

## Comparison Of Antimicrobial Activity Of Crude Extracts Of *Mangifera indica*, *Psidium guajava*, *Piper nigrum*, *Anacardium occidentale* and *Syzygium aromaticum* Against Dental Cariogenic *Streptococcus sp.*

A. Raimol Baby, B. Nimisha K U, C. Shehinas R S, and D. Reshna S N

**Abstract** One of the most common trouble that people of all age group suffer is that of dental caries. Without any age bar from the young to the aged suffer from this ailment. Around the world researches are going on to resolve this issue. Mouth harbors a diverse microbial community and hence is prone to many microbial activities. The major causative organisms of dental caries include *Streptococcus* species. Many treatments are currently being used but only with side effects. As an attempt to overcome the side effects of the treatment using chlorohexidine, povidone-iodine the rich herbal resources with antibacterial activity was examined. In this study the plants that were considered include *Mangifera indica*, *Psidium guajava*, *Piper nigrum*, *Anacardium occidentale* and *Syzygium aromaticum*. By carrying out disc diffusion assay antimicrobial activity of each plant was assessed and results were compared to that of clove oil. This study mainly focus on antibacterial activity of pepper, cashew leaves, guava and mango leaves due to the age old tradition of using these for dental issues and low side effects.

**Index Terms**— antibacterial activity, clove oil, microbial community, *Streptococcus* species

### I. INTRODUCTION

THIS paper deals with comparison of antibacterial activity of certain medicinal plants with that of clove oil against cariogenic *Streptococcus sp* which are primarily responsible for dental caries also known as tooth decay which results in localized dissolution and destruction of calcified tissues of the teeth<sup>[1]</sup>. Cavities are of different types it may vary in colour from yellow to black. Pain and difficulty while eating are its main symptoms however severe complications include inflammation of tooth tissue, tooth loss infection or puss formation. Caries are formed when hard tissues of teeth like enamel, dentin are broken down due to bacterial activity<sup>[2]</sup>.

Mouth harbors diverse bacteria of which only a few specific species of bacteria are believed to cause dental caries: *Streptococcus mutans* and *Lactobacillus* species are few among them<sup>[18]</sup>. *Streptococcus salivariu*, *Streptococcus mitis*, and *Streptococcus sanguis*, *Streptococcus pneumonia* *Streptococcus pyogens*, *Fusospirachetes*, *veillonella*, *Actinobacillus actinomycen-* *temcomitans*, *Staphylococcus aureus*, *Staphylococcus epidermis* *Corynebacterium* are also involved in the development of dental caries.

Recently many modern medicines exist side by side with traditional practice. Dental infections are treated using Pencillin G or Pencillin V<sup>[7]</sup>. Erythromycin is also a common choice. Using antibiotics to fight dental issues can cause further problems as bacteria mutate very easily and gain resistance to antibiotics. Yet another fact is that when used for a long time some of the antibiotics like aureomycin and vancomycin cause hearing loss and kidney damage.

Medicinal plants represent a rich source of antimicrobial activity. Plants have attained a significant role in maintaining the oral health. In this study the antimicrobial activity of few the plants that we see around is taken for the study. This includes mango leaves, cashew leaves, guava leaves and pepper. From the good olden days mango leaves were used for cleaning mouth. Chewing cashew leaves and allowing the juice to remain in the mouth was also a common practice. Chewing tender leaves of guava to prevent bleeding of gums and bad breath was a tradition in our country. Black peppercorns are useful if chewed as they can help clean out the bacteria and relieve pain. The composition of these also revealed antimicrobial activity and hence were considered for the study.

The main objective of the study include

- To obtain extract from *Mangifera indica*, *Psidium guajava*, *Piper nigrum*, *Anacardium occidentale*
- To compare the antimicrobial activity of these extracts by carrying out disc diffusion assay
- To compare the antimicrobial activity of all four extracts with clove oil.

Procedures For Paper Submission

A. *Materials and method*

ISOLATION OF Streptococcus sp. FROM DENTAL CAVITY

Sample collection

Four patients were taken for the study. A cotton swab was introduced into the cavities of the patient and the sample was inoculated into agar medium by streaking on nutrient agar plates. One plate was kept as control. The plates were incubated at 37°C overnight and the colonies obtained were taken for identification of species.

B. *Media Used*

TABLE I  
 Media used and its composition

Media	Composition (grams/litre)
Nutrient agar	Peptone (5), beef extract(1.5), agar(15),ph-7.4
Nutrient broth	Peptone (5), beef extract (1.5), ph-7.4
Blood agar	Peptone (5), beef extract(1.5), agar(15),blood(7.5), ph-7.4

Cultural characteristics of isolated bacteria such as size, shape, pigmentation, elevation and margin of colony were recorded by culturing them on nutrient agar medium and incubated for 24 - 48 hours at 37°C ± 1°C. The colonies are round, raised, slimy, in chains and not pigmented. These are fastidious organisms and show alpha-hemolytic behavior when cultured in blood agar media. Alpha hemolysis shows greenish coloration due to hydrogen peroxide released from the colonies and inducing oxidation of hemoglobin to methemoglobin.

C. *Morphological characteristics*

Morphological characteristics are identified using Simple staining procedure and Gram staining procedure.

D. *Disc diffusion assay (Kirby-Bauer method)*

Select about 4 to 5 colonies with same morphological type from an agar plate. Using a wire loop transfer the growth to a tube containing 4 to 5 mL of a suitable broth medium, such as nutrient agar broth. Incubate this for at 37°C until it reaches the standard. Using a L-rod spread .5ml of broth over the sterile agar medium by micropipette and cover using the plate top for 15 minutes and ensure to remove the moisture content. Dip sterile sensitivity discs in each extract and place appropriately on the surface of agar using sterile forceps. Invert the plates and incubate at 35°C for 16 to 18 hours and examine the zone of inhibition and take down the measurements in millimetres.

E. *Solvent Extraction*

Samples are extracted using soxhlet apparatus. A Soxhlet Extractor has three main sections: A percolator (boiler and reflux) which circulates the solvent, a thimble which retains the solid to be laved, and a siphon mechanism, which periodically empties the thimble.

The plant parts taken for study includes leaves of *Mangifera indica*, *Anacardium occidentale* and *Psidium guajava* and dried seeds of *Piper nigrum*. Each sample was wrapped in cotton cloth and placed inside the thimble. The solvent is heated and the solvent vapour travels up a distillation arm. This moves into a chamber. The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material. The chamber containing the solvent is returned to the distillation flask. This cycle may be allowed to repeat many times, over hours or days. The extract finally obtained was transferred to plastic zip-lock bags, labelled and stored.

F. *Comparison of antimicrobial activity*

The diameter of inhibition zone corresponds to the antimicrobial activity of each extract against the organism inoculated during disc diffusion assay. The diameter of zone of clearance of each extract was compared with each other. The antimicrobial activity of each sample was compared with antimicrobial activity of clove. The comparison may be inferred from the chart or from the percentage increase or decrease value in the antimicrobial activity of each extract from that of clove which can be calculated by;

Percentage of antimicrobial activity with respect to clove  $\frac{\text{Diameter of zone of extract} - \text{Diameter of zone of clove}}{\text{Diameter of zone of clove}} \times 100$

Diameter of zone of clove

## II. RESULTS

The study on antimicrobial assay of various plant extracts against human caryogenic microorganism (*streptococcus sp.*) was carried out and compared with each other and also with clove. The comparison was made based on the diameter of zone of clearance obtained from the disc diffusion assay. Observations are tabulated as below:

TABLE II  
 ZONE OF INHIBITION BY DIFFERENT EXTRACTS  
 AGAINST *STREPTOCOCCUS SP*

Extract used	Diameter of zone of inhibition (in mm )
Clove	15.33
Mango	15.66
Guava	3.3
Cashew	6.33
Pepper	5.3

Through the study it was observed that extract of mango leaf had comparable antimicrobial activity to that of clove. It could also be observed that cashew have better antibacterial activity against *streptococcus sp.* than pepper and guava. Guava has least antimicrobial activity than all other extracts.

TABLE III  
 COMPARISON OF ANTIMICROBIAL ACTIVITY WITH  
 CLOVE

Extract	Antimicrobial activity in comparison with clove
Mango	0.021
Guava	-0.78
Cashew	-0.58
Pepper	-0.65

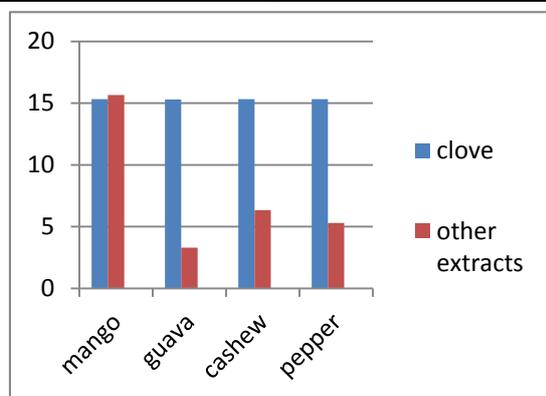


Fig. 1. Comparison of antimicrobial activity of other extracts with clove

## III. CONCLUSION

Dental caries are caused by the opportunistic pathogens in the oral flora. Many plants that see around us have antimicrobial activity and our study revealed that *Mangifera indica* have comparable antimicrobial activity to that of clove oil against *Streptococcus* species. The natural C-glucoside xanthone mangiferin [2-C-β-Dgluco-pyranosyl-1,3,6,7-tetrahydroxyxanthone ] reported in mango leaves and the presence of a phenolic compound from leaves of *Mangifera indica* named as homomangiferin [41] have antimicrobial effect which may be responsible for inhibiting the growth of *Streptococcus sp.* in a higher level than other extracts taken for the study and after further studies it can be used as an ingredient in tooth pastes and other oral care products.

## REFERENCES

- [1] W. J. Loesche, "Role of Streptococcus mutans in human dental decay," Microbiological Reviews, vol. 50, no. 4, pp. 353–380, 1986.
- [2] I. Kleinberg, "A mixed-bacteria ecological approach to understanding the role of the oral bacteria in dental caries causation: an alternative to Streptococcus mutans and the specific-plaque hypothesis," Critical Reviews in Oral Biology and Medicine, vol.13, no. 2, pp. 108–125, 2002.
- [3] J. M. Hardie, "Oral microbiology: current concepts in the microbiology of dental caries and periodontal disease," British Dental Journal, vol. 172, no. 7, pp. 271–281, 1992.
- [4] G. W. Taylor, M. C. Manz, and W. S. Borgnakke, "Diabetes, periodontal diseases, dental caries, and tooth loss: a review of the literature," Compendium of Continuing Education in Dentistry, vol. 25, no. 3, pp. 179–184, 186–188, 190, 2004.
- [5] J. T. Wright and T. C. Hart, "The genome projects: implications for dental practice and education," Journal of Dental Education, vol. 66, no. 5, pp. 659–671, 2002.
- [6] V. Baelum, W. M. Luan, X. Chen, and O. Fejerskov, "Predictors of tooth loss over 10 years in adult and elderly Chinese," Community Dentistry and Oral Epidemiology, vol. 25, no. 3, pp. 204–210, 1997.
- [7] I. Kubo, H. Muroi, and M. Himejima, "Antimicrobial activity against Streptococcus mutans of mate tea flavor components," Journal of Agricultural and Food Chemistry, vol. 40, pp. 245–248, 1992.

[8] E. K. Jonathan, K. J. Anna, and V. S. Johannes, "Zulu medicinal plants with antibacterial activity," *Journal of Ethnopharmacology*, vol. 69, no. 3, pp. 241–246, 2000.

[9] J.

- Antidiabetic effects of extracts from *Psidium guajava*. *J. Ethnopharmacol.*, 96, 411–415 (2005).
- [33] I.A. Ross, *Medicinal Plants of the World: Chemical Constituents, traditional, and Modern Medicinal Uses*. Humana Press, Totowa (1999).
- [34] J.D. da Silva, A.I.R. Luz, M.H.L. da Silva, E.H.A. Andrade, M.G.B. Zoghbi and J.G.S. Maia, Essential oils of the leaves and stems of four *Psidium* spp. *Flavour Fragr. J.*, 18, 240–243 (2003).
- [35] F. Adam, I. Vahirua-Lechat, E. Deslandes and C. Menut, Aromatic plants of French Polynesia. V. Chemical composition of essential oils of leaves of *Psidium guajava* L. and *Psidium cattleianum* Sabine. *J. Essent. Oil Res.*, 23, 98–101 (2011).
- [36] R. Porat, Z. Tietel, I. Zippori and A. Dag, Aroma volatile compositions of high- and low-aromatic guava varieties. *J. Sci. Food Agr.*, 91, 2794–2798 (2011).
- [37] S. Tachakittirungrod, S. Okonogi and S. Chowwanapoonpohn, Study on antioxidant activity of certain plants in Thailand: Mechanism of antioxidant action of guava leaf extract. *Food Chem.*, 103, 381–388 (2007).
- [38] Michalek SM, McGhee JR, Shiota T, *et al.* Low sucrose levels promote extensive *S. mutans*-induced dental caries. *Infect Immun* 1977; 16 :712-4.
- [39] Limsong J, Benjavongkulchai E, Kuvatanasuchati J. Inhibitory effect of some herbal extracts on adherence of *S. mutans*. *J Ethnopharmacol* 2004; 92: 281-9.
- [40] Fathilah AR, Rahim ZHA. The anti-adherence effect of *Piper betle* and *Psidium guajava* extracts on the adhesion of early settlers in dental plaque to saliva-coated glass surfaces. *J Science* 2003; 45: 201-6.
- [41] Subbarayan C, Cama HR. Isolation & characterization of a carotenoid-protein complex from *Mangifera indica* (mango) *Indian J Biochem.* 1966;3:225–7. [[PubMed](#)]
- [42] [biolabs.tmcc.edu/Micro%20Web/Strep.pdf](http://biolabs.tmcc.edu/Micro%20Web/Strep.pdf)
- [43] *Medical Microbiology*. 4th edition Maria Jevitz Patterson
- [44] [[www.microbiologyinfo.com/cultural-characteristics-of-streptococcus/](http://www.microbiologyinfo.com/cultural-characteristics-of-streptococcus/)]