DOI: 10.7860/JCDR/2017/22143.9160

Original Article

Dentistry Section

Antimicrobial Effects of *Garcinia Mangostana* on Cariogenic Microorganisms

SUNITHA JANARDHANAN[1], JAIDEEP MAHENDRA[2], A. S. SMILINE GIRIJA[3], LITTLE MAHENDRA[4], VIJAYASHREE PRIYADHARSINI[5]

ABSTRACT

Introduction: *Garcinia mangostana* commonly called as Mangosteen fruit has been used as an antibacterial agent since age old times. The mangosteen pericarp has proven to have antibacterial effect, but the effect of the same on cariogenic organisms has not been explored. The present study was an attempt to gain a better understanding of the antibacterial effect of mangosteen pericarp on the cariogenic bacteria, to unravel the therapeutic potential for the same.

Aim: The aim of the study was to assess the antibacterial efficacy of the crude chloroform extract of mangosteen pericarp against cariogenic bacteria.

Materials and Methods: The study was done under laboratory settings using an in vitro design. The microorganisms namely *Streptococcus mutans, Streptococcus sanguis, Streptococcus salivarius, Streptococcus oralis* and *Lactobacillus acidophilus* were procured from American Type Cell Culture (ATCC) and Microbial Type Culture Collection (MTCC) were revived and lawn cultured. The antibacterial effect of mangosteen pericarp was tested using agar well diffusion method on Trypticase Soy Agar-Blood Agar (TSA-BA) and de Man, Rogosa and Sharpe (MRS) agar media. The standard antiplaque agent chlorhexidine was used as the positive control. This cross-sectional, experimental study was done in Central Research laboratory, Meenakshi Ammal Dental College for period of eight weeks. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values were determined by microbroth dilution method. Statistical analysis was done by calculating the mean of the zones of inhibition on tested microorganisms. Mann-Whitney test was done to compare the zones of inhibition of mangosteen and chlorhexidine.

Results: The antibacterial bioassay showed the highest activity for *Lactobacillus acidophilus* (13.6 mm) and *Streptococcus sanguis* (13.6 mm), whereas, it showed a medium and low activity for *Streptococcus oralis* (11.3 mm), *Streptococcus mutans* (10.6 mm) and *Streptococcus salivarius* (3 mm) respectively. The MBC and MIC values were lowest for *Lactobacillus acidophilus* (MIC 25 mg/ml, MBC 50 mg/ml) and *Streptococcus oralis* (MIC 50 mg/ml, MBC 100 mg/ml).

Conclusion: Mangosteen pericarp extract had a higher zone of inhibition against the tested microorganisms which suggests its potent antibacterial action against cariogenic organisms. However, further analytical studies are needed to isolate the key molecules of mangosteen pericarp, to explore its anticariogenic therapeutic potential on gram negative oral microorganisms.

Keywords: Herb, In vitro, Mangosteen

INTRODUCTION

Mangosteen with the botanical name *Garcinia mangostana* is regarded as a queen of fruits or a superfruit since it is endowed with properties, potentially beneficial to overall health of an individual. The fruit which is native to Southeast Asia has been an integral part of Chinese medicine and ayurveda since ancient times [1]. Mangosteen plant's leaves and bark have been used in oral care in some African countries, as chew sticks and an astringent [2]. The pericarp has also been proved in numerous studies, that it is endowed with antibacterial potential [3]. In India, the usage of ayurvedic and siddha based medicine is especially huge among urban and rural population for its antiseptic, anti-inflammatory, analgesic, antiparasitic, antipyretic, anticarcinogenic effect [4]. Mangosteen pericarp is rich in compounds called xanthones which includes compounds like α-mangostin, β-mangostin, γ-mangostin, garcinone B, garcinone E, along with mangostin one [5].

Dental plaque is a tooth associated biofilm which plays an important role in the causation of dental caries. The cariogenic organisms such as *Streptococcus mutans, Streptococcus sanguis, Streptococcus salivarius, Streptococcus mitis, Streptococcus oralis* and *Lactobacillus acidophilus* play a vital role in the aetiology of the caries. The growth of these organisms would have a profound influence on the initiation and progression of disease [6]. Conventional anticariogenic agents are although numerous, majority of them are known to have side effects such as multiple drug resistance [1]. Herbal medicine has gained importance in the field of dentistry due to its minimal adverse effects on the oral tissue and is, as effective as the conventional anticariogenic agents [7]. World Health Organisation (WHO) proposed that, any medicinal herb has to go through various scientific studies to establish the potential for its therapeutic use [8]. The purpose of this study was to find the antimicrobial properties of mangosteen pericarp on cariogenic organisms by measuring the zones of inhibition. The MIC of the mangosteen extracts was also estimated to find out the minimal concentration at which the mangosteen pericarp will inhibit the growth of the bacteria. MBC was also estimated to identify the bacterial lysis. MBC is the lowest concentration at which 99.99% of the bacteria analyzed, show lysis [9]. These would open up newer avenues in using mangosteen as an antibacterial agent.

MATERIALS AND METHODS

The cross-sectional in vitro study was performed in Central Research Laboratory, Meenakshi Ammal Dental College and Research Institute, Chennai, Tamil Nadu, India for a period of eight weeks. The institutional ethical committee and Institutional Review Board, MAHER University approved the study. (MAHER-MU-004-IEC/2016)

Test Organisms used for the Study

Standard strains of the microorganisms *Streptococcus oralis*

MTCC 2696 *Streptococcus mutans* MTCC no. 890, *Lactobacillus acidophilus* MTCC no. 10307, *Streptococcus salivarius* ATCC no. 13419 and *Streptococcus sanguis* ATCC no. 10556 for the study were procured from MTCC and ATCC.

Crude Solvent Extraction

The pericarp was extracted from the fruit of *Garcinia mangostana* by drying in shade and then powdered. The resulting powder was then stored in air tight wide mouth containers. The crude extract of mangosteen pericarp powder was prepared using an organic solvent, chloroform at room temperature. Almost 25 mg of the mangosteen powder was dissolved in 75 ml of chloroform (1:3 w/v) [10]. The resulting mixture was placed in an orbital shaker for three consecutive days. The solution obtained was then filtered using a whatman filter paper. The filtrate was allowed to evaporate in a rotary evaporator. Almost 100 mg of the crude extract was mixed with 1 ml of Dimethyl Sulphoxide (DMSO) and the product was stored in amber coloured storage containers for further use.

Antibacterial Assay of Mangosteen

The antibacterial effect of the pericarp was tested by using agar well diffusion method [11]. The bacterial inoculums were prepared and were adjusted to 0.5 McFarland turbidity standards [12]. Nearly, 100 µl of the suspension was then streaked uniformly on TSA-BA and MRS agar to form lawn cultures. Three wells were cut on the surface of each culture plate with a sterile borer of 0.5 cm diameter, in all the five plates streaked with the respective organisms. Nearly, 50 µl of pericarp extract was poured into the wells that were seeded with the bacterium to be tested. The plates were then placed at room temperature for an hour, followed by incubation for 24-48 hours at 37ºC. The degree of antibacterial activity against tested bacteria was assessed by measuring the diameter of zone of inhibition. The zones of inhibition produced were recorded at the end of 24-48 hours. Chlorhexidine in 0.2% was used as positive control, while DMSO without the test ingredient was taken as a negative control. The test was repeated for each microorganism thrice. The results of the tests were recorded in triplet culture plates.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MIC was done by a broth dilution method, where in pure cultures of each microorganisms was grown in their respective broth 1:1 dilutions were made in 96 well microtitre plates [12]. Nearly, 100 µl of the organisms obtained from the logarithmic phase of growth were mixed with 100 µl of the herbal extract. Once diluted, the volume of the diluted herbal extract was added to each dilution wells. The inoculated, serially diluted mangosteen pericarp extract was then incubated for about 24-48 hours at 37ºC. The dilution wells were observed for microbial growth indicated by turbidity at the bottom of the well. The final tube devoid of any turbidity determines the MIC of the mangosteen crude extract. The lowest concentration of the extract which causes the lysis of majority of bacteria was recorded as MBC. The turbidity control was the organism with DMSO and the drug turbidity control was drug along with DMSO for determination of MIC and MBC.

STATISTICAL ANALYSIS

Statistical analysis was done by using SPSS Software version 16 for calculating the mean of the zones of inhibition on tested microorganisms. Mann-Whitney test was done to compare the zones of inhibition of mangosteen and 0.2% chlorhexidine.

RESULTS

Mangosteen pericarp extract showed a maximum zone of inhibition for *Lactobacillus* [Table/Fig-1] and *Streptococcus sanguis* [Table/Fig-2] as compared to *Streptococcus oralis* [Table/Fig-3], and *Streptococcus mutans* [Table/Fig-4]. It was least for *Streptococcus salivarius* [Table/Fig-5]. When compared with standard antiplaque agent such as chlorhexidine at 0.2%, it showed a higher zone of inhibition and was found to be statistically significant [Table/Fig-6].

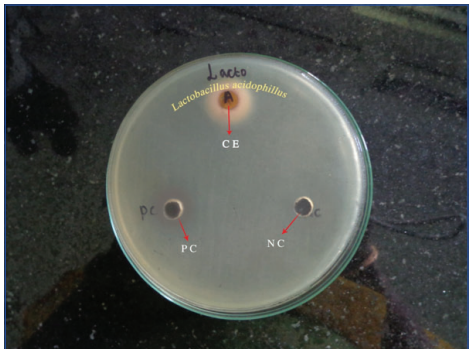
The MIC and MBC values of the mangosteen pericarp was lowest for *Lactobacillus acidophilus* (MIC 25 mg/ml, MBC 50 mg/ml) and *Streptococcus oralis* (MIC 50 mg/ml, MBC 100 mg/ml) compared to the other tested organisms namely *Streptococcus mutans*, *Streptococcus salivarius* and *Streptococcus sanguis* showing the mangosteen pericarp as an effective antibacterial agent at low concentrations [Table/Fig-7].

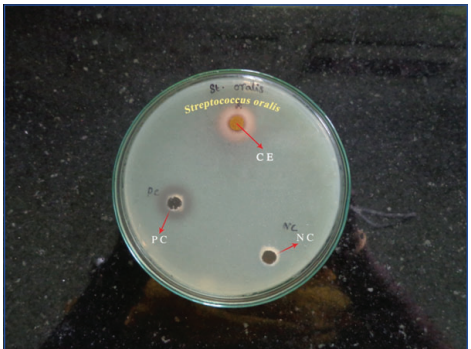
DISCUSSION

Herbal medicine is an inherent part of our ancient culture and is being used in ayurveda and siddha systems of medicine for decades. Currently, a number of herbs are also being used in oral health care. Dental caries is a common microbial disease affecting the tooth predominantly caused by the *Streptococcus* and *Lactobacillus* group of bacteria. Since, microorganisms play a key role in the aetiology of this disease, agents impeding the growth of these cariogenic organisms would have a significant role in inhibiting the disease [6]. Today various conventional anticariogenic agents are available, but they have numerous side effects [13].

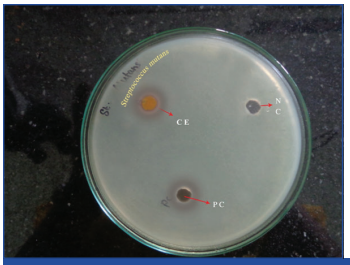
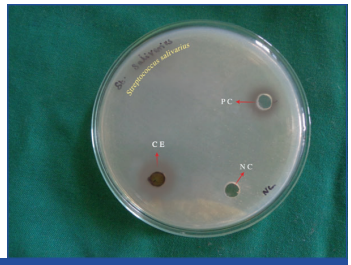
Herbal medicine has proven to be more promising with respect to antimicrobial activity [14]. The chemotherapeutic property of certain herb derived drugs is capable of restraining the antibiotic resistance as well as the side effects caused by the conventional drugs, leading to a new revolution using these herbal molecules. Thus, the herb derived molecule used as antibacterial therapy is an added advantage to the modern medicine [15,16].

Mangosteen pericarp is an inherent part of this herbal medicine and is widely used in treatment of numerous disorders ranging from, stomach ailments, diarrhoea, and dysentery. It is also being used as an anti-inflammatory, antibacterial and an antifungal agent to treat thrush [4]. It also has an antibacterial property which





[Table/Fig-1]: Antibacterial zones of inhibition produced by chloroform extract of mangosteen against *Latobacillus acidophilus*. **[Table/Fig-2]** Antibacterial zones of inhibition by chloroform extract of mangosteen against *Streptococcus sanguis*. **[Table/Fig-3]** Antibacterial zones of inhibition by chloroform extract of mangosteen against *Streptococcus oralis*.
CE- Chloroform extract of mangosteen, PC- positive control, NC- negative control (Images left to right)

[Table/Fig-4]: Antibacterial zones of inhibition by chloroform extract of mangosteen against *Streptococcus mutans*. **[Table/Fig-5]** Antibacterial zones of inhibition by chloroform extract of mangosteen against *Streptococcus salivarius*.
CE- Chloroform extract of mangosteen, PC- positive control, NC- negative control

Organism	Group	Mean	Mean Rank	p-value
Streptococcus sanguis	Mangosteen	13.6	3.83	0.658
	Chlorhexidine	15	3.17	
Streptococcus salivarius	Mangosteen	3	2.00	0.049*
	Chlorhexidine	13	5.00	
Streptococcus oralis	Mangosteen	11.3	2.00	0.046*
	Chlorhexidine	13	5.00	
Streptococcus mutans	Mangosteen	10.6	2.00	0.043*
	Chlorhexidine	14	5.00	
Lactobacillus acidophillus	Mangosteen	13.6	2.33	0.099
	Chlorhexidine	14	4.67	

[Table/Fig-6]: Mann-Whitney test to compare the effect of *Garcinia mangostana* pericarp extract and chlorhexidine on oral microorganisms.
*-Significant

Organisms	MIC mg/ml	MBC mg/ml
Streptococcus sanguis	100	200
Streptococcus salivarius	100	200
Streptococcus oralis	50	100
Streptococcus mutans	100	200
Lactobacillus acidophilus	25	50

[Table/Fig-7]: Determination of MIC and MBC of *Garcinia mangostana* on microorganisms.

remains unexplored on oral bacteria [10]. We hypothesize that the mangosteen pericarp extract is as effective as chlorhexidine in inhibiting the growth of the cariogenic organisms grown in vitro. This was determined by evaluating the zones of inhibition produced by the mangosteen pericarp when grown in the presence of the tested microorganisms. For any agent with proven antibacterial effect, the zone of inhibition should be 3 mm or more. Larger the zone, the more effective is the antimicrobial potential [17].

In our study, the majority of the microorganisms showed antimicrobial zones above 3 mm [Table/Fig-6] showing considerable antibacterial inhibitory effect of the pericarp against these microorganisms. The mangosteen extract gave a range of zone of inhibition from 3-13.6 mm at an extract concentration of 100 mg/ml on the tested bacteria. *Streptococcus salivarius* showed a 3 mm wide zone. The zones of inhibition were compared to standard chlorhexidine at 0.2% [13-15] which gave a comparable range and it was statistically significant for *Streptococcus salivarius, Streptococcus mutans* and *Streptococcus oralis*. Similar results have been reported by Linuma et al., who showed that the pericarp extracts of mangosteen had an antibacterial effect on methicillin resistant *Staphylococcus aureus* [18]. Similarly, Fernando KMEP et al., evaluated the antibacterial activity of methanolic extracts of mangosteen, which showed inhibitory activity on *Bacillus subtilis, Staphylococcus aureus* and *Streptococcus faecalis* [19].

The MIC and MBC were lowest for *Lactobacillus acidophilus* and *Streptococcus oralis* when compared to the other microorganisms inferring that the mangosteen was effective, even at very low concentration in inhibiting these above organisms among the five tested bacteria [Table/Fig-7]. The results were in agreement with Torrungruang K et al., who showed the similar values for MIC and MBC of mangosteen on two different strains of *Streptococcus mutans* [9]. In our study, the MIC and MBC of the crude extract of mangosteen pericarp for *Lactobacillus acidophilus* (25 mg/ml) was comparable to that of gold standard chlorhexidine having an MIC of 20 mg/ml. Similar study was reported by Linuma et al., who showed the presence of one active isolate, alpha mangosteen, a xanthone derivative with a MIC of 1.57 -1.5 µg/ml [18]. Our study was one of the few study to assess the effect of mangosteen extract on a group of oral cariogenic microorganisms.

LIMITATION

The crude form of mangosteen pericarp was used in our study. The major active compound in mangosteen pericarp was not isolated and identified. It is possible that a combination of active compounds may be responsible for inhibition rather than a single compound giving the synergistic effect as a whole.

CONCLUSION

We concluded that the crude chloroform extract of mangosteen pericarp showed an effective zone of inhibition against *Streptococcus mutans, Streptococcus sanguis, Streptococcus salivarius, Streptococcus oralis and Lactobacillus acidophilus*. Thus, the mangosteen pericarp extract showed promising activity against the dental pathogens. Further purification studies to analyze the active biocompound from chloroform extract would aid in designing a novel drug in the treatment of dental infections like caries.

ACKNOWLEDGEMENTS

The authors would like to thank Mrs. Chitra, Assistant, Department of Microbiology, Meenakshi University, Chennai, India, for the technical support.

REFERENCES

[1] Pedrasa-Chaverri J, Rodroguez NC, Arozco-Ibarra M, Perez-Rojas JM. Medicinal properties of mangosteen. Food Chem Toxicol. 2008;46:3227-29.
[2] Sagar S. Role of natural toothbrushes in containing oral microbial flora-A review. Asian J Pharm Clin Res. 2015;8(4):29-33.
[3] Gutierrez-Orozco F, Failla ML. Biological activities and bioavailability of mangosteen xanthones: A critical review of the current evidence. Nutrients. 2013;5:3163-83.
[4] Ibrahim MY, Hasim NM, Mariod AM, Mohan S, Abdulla MA , Abdelwahab SI, et al. α-Mangostin from *Garcinia mangostana* Linn: An updated review of its pharmacological properties. Arab J Chem. 2014;4:123-29.
[5] Shibata MA, Matoba Y, Tosa H, Iinuma M. Effects of mangosteen pericarp extracts against mammary cancer. AlternInteg Med. 2013;8(2):1-5.
[6] Sivapathasundharam B, Raghu AR. Microbiology of dental caries. In: Sivapathasundharam B, Rajendran R Editors. Shafer's textbook of Oral Pathology. 6th Ed: Elsevier publications; 438–43.
[7] Bhat N, Mitra R, Oza S, Mantu VK, Bishnoi S, Gohil M, et al. The antiplaque effect of herbal mouthwash in comparison to chlorhexidine in human gingival disease: A randomized placebo controlled clinical trial. J Complement Integr Med. 2014;11(2):129-37.
[8] Parameswari K, Aluru S, Kishori. B. In vitro antibacterial activity in the extracts of solanum nigrum. Indian Streams Research Journal. 2012;7(2):1-4.
[9] Torrungruang K, Vichienroj P, Chutimaworapan S. Antibacterial activity of mangosteen pericarp extract against cariogenic *Streptococcus mutans*. CU Dent J. 2007;30:1-10.
[10] Vaishnavi S, Chaly PE, Girija AS, Raghuraman R. Antimicrobial activity of gotukola leaves and neem leaves- A comparative in vitro study. J Ayurveda Hol Med. 2015;3(3):11-15.
[11] Sridhar TM, Josthna P, Naidu CV. In vitro antibacterial activity and phytochemical analysis of solanumnigrum (Linn.)- An important antiulcer medicinal plant. J Exp Sci. 2011;2(8):24-29.
[12] Laboratory Methods and Strategies for Antimicrobial Susceptibility Testing. In: Forbes B A, Sahm DF, Weissfeld AC. (ed.) Bailey and Scott's Diagnostic Microbiology. 12th Ed. St. Louis, MO: Mosby Elsevier; 2007:187-214.
[13] Mathur S, Mathur T, Shrivastava R, khatri R. Chlorhexidine: The gold standard in chemical plaque control. Natl J Physiol Pharm Pharmacol. 2011;1(2):45-50.
[14] Yogananth N, Buvaneswari S, Muthezhilan R. Larvicidal and antibacterial activities of different solvent extracts of solanumnigrum. Global J Biotech Biochem. 2012;7(3):86-89.
[15] Suksamrarn S, Suwannapach N, Ratananukul P, Aroonlerk N, Suksamrarns

A. Xanthones from green fruit hulls of *Garcinia mangostana*. J Nat Prod. 2002;65(5):448-56.
[16] Pandey MM, Rastogi S, Rawat AKS. Indian traditional Ayurvedic system of medicine and nutritional supplementation. Evid Based Complement Alternat Med. 2013;37:1-12.
[17] Collee JG, Dunguid JP, Fraser AG, Marmion BP, Simmons A. Laboratory control of antimicrobial therapy. In: Mackie and McCartney, editors. Practical Medical Microbiology. 14th Ed. Churchill Livingstone: Edinburgh, London, Melbourne and New York 1998:151-78.
[18] Linuma, Isoa H, Inaka T, Kobayashi Y, Shimano H, Miyauchi KI. Antibacterial activity of xanthones from guttiferaeous plants against methicillin resistant *Straphylococus aureus*. J Pharm Pharmacol. 2007;48:861-65.
[19] Fernando KMEP, Dasanayake PN. Antibacterial activity of extracts of pericarp of *Garcinia mangostana*. Vidyodaya Jcf Sci. 2006;3:99-107.

PARTICULARS OF CONTRIBUTORS:
1. Research Scholar, Meenakshi Academy of Higher Education and Research, Chennai, Tamil Nadu, India.
2. Professor, Department of Periodontology, Meenakshi Ammal Dental College, Chennai, Tamil Nadu, India.
3. Reader, Department of Microbiology, Meenakshi Ammal Dental College, Chennai, Tamil Nadu, India.
4. Associate Professor, Department of Periodontology, Rajamuthaiah Dental College, Chennai, Tamil Nadu, India.
5. Scientist III, Central Research Laboratory, Meenakshi Academy of Higher Education and Research, Chennai, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Jaideep Mahendra,
Professor, Department of Periodontology, Meenakshi Ammal Dental College, Chennai - 600095, Tamil Nadu, India.
E-mail: jaideep_m_23@yahoo.co.in

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Jun 17, 2016**
Date of Peer Review: **Jul 22, 2016**
Date of Acceptance: **Oct 17, 2016**
Date of Publishing: **Jan 01, 2017**