Original Article

# Antimicrobial activity of Curcuma amada extract on Streptococcus mutans—An *in vitro* study

Jessica Maria J, Karpagaselvi Karuppiah, Matheen Shariff, Aneek Mukherjee

Department of Oral Pathology and Microbiology, Vydehi Institute of Dental Sciences and Research Centre, Bengaluru, Karnataka, India

## Abstract

**Context:** Dental caries is prevalent in spite of widespread use of mechanical and chemical plaque control methods. Streptococcus mutans is said to have a strong background in initiation of dental caries. Hence, exceptional methods are required which would be effective against dental caries. Current era is taking people back to traditional or herbal medicine, which is said to have comparatively better healing effects than synthetic drugs in the market.

**Aim:** Determine and analyse the minimum zone of inhibition of Curcuma amada against Streptococcus mutans.

**Settings and Design:** An *In vitro* Study.

**Methods, Statistical Analysis Used:** The well diffusion method using blood agar plates was used to evaluate the antibacterial activity of 5%, 10% and 25% concentration of C. Amada extract against Streptococcus mutans in comparison with 0.2% chlorhexidine. Results were statistically analysed using independent sample t-test or Mann–Whitney U test to compare mean or median zone of inhibition between two groups. Thus, the zone of inhibition (in mm) was analysed using the mean of all the readings obtained and the level of significance at $<0.05$ was considered statistically significant at 5% of level of significance.

**Results:** Maximum zone of inhibition was found to be with C. amada compared to corresponding concentration of 0.2% chlorhexidine. Thus, inhibitory effect of C. amada is significantly better than 5%, 10% and 25% chlorhexidine mouthwash. The inhibitory effect increases as the concentration increases.

**Conclusions:** The antibacterial activity of C. amada against Streptococcus mutans raises the possibility of incorporating it in various dental therapeutic agents.

**Keywords:** Antimicrobial activity, Curcuma amada, ethanolic extract, Streptococcus mutans

**Address for correspondence:** Dr. Karpagaselvi Karuppaih, Professor and HOD, Department of Oral Pathology and Microbiology, Vydehi Institute of Dental Sciences and Research Centre #82 EPIP Area, Whitefield, Bengaluru - 560 066, Karnataka, India.
E-mail: selvisanjai@gmail.com

**Submitted:** 28-Dec-2022, **Revised:** 14-Mar-2023, **Accepted:** 13-Apr-2023, **Published:** 13-Jul-2023

## INTRODUCTION

Dental caries is a microbial disease of the calcified tissues of the teeth and characterized by demineralization of inorganic portion and destruction of the organic substances of tooth.[1]

Dental caries remains the most prevalent disease of both children and adults even after widespread use of toothbrushes and toothpaste.[2] Hence, there is an emerging need to find a novel method which is more effective against

| Access this article online | |
|---|---|
| Quick Response Code: | Website: www.jomfp.in |
| | DOI: 10.4103/jomfp.jomfp_551_22 |



**How to cite this article:** Maria JJ, Karuppiah K, Shariff M, Mukherjee A. Antimicrobial activity of curcuma amada extract on streptococcus mutans—An *in vitro* study. J Oral Maxillofac Pathol 2023;27:302-6.

dental caries. Literature suggests a strong involvement of Streptococcus mutans (S. mutans) in the initiation of dental caries.[1]

Chlorhexidine gluconate is one of the commercially available chemical mouthwashes, which is already proven to be an antibiotic and disinfectant. It is effective antimicrobial at 0.12 to 0.2% concentration and is used as gold standard control in various studies though it does have some side effects like permanent staining of teeth, mucosal erosion and dysgeusia.[3]

Medicinal plants have been used worldwide, since ages, with people preferring them due to easy availability, low cost, biocompatibility and lesser side effects compared to synthetic drugs. These include plants like neem, clove, babool, turmeric, etc., which have shown effective antimicrobial activity. Recently, there is a trend for use of herbal products in oral health care due to the presence of phytochemicals like alkaloids, essential oils, resins, glycosides, tannins and steroids.[4] One such plant with wide range of phytochemicals is Curcuma amada.

Curcuma amada Roxb. (Mango ginger) is a unique rhizome with morphological resemblance to ginger (zingiber *officinale*) but imparts a mango (*mangifera indica*) flavour. It belongs to the Zingiberaceae family and is known for preservative and medicinal values. The Ayurveda and Unani system of medicine has described its importance as appetizer, alexiteric, antipyretic, aphrodisiac and laxative. The name of this yellow-coloured spice is derived from Arabic word Kurkum which means yellow.[5-7]

Mango ginger when subjected to antibacterial guided purification by repeated silica gel chromatography yielded a pure compound after which it was named as amadaldehyde.[7] Various studies have suggested it as a novel compound which exhibits antibacterial activity against a wide range of organisms like Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi, Klebsiella pneumoniae, Enterobacter aerogenes, Proteus mirabilis, Yersinia enterocolitica, Micrococcus luteus, Staphylococcus aureus, Enterococcus faecalis, Bacillus subtilis, Bacillus cereus and Listeria monocytogenes.[6,7]

To our best of knowledge, there are no studies on the effect of C. amada on S. mutans. So, the aim of this study is to find the antimicrobial property of C. amada against Streptococcus mutans.

## SUBJECTS AND METHODS

An *in vitro* study to compare the effectiveness of 5%, 10% and 25% Curcuma amada extract with 5%, 10% and 25% of 0.2% chlorhexidine gluconate on S. mutans was conducted after the approval of institutional ethics committee.

Raw C. amada [Figure 1a]. The ethanol extract of C. amada was prepared by drying 200 gm of C. amada for 15 days [Figure 1b]. Then it was blended to get the powder. This was weighed into 5g, 10g and 25g and transferred into a sterile beaker. To each beaker, 100 ml of ethanol was added and soaked for 48 hrs. The solution was centrifuged at 2000 rpm for 10 minutes [Figure 2a], and then the supernatant was filtered using a 0.45 mm membrane filter [Figure 2b]. The ethanol extract was prepared at 5%, 10%, 25% concentration and is stored at 20 degrees Celsius.[8]

### Preparation of culture media

The strains of S. mutans (ATCC 25175 HIMEDIA) were cultured on nutrient broth [Figure 3a] by incubation at 37 degrees Celsius for 24 hrs and then were streaked onto the nutrient agar plate [Figure 3b] using loop for a nutritious growth.[8]

Preparation of 0.2% chlorhexidine gluconate aqueous extract

The 0.2% chlorhexidine gluconate mouthwash was weighed into 5 ml, 10 ml and 25 ml amounts and was transferred into labelled beakers, to which 95 ml, 90 ml and 25 ml of distilled water were added, respectively. The mixture was then shaken well.[8]

### Ditch plate method

The solid agar plate (60 numbers) was punched with wells having a diameter of 7 mm [Figure 3c] streaked under laminar flow cabinet, and the wells were filled with extracts of various concentrations. Separate plates for C. amada and 0.2% chlorhexidine gluconate were used, with different concentrations. The plates were incubated at 37°C for 48h. After incubation, the zone of inhibition [Figure 4] was measured in millimetres


**Figure 1:** (a) Dried 200 gm of C. amada for 15 days. (b) Curcuma amada powder is diluted with ethanol and kept aside for 48 hrs

using Vernier callipers.[8] Triplicate methods were used to eliminate all the possible errors.

## RESULTS

For statistical analysis—Data was analysed using SPSS software (version 19). *P* value <0.05 is considered as statistically significant. Since the data is not normal, Nonparametric test, Mann–Whitney U test is performed to compare median values of minimum zone of inhibition at 5%, 10% and 25% between Curcuma amada and chlorhexidine mouthwash.

After incubation of the plates at 37 degrees for 48 hours, the zone of inhibition was measured in millimeter using Vernier calliper. The following readings were obtained. The inhibitory effect of 5%, 10%, 25% C. amada is significantly better than chlorhexidine mouthwash (P < 0.001).

The minimum zone of inhibition at 5% of C. amada the median value was found to be 4.90, which is within the range of 4.40–5.00. The minimum zone of inhibition at 10% of C. amada the median value was found to be 8.60, which is within the range of 8.20–8.70. The minimum zone of inhibition at 25% of C. amada the median value was found to be 11.9, which is within the range of 11.50–12.0. The minimum zone of inhibition at 5% of chlorhexidine mouthwash the median value was found to be 1.90, which is within the range of 1.40–2.00. The minimum zone of inhibition at 10% of chlorhexidine mouthwash the median value was found to be 3.60, which is within the range of 3.30–3.90. The minimum zone of inhibition at 25% of chlorhexidine mouthwash the median value was found to be 5.80, which is within the range of 5.60–6.10. The data obtained is tabulated in Table 1a.

The minimum zone of inhibition at 5% median value is 4.90 for the Curcuma amada group and 1.90 for the mouthwash group, which is found to be statistically significant.

The minimum zone of inhibition at 10% median value is 8.60 for the Curcuma amada group and 3.60 for the mouthwash group, which is found to be statistically significant. The minimum zone of inhibition at 25% median value is 11.90 for the Curcuma amada group and 5.80 for the mouthwash group, which is found to be statistically significant [Table 2a]. The inhibitory effect increases as the concentration increases (P < 0.001) [Graph 1a].

## DISCUSSION

The literature review describes the use of many plants with beneficial antimicrobial activity against Streptococcus

**Figure 2:** (a) The solution was centrifuged at 2000 rpm for 10 minutes. (b) The supernatant was filtered using a 0.45 mm membrane filter

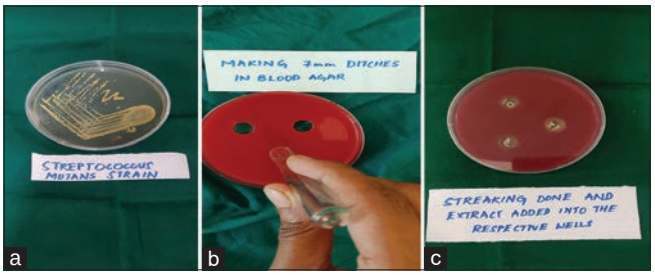



**Figure 3:** (a) S. mutans streaked onto nutrient agar plate. (b) Onto the solid agar plate (60 numbers) wells of diameter 7 mm were punched under laminar cabinet flow. (c) Wells were filled with extracts of various concentrations

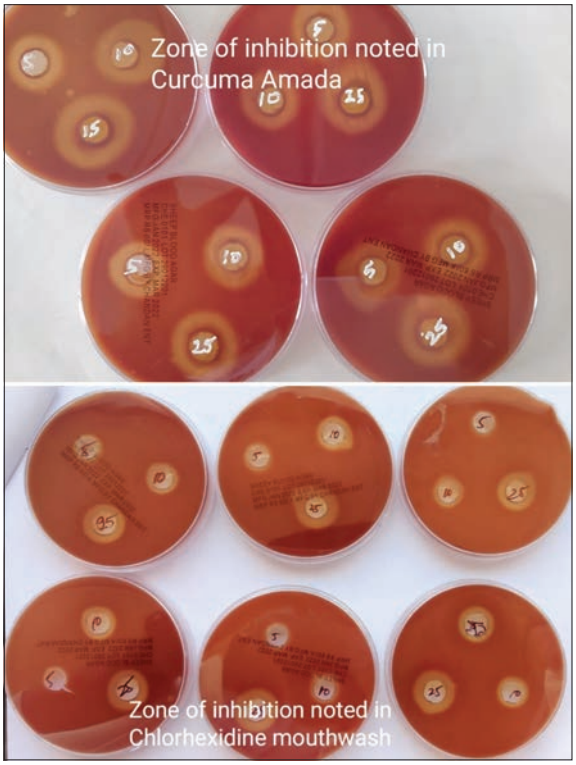


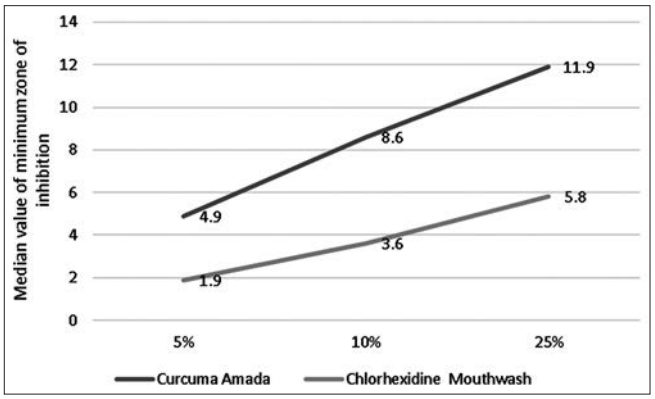

**Figure 4:** Zone of inhibition noted and measured

**Table 1a: Descriptives of minimum zone of inhibition across the groups**

| Minimum zone of inhibition | Curcuma amada (*n*=30) | | | | | Chlorhexidine mouthwash (*n*=30) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $Q_1$ | Median | $Q_3$ | Minimum | Maximum | $Q_1$ | Median | $Q_3$ | Minimum | Maximum |
| 5% | 4.80 | 4.90 | 4.90 | 4.40 | 5.00 | 1.80 | 1.90 | 2.00 | 1.40 | 2.00 |
| 10% | 8.50 | 8.60 | 8.70 | 8.20 | 8.70 | 3.50 | 3.60 | 3.80 | 3.30 | 3.90 |
| 25% | 11.8 | 11.9 | 12.0 | 11.50 | 12.0 | 5.70 | 5.80 | 5.90 | 5.60 | 6.10 |

**Table 2a: Comparison of minimum zone of inhibition at 5%,10 %and 25% across the groups**

| Statistic | Minimum zone of inhibition | | |
|---|---|---|---|
| | 5% | 10% | 25% |
| Mann–Whitney U | 0 | 0 | 0 |
| *Z* | -6.72 | -6.71 | -6.72 |
| *P* | <0.001 | <0.001 | <0.001 |




**Graph 1a:** Median value of minimum zone of inhibition between the groups

mutants.[9,10] C. amada has been described as promising spice with various biologic activities as anti-inflammatory, antimicrobial, antifungal, anticancer, antitubercular and platelet inhibitory properties due to the presence of multiple bioactive phytochemicals.[5-7]

The studies have shown that ethanolic extract of C. amada against various tested microorganisms was most effective compared to other phenolic extracts (acetone, chloroform, ether and carbon tetrachloride) and aqueous extract.[6,7,11]

- While there are studies that have demonstrated *in vitro* antimicrobial activity of Curcuma amada Roxb. against Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli and Pseudomonas aeruginosa, there is no study in literature against S. mutans.[11]

So, in this present study ethanol extract C. amada was used to assess its antimicrobial property against Streptococcus mutans in comparison with 0.2% chlorhexidine mouthwash as a control. Ditch plate method of bacterial culture was used and obtained mean zone of inhibition at value of 5%, 10% and 25% C. amada and 0.2% chlorhexidine mouthwash were 4.8133 and 1.8533 mm, 8.5600 mm and 3.6600,11.87 mm and 5.84 mm, respectively.

In the present study, as the concentration of C Amada ethanolic extract increases, its antimicrobial effect also increases.

The present study demonstrates antimicrobial activity of C. amada against S. mutans, which is similar to findings of previous studies against other microorganisms that have shown the presence of its bioactive compound amadaldehyde, being responsible for its antibacterial effect.[6,11,12]

This study done using the ethanolic extract is a basic one. Bioactive compounds can be obtained in a pure form using high-performance liquid chromatography, and henceforth, further *in vivo* studies can be planned to explore the vast antimicrobial activity of Curcuma amada.

Furthermore, in the present study, the median value of minimum zone of inhibition of C. amada against S. mutans was found to be much higher than 0.2% chlorhexidine, which validates it being potent antimicrobial, a fact known from previous studies against other microorganisms.[12]

## CONCLUSION

From the present *in vitro* study, it can be concluded that ethanolic extract of C. amada has potent antibacterial activity against S. mutans. Future, *in vivo* studies can be planned for demonstrating effectiveness of C. amada as it is more potent than chlorhexidine mouthwash which is commonly referred to as the 'The gold standard' in anti-plaque agents and hence can be used as an alternative in lieu of synthetic drugs. Furthermore, the mango taste of C. amada can be a refreshing change when used in mouthwashes and dentifrices compared to chemically available strongly flavoured synthetic mouthwashes and dentifrices.

**Acknowledgement**

The authors would like to acknowledge Mrs. Vidya Lokesh from the department of pharmacy, Mrs. Clara from the department of microbiology and Ms. Supriya (biostatistician) from the department of community medicine.

**Financial support and sponsorship**

All authors would like to acknowledge RGUHS Karnataka for providing UG grant (Proposal number-UG21DEN117, Year-21-22).

**Conflicts of interest**

There are no conflicts of interest.

## REFERENCES

1. Sivapathasundharam B, Raghu AR. Dental caries. In: Sivapathasundharam B, editor. Shafer's Textbook of Oral Pathology, 9th Edition, [An adaptation of A Textbook of Oral Pathology, 1983, 4e, Elsevier Inc]. India: Elsevier RELX India Pvt Ltd; 2020. p. 369-403.
2. Pandey P, Nandkeoliar T, Tikku AP, Singh D, Singh MK. Prevalence of dental caries in the Indian population: A systematic review and meta-analysis. J Int Soc Prev Community Dent 2021;11:256-65.
3. Balagopal S, Arjunkumar R. Chlorhexidine: The gold standard antiplaque agent. J Pharm Sci Res 2013;5:270.
4. Salman BN, Vahabi S, Rad MM. Use of herbs and medicinal plants in dentistry: A review. J Dent Sch 2017;35:133-49.
5. Rao MR, Reddy IB, Gopal SR, Bhaskar D, Ramana T. A comparative study of antimicrobial activity of Curcuma amada and Alpinia galanga of Zingiberaceae family. Asian J Chem 2008;20:5293-300.
6. Policegoudra RS, Rehna K, Rao LJ, Aradhya SM. Antimicrobial, antioxidant, cytotoxicity and platelet aggregation inhibitory activity of a novel molecule isolated and characterized from mango ginger (Curcuma amada Roxb.) rhizome. J Biosci 2010;35:231-40.
7. Policegoudra RS, Aradhya SM, Singh L. Mango ginger (Curcuma amada Roxb.)–A promising spice for phytochemicals and biological activities. J Biosci 2011;36:739-48.
8. Sajankumar RP, Hegde V, Shetty PJ. Antimicrobial effectiveness of Neem (Azadirachta indica) and Babool (Acacia nilotica) on Streptococcus mutans: An *in vitro* study. J Indian Assoc Public Health Dent 2015;13:517.
9. Mathai K, Anand S, Aravind A, Dinatius P, Krishnan AV, Mathai M. Antimicrobial effect of ginger, garlic, honey, and lemon extracts on streptococcus mutans. J Contemp Dent Pract 2017;18:1004-8.
10. Azizi A, Aghayan S, Zaker S, Shakeri M, Entezari N, Lawaf S. *In vitro* effect of zingiber officinale extract on growth of streptococcus mutans and streptococcus sanguinis. Int J Dent 2015;2015:489842. doi: 10.1155/2015/489842.
11. Kaur R, Kaur B, Suttee A, Kalsi V. Comparative assessment of *in vitro* antimicrobial activity of Curcuma caesia Roxb. and Curcuma amada Roxb. Asian J Pharm Clin Res 2018;11:94-7.
12. Mahadevi R, Kavitha R. Phytochemical and pharmacological properties of curcuma amada: A review. Int J Res Pharm Sci 2020;11:3546-55.