subjects when compared with the Chlorhexidine mouth rinse. It can be concluded based on the results of this study that *S. acmella* has beneficial effects against *S. mutans* and can be a potential option for preventive measures against dental caries, although more studies are needed for its validation.

Keywords: Chlorhexidine; Spilanthes acmella; mouthwash; dental caries; Streptococcus mutans.

1. INTRODUCTION

Lately, an increased popularity of natural and plant-based products has been noticed in the healthcare field globally. Evidence shows that as high as 80% of the population worldwide benefit from medicinal plants and indigenous systems of medicine to meet their primary healthcare requirements [1]. The growing interest in drugs of plant origin are attributed to the perceived advantages of greater biocompatibility, lesser toxicity and better access compared to synthetic medicines [2,3]. The field of dentistry is not excluded from this phytotherapeutic upsurge. Role of phytochemical mouthwashes as anti-cariogenic or cariostatic agents have been widely studied [4,5,6].

Spilanthes acmella, is a plant which is widely used in various indigenous healthcare practices in the Indian subcontinent. It is known as the anti-toothache plant owing to the anaesthetic effect produced on chewing or placing crushed flower heads at the site of pain [7,8]. Phytochemical analysis has shown the presence of the isobutylamide constituent 'spilanthol' and triterpenoids [7,8]. The anaesthetic property is attributed to alkamides which are similar to certain endogenous cannabinoid cerebral neurotransmitters [8]. Despite the time-tested benefits on oral diseases affirmed by alternative systems of medicine, it is important to scientifically validate the efficacy and safety as a preliminary step in adoption of the product to mainstream dentistry.

Factors like lack of dexterity, individual motivation and monitoring, limit the effectiveness of tooth brushing which necessitates the use of chemotherapeutic agents for control of plaque in children [9]. Chlorhexidine is considered as the 'gold standard' mouth rinse due to its broad-spectrum anti-microbial effect and substantivity [10].

The most common plaque-mediated disease in children is dental caries, which is also one of the most common childhood diseases. Oral microorganisms present in dental plaque are considered crucial for the initiation and progression of dental caries. The most commonly implicated bacterial species in its etiology is *Streptococcus mutans* for its initiation.

The aim of this study is to assess and compare the effects and acceptance of *Spilanthes acmella* with Chlorhexidine (0.2%) mouth rinse on dental caries causing pathogen *Streptococcus mutans* colonizing the oral cavity of children.

2. METHODOLOGY

The investigation was a double-blind, comparative interventional study on two randomized parallel groups of children who used either a *Spilanthes acmella* mouth rinse or a commercially available Chlorhexidine (0.2%) mouth rinse twice daily for a 15-day period. Healthy children aged 8-12 years with four or more restored, decayed and/or missing teeth (DMFT/dmft ≥4), no history of recent antibiotic usage or fluoride therapy were selected from a residential school which ensured a uniform dietary pattern for the participants. Subjects who did not meet these criteria were excluded from the study. The participants were randomly divided into two groups of 20 each by lottery method. Commercially available Chlorhexidine (0.2%) mouth rinse (Periex from Dentaids) and *Spilanthes acmella* mouth rinse prepared using Soxhlet extraction were used in the study. Unlabelled identical bottles containing the mouth rinses and 10ml measuring cups were provided to each subject for easy dispensing of the solution. The children were trained to rinse the mouth for one minute using five milliliters of the respective mouth rinse twice daily for 15 days, once after breakfast in the morning and once at night after dinner. Non-stimulated whole salivary samples were tested for the colony forming units (CFU) of *Streptococcus mutans* both at baseline and at the end of 15 days using the same technique.

2.1 Preparation of *Spilanthes acmella* Mouth Rinse

Spilanthes acmella flowers were collected from the medicinal garden of KMCT Ayurveda Medical College. Identification and authentication of the

specimens were done at the same institution (Fig. 1).

Flower heads of *S. acmella* were air dried and subjected to coarse grating to produce a coarse powder of uniform texture at the National Institute of Pharmacy, Kozhikode. The extract was then obtained with ethanol using a Soxhlet apparatus (Fig. 2).

The desired concentration of the plant extract (10%) was made by dissolving in dimethyl sulfoxide. Authorized additive, peppermint flavour (1g/l), and sodium saccharine (1g/l) a sweetening agent were used to formulate the mouth rinse [11].

2.2 Microbiological Method

The saliva samples were collected in the morning on an empty stomach, adhering to precautions to avoid any bias in concentration due to circadian rhythm or contamination with food debris [12,13]. The samples were inoculated within 30 minutes after collection. All the saliva samples were serially diluted using physiological saline to obtain minus three (-3) concentration in order to obtain working salivary samples. Using a reusable inoculating loop, 0.1 ml saliva sample was spread by the streaking method on Mitis Salivarius agar enriched with potassium tellurite. The inoculating loop was sterilized by flaming the loop. The plates were incubated for 48 hours at 37ºC in the incubation chamber to obtain maximum growth of microbial colonies. The colony forming units (CFU) of *S. mutans* were identified by the morphology, size and colour. The colonies had blue (gum drop) appearance. Counting was done with the help of a microbiologist using a digital colony counter. The colony count was expressed as the number of colony forming units per millimeter (CFU/ml) of saliva. Semi-quantification of the number of colonies was done by multiplying the actual colony count with 1x 10^3 to adjust for the dilution factor [14]. The process was done at baseline and repeated on the 15th day after commencing the intervention.

2.3 Statistical Analysis

The data was entered into Microsoft Excel and analyzed using SPSS software version 25 and subjected to descriptive statistics, paired t-test (for comparison of intragroup differences in pre-rinse and post-rinse phases.

3. RESULTS

Table 1 shows the descriptive statistics of study subjects.

Table 2 assesses the intragroup variation in pre and post rinse CFU counts by paired t test. Mean colonies of *S.mutans* at baseline for Chlorhexidine was 5.570 X 10^7 ±2.86 which reduced to 0.510 X 10^6 ±0.22 after 15 days of intervention. This difference was statistically highly significant (p<0.001). *S. acmella* mouth rinse showed reduction of colony forming units from 4.736 X 10^7 ±1.63 to 3.200 X 10^6 ±1.33 within the same time frame , which was also statistically highly significant (p <0.001). So, when pre-rinse and post-rinse CFU were compared, there was a highly significant reduction of colony forming units for both Chlorhexidine and *S. acmella* groups (p <0.001).

Pre-rinse mean CFU for *S. acmella* mouth rinse was 4.736 X107 ±1.63 against 5.572 X107 ±2.86 for Chlorhexidine. Post-rinse values in both groups showed lesser mean CFU count, 3.200 X106 ±1.33 for *S. acmella* and 0.510 X106 ±0.22 for Chlorhexidine. This difference was statistically significant (p=0.001).

Table 4 shows the percentage of the study subjects in each mouth rinse group giving a positive (YES) and negative (NO) response to the questions asked regarding flavour, smell and willingness to rinse respectively.

The subjects in the *S. acmella* group showed better acceptance for the flavour (60%) and willingness to use (65%) compared to 45% and 40% respectively for Chlorhexidine. With regards to smell of mouth rinses, Chlorhexidine showed better acceptance with 75% participants favouring this over *S. acmella* mouth rinse (60%).

To summarize the results, mean differential colony counts in pre-rinse and post-rinse phases showed very highly significant fall in the colony counts of *Streptococcus mutans* in both the mouth rinse groups (P<0.001) though reduction in Chlorhexidine group was greater compared to *S. acmella* group.

Fig. 1. *Spilanthes acmella* flowers

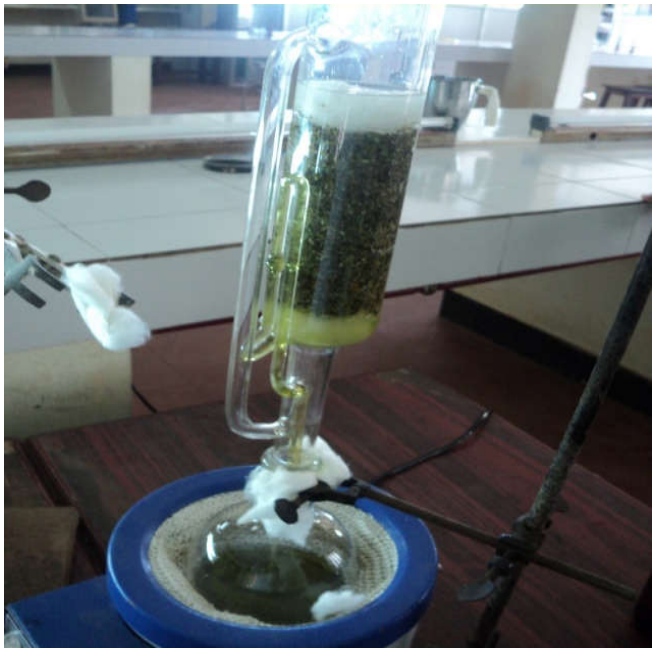

Fig. 2. Preparation of ethanolic extract of *S. acmella* using a Soxhlet apparatus

Table 1. Descriptive statistics of study subjects

Group	Age Mean and Standard deviation values	DMFT+dmf score Mean and Standard deviation values
Chlorhexidine	10.8 ± 1.04	14.51 ± 5.47
S. acmella mouth rinse	10.20 ± 1.40	11.75 ± 4.52

Table 2. Comparison of the CFU observed in pre and post rinse phases(paired t test)

Group	N	Pre-rinse mean±SD (10^7 CFU/ml)	Post-rinsemean±SD (10^6 CFU/ml)	P value
Chlorhexidine	20	5.570±2.86	0.510±0.22	<0.001**
S. acmella mouth rinse	20	4.736±1.63	3.200±1.33	<0.001**

(p< 0.05 - Significant, p < 0.001 - Highly significant**)*

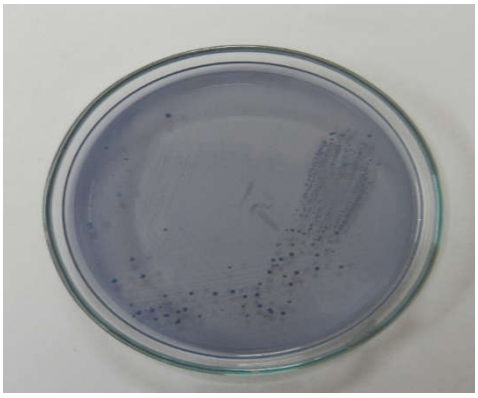

Fig. 3. Baseline sample(Chlorhexidine)

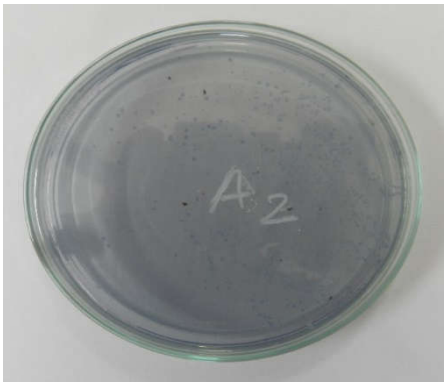

Fig. 4. Post-rinse sample (Chlorhexidine)

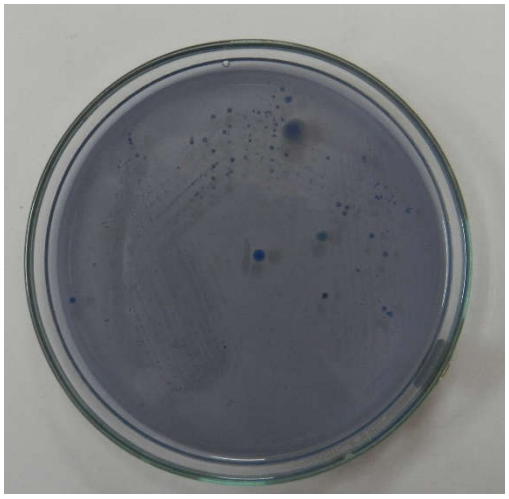

Fig. 5. Baseline sample(*S. acmella*)

Fig. 6. Post-rinse sample (*S. acmella*)

Table 3. Comparison of chlorhexidine mouth rinse with *S. acmella* mouth rinse using the unpaired t-test

Group	N	Pre-rinse mean±SD (107 CFU/ml)	Post-rinse mean±SD (106 CFU/ml)
Chlorhexidine	20	5.572±2.86	0.510±0.22
S. acmella mouth rinse	20	4.736±1.63	3.200±1.33
t value		5.038	2.513
P value		<0.001**	0.001*

Table 4. Responses of the children to various questions regarding the acceptability of mouth rinses

Question	Mouth rinse groups and the responses	
	Chlorhexidine N=20	***S. acmella* N=20**
Flavour	YES=09 (45%)	YES=12 (60%)
	NO=11 (55%)	NO=08 (40%)
Smell	YES=15 (75%)	YES=12 (60%)
	NO=5 (25%)	NO=8 (40%)
Willingness to rinse	YES=08 (40%)	YES=13 (65%)
	NO=12 (60%)	NO=7 (35%)

4. DISCUSSION

Chlorhexidine has been widely used as an antiplaque and antigingivitis agent since many decades [15]. The results of this study were in accordance with a similar study assessing the effectiveness of 0.2% Chlorhexidine mouthwash on *Streptococcus* colony counts [9]. Many studies over the years have affirmed the effectiveness of Chlorhexidine mouthwash against *S.mutans* species [9,16,17,18]. Brookes et al. state that Chlorhexidine mouthwashes can be bacteriostatic or bactericidal depending upon the concentration [19]. Despite being a popular antiseptic mouthwash, there are many disadvantages for Chlorhexidine. Long term use has been found to result in brownish discoloration of the teeth, irritation of oral mucosa, and bitter taste [9,19]. Hence, there is a need to develop an alternative mouth wash which will have comparable benefits as Chlorhexidine without having its adverse effects.

The results of this comparative interventional study showed that both *S. acmella* mouth rinse and Chlorhexidine exhibited significant reductions in salivary *Streptococcus mutans* counts upon comparing the baseline values with the posttreatment values after the 15 day intervention.

Spilanthes acmella is a plant of great importance in the African and Indian traditional pharmacopoeia [7,8,20]. Phytochemical studies have reported the presence of various bioactive metabolites which have antipyretic, anti-inflammatory, analgesic, local anesthetic and antimicrobial activities [7,8]. Despite its widespread use in traditional systems of medicine as the anti-toothache plant, scientific studies validating its use in dentistry are scarce. A comparative evaluation of the antibacterial and antifungal activity of *S. acmella* and calcium hydroxide on root canal pathogens have exhibited remarkable results, thus suggesting the potential use of *S. acmella* as an intracanal medicament in endodontics [21]. To the best of our knowledge, this is the first study to scientifically extrapolate the effect of *S. acmella* on the dental caries causing pathogen *Streptococcus mutans.* Our study shows that significant reduction was noted in the colony forming units of salivary *Streptococcus mutans.* It was also found that the general acceptance to use was greater for *S. acmella* mouth rinse in comparison with Chlorhexidine.

5. CONCLUSION

Although the reduction of the *Streptococcus mutans* colony count in the chlorhexidine group was greater compared to the *S. acmella* group, in the current scenario where researchers are interested in scientifically validating natural substances as alternatives for the control of caries in terms of antimicrobial response and lower associated risks, this study provides a foundation for further scientific and clinical research with regards to the anti-cariogenic properties of *S. acmella.* However, more studies with higher quality is required before full recommendations can be made with regards to incorporating this product into preventive protocols.

CONSENT AND ETHICAL APPROVAL

The study protocol was reviewed and approved by the Institutional Ethical Committee, KMCT Dental College, Kerala, India and registered with the Clinical Trial Registry of India. An informed consent was obtained from the caretakers and authorities of a residential school after detailed explanation of the nature of the study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kumar G, Jalaluddin MD, Rout P, Mohanty R, Dileep CL. Emerging trends of herbal care in dentistry. Journal of clinical and diagnostic research: JCDR. 2013;7(8): 1827.
Available:https://doi.org/10.7860/JCDR/2013/6339.3282

2. Dick TN, Marques LC, Lopes AD, Candreva MS, Santos LR, Picciani BL. Phytotherapy in Dentistry: A Literature Review Based on Clinical Data. European Journal of Medicinal Plants. 2020;29:1-3. Available:https://doi.org/10.9734/ejmp/2020/v31i1030276
3. Salman BN, Vahabi S, Rad MM. Use of herbs and medicinal plants in dentistry: a review. Journal Dental School. 2017;35(2): 133-49. Available:https://doi.org/10.22037/jds.v35i2.24622
4. Philip N, Leishman S, Walsh L. Potential role for natural products in dental caries control. Oral Health Prev. Dent. 2019; 1;17:479-85. Available:https://doi.org/10.3290/j.ohpd.a42741.
5. Karadaglioglu OI, Alagoz LG. Herbal products against dental caries. Annals of Dental Specialty Vol. 2020;8(4):55.
6. Sheik R, Lakshmi T. Awareness of herbal mouthwash for dental caries among school children: A cross-sectional study. Journal of Advanced Pharmacy Education & Research. 2017;7(3).
7. Dubey S, Maity S, Singh M, Saraf SA, Saha S. Phytochemistry, pharmacology and toxicology of Spilanthes acmella: a review. Advances in Pharmacological Sciences. 2013;1;2013. Available:https://doi.org/10.1155/2013/423750
8. Prachayasittikul V, Prachayasittikul S, Ruchirawat S, Prachayasittikul V. High therapeutic potential of Spilanthes acmella: a review. EXCLI Journal. 2013;12:291. PMID: 27092032
9. Lakade LS, Shah P, Shirol D. Comparison of antimicrobial efficacy of chlorhexidine and combination mouth rinse in reducing the Mutans streptococcus count in plaque. Journal of Indian Society of Pedodontics and Preventive Dentistry. 2014;32(2):91. Available:https://doi.org/10.4103/0970-4388.130780
10. Kaur H, Jain S, Kaur A. Comparative evaluation of the antiplaque effectiveness of green tea catechin mouthwash with chlorhexidine gluconate. Journal of Indian Society of Periodontology. 2014;18(2):178. Available:https://doi.org/10.4103/0972-124X.131320
11. Hajenorouzali Tehrani M, Asghari G, Hajiahmadi M. Comparing *Streptococcus mutans* and *Lactobacillus* colony count changes following green tea mouth rinse or sodium fluoride mouth rinse use in children (Randomized double-blind controlled clinical trial). Dental Research Journal. 2012;8(5). PMID: 23372597
12. Thomas A, Thakur SR, Shetty SB. Anti-microbial efficacy of green tea and chlorhexidine mouth rinses against Streptococcus mutans, Lactobacilli spp. and Candida albicans in children with severe early childhood caries: A randomized clinical study. Journal of Indian Society of Pedodontics and Preventive Dentistry. 2016;34(1):65. Available:https://www.jisppd.com/text.asp?2016/34/1/65/175518
13. Pratten J, Wills K, Barnett P, Wilson M. In vitro studies of the effect of antiseptic-containing mouthwashes on the formation and viability of Streptococcus sanguis biofilms. J Appl Microbiol. 1998;84:1149-55 Available:https://doi.org/10.1046/j.1365-2672.1998.00462.x
14. Wan AK, Seow WK, Walsh LJ, Bird PS. Comparison of five selective media for the growth and enumeration of *Streptococcus mutans*. Aust Dent J. 2002;47:21-6 Available:https://doi.org/10.1111/j.1834-7819.2002.tb00298.x
15. Corbet EF, Tam JO, Zee KY, Wong MC, Lo EC, Mombelli AW. Therapeutic effects of supervised Chlorhexidine mouthrinses on untreated gingivitis. Oral Dis. 1997; 3:9–18. Available:https://doi.org/10.1111/j.1601-0825.1997.tb00003.x
16. Spets-Happonen S, Markkanen H, Pöllänen L, Kauppinen T, Luoma H. Salivary Streptococcus mutans count and gingivitis in children after rinsing with a chlorhexidine- fl uoride solution with and without strontium. Scand J Dent Res. 1985;93:329-35. Available:https://doi.org/10.1111/j.1600-0722.1985.tb01977.x
17. Neeraja R, Anantharaj A, Praveen P, Karthik V, Vinitha M. The effect of povidone-iodine and chlorhexidine mouth rinses on plaque Streptococcus mutans count in 6- to 12-year-old school children: An *in vivo* study. J Indian Soc Pedod Prev Dent. 2008;26:S14-8 Available:https://www.jisppd.com/text.asp?2008/26/5/14/41748

18. Kulkarni VV, Damle SG. Comparative evaluation of efficacy of sodium fluoride, chlorhexidine and triclosan mouth rinses in reducing the mutans streptococci count in saliva: An in vivo study. J Indian Soc Pedod Prev Dent. 2003;21:98-104.
PMID: 14703215
19. Brookes ZL, Bescos R, Belfield LA, Ali K, Roberts A. Current uses of chlorhexidine for management of oral disease: a narrative review. Journal of Dentistry. 2020;17:103497.
Available:https://doi.org/10.1016/j.jdent.2020.103497
20. Spelman K, Depoix D, McCray M, Mouray E, Grellier P. The traditional medicine Spilanthes acmella, and the alkylamides spilanthol and undeca-2e-ene-8, 10-diynoic acid isobutylamide, demonstrate *in vitro* and *in vivo* antimalarial activity. Phytotherapy Research. 2011;25(7):1098-101.
Available:https://doi.org/10.1002/ptr.3395
21. Sathyaprasad S, Jose BK, Chandra HS. Antimicrobial and antifungal efficacy of Spilanthes acmella as an intracanal medicament in comparison to calcium hydroxide: An in vitro study. Indian Journal of dental research. 2015;26(5):528.
Available:https://www.ijdr.in/text.asp?2015/26/5/528/172081

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