

**QUANTIFICATION AND PURIFICATION OF MANGIFERIN FROM 11 INDIAN
MANGO (*MANGIFERA INDICA* L.) VARIETIES AND ASSESSMENT OF
ANTIMICROBIAL EFFICACY OF MOST QUANTIFIED VARIANT OF MANGO
AGAINST THE PERIODONTAL MICROBES: AN INVITRO AND INVIVO STUDY**

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Received: Nov 29, 2025

Accepted: Dec 30, 2025

Published: Jan 3, 2026

ABSTRACT:

Aim: The aim of the present study was to quantify the amount of Mangiferin in 11 different varieties of Indian mango to assess the antimicrobial efficacy of Mangiferin extract from the most quantified variant of mango against the periodontal microbes in chronic gingivitis.

Material and Method: Quantification of mangiferin in fruit peel, pulp, and seed kernel was carried out in 11 Indian mango cultivars. Maximum mangiferin was recorded in Ladvo(47.02mg/g) variety. Efficient purification of mangiferin from fruit peel of Ladvo variety was then established using combination resin chromatography. The Purified extract was tested against standard ATCC Strains of *S. mutans*, *P. gingivalis*, AAC. The Minimum Inhibitory Concentration was determined by serial broth dilution. The Minimum Bactericidal Concentration was obtained by sub-culturing method. Twenty systemically healthy patients were randomly allocated to test group [(Ladvo mouthwash = 10 patients)] or the control group [(Chlorhexidine mouthwash = 10 patients)]. Clinical parameters recorded were: Plaque Index (PI) Gingival Index (GI) at baseline and 6 weeks post therapy.

Results: The extract showed satisfactory inhibitory (13.5 % , 6.45 % and 3.4 % respectively) and bactericidal effect (95% , 35% and 65 % respectively) against all test pathogens. The mean PI score at baseline for Test sites was 0.89 ± 0.13 and after 6 weeks was 0.63 ± 0.14 . The mean PI score at baseline for Control sites was 0.95 ± 0.08 and after 6 weeks was 0.74 ± 0.16 . The mean GI score at baseline for Test sites was 0.86 ± 0.11 and after 6 weeks was 0.68 ± 0.13 The mean GI score at baseline for Control sites was 0.94 ± 0.05 and after 6 weeks was 0.76 ± 0.07 .

Conclusion: The study confirms the antimicrobial effect of Mangiferin against the periodontal microbes and thus its potential usage as a therapeutic agent in chronic gingivitis.

KEY WORDS : Mangiferin extract , Ladvo , Chronic gingivitis, Periodontal microbes

INTRODUCTION:

Periodontal diseases are long-lasting inflammatory conditions marked by the erosion of connective tissue, absorption of alveolar bone, and the development of periodontal pockets due to a complex interplay between pathogenic bacteria and the body's immune reaction. Currently, periodontitis, its severe stage, is thought to be triggered and sustained by a limited group of mainly Gram-negative, anaerobic, or micro-aerophilic bacteria that inhabit below the gum line. These bacteria lead to tissue damage through direct release of toxins and by stimulating the body's defense mechanisms, resulting in inflammation. ⁽¹⁾

Oxidative stress stands out as a key factor in understanding the pathophysiology of inflammation seen in periodontal diseases, as highlighted in recent comprehensive reviews. Reactive oxygen species (ROS) play crucial roles in various bodily functions. However, when the antioxidant system fails to effectively neutralize their effects, it can lead to tissue and cell damage, exacerbating periodontal deterioration. ⁽²⁾

As a result, inflamed tissues show a range of molecular oxidative species, such as reactive nitrogen species (RNS) and reactive oxygen species (ROS). This is particularly observed in various types of periodontal disease, where functionally activated polymorphonuclear leukocytes (PMN) display increased production of ROS. Consequently, the heightened activity of pro-inflammatory transcription factors in inflamed periodontal tissues leads to a depletion of antioxidants and the generation of reactive molecular species. Furthermore, some oxidation products can enhance neutrophil adhesion chemotaxis, and priming in highly reactive neutrophils, potentially amplifying the damaging effects of oxidative stress. ⁽³⁾

Considering the growing awareness of oxidative stress's impact on periodontal disease, exploring host-modulatory therapies with antioxidants could prove intriguing in halting or mitigating the degradation of both soft and hard periodontal tissues. Antioxidants, characterized by their ability to delay or prevent oxidation of susceptible substrates when present in lower concentrations than those substrates, serve as a robust defense mechanism against oxidative damage triggered by reactive molecular species. Their primary function involves eliminating or deactivating these reactive molecular species, as well as repairing any resulting damage. ⁽⁴⁾

Many natural products hold significant potential in preventing and treating periodontal diseases, as they often possess both anti-oxidative and anti-inflammatory properties. ⁽⁵⁾

Contemporary global complementary and alternative practices in dentistry envisage to manipulate molecules from traditionally used plant derivatives as adjuncts. *Mangifera indica* L. belongs to Anacardiaceae. Fully ripe mango has high amount of bioactive compounds such as phenolic compounds, β Carotene, Vitamin C. Among the various phenolic compounds, Mangiferin is a distinct one as it contains anti-inflammatory, immunomodulatory and antioxidant properties.

Among the plethora of oral hygiene products, chlorhexidine has been the mouthwash of choice but has various side effects such as taste alteration, staining etc. Alternative agents are therefore of particular interest which will act effectively against the causative organisms. ⁽⁶⁾

The aim of the present study was to quantify the amount of Mangiferin in 11 different varieties of Indian mango to assess the antimicrobial efficacy of Mangiferin extract from the most quantified variant of mango against the periodontal microbes in chronic gingivitis.

METHOD AND MATERIAL:

The participants for this study were recruited from the outpatient department of periodontology at Swargiya Dadasaheb Kalmegh Smruti Dental college and Hospital Hingna, Nagpur, India, following approval from the institutional ethics committee. Each participant provided both verbal and written informed consent prior to inclusion in the study. The study was designed as a single blinded randomized clinical trial. Twenty systemically healthy patients were randomly allocated to either the test group [G1 (Ladvo mouthwash = 10 patients)] or the control group [G2 (Chlorhexidine mouthwash = 10 patients)].

Inclusion criteria:

Both male and female patients of age Group 30-50 years having chronic gingivitis after SRP and willing to give informed consent are included in the study.

Exclusion criteria:

Patients taking medications or having treatment which may affect mucosal healing and are on antibiotic therapy since past 6 months were excluded from the study.

Quantification:

the mangoes were dissected and their peels were separated

the peel sample were washed shade dried for a day and dried completely in an oven at 38 c

the dried peels were coarsely powdered using a rotary grinder sieved through BSS mesh 85, and stored in airtight plastic container to be used for HPLC analysis.

Solvent was removed under reduced pressure and the residue was reconstituted in HPLC grade water making the total volume 10 ml in a volumetric flask

Exactly 0.5 ml of this diluted extract was pipetted out in a 5 ml volumetric flask and the volume was made up using HPLC grade water and used directly for HPLC analysis

Preparation of bacterial suspension:

A total of three periodontal pathogens were used in this study. *Streptococcus mutans* (MTCC25175) from microbial type culture collection and gene bank Chandigarh India and *Aggregatibacter Actinomycetemcomitans* (ATCC 29522) and *Porphyromonas gingivalis* (ATCC 33277) from American type cell culture.

Microbial subculturing:

Isolated microorganism were kept on glycerol broth at -80 c

Before testing antibacterial activity *P. gingivalis* and *Aa* was subcultured on blood agar supplemented with hemin and vit K (Himedia Mumbai India) and *S. mutans* was subcultured on brain heart infusion agar plate

Determination of MIC:**Serial broth dilution method**

200 µl of the following media was added from 2nd to 10th tube by using a micropipette

Brain heart infusion broth was added to streptococcus mutans (MTCC)

200 µl of BHI broth supplemented with 2% horse serum was added for Porphyromonas gingivalis (ATCC-33277) and Aggregatibacter Actinomycetemcomitans (ATCC 29523)

Determination of MBC:

Agar plate sub culture streaking method

The minimum bactericidal concentration value was determined by sub culturing on BHI or S-BHI agar plates of each well component that was not showing visible indicator changes

The least concentration which showed no visible growth on the agar plate after incubation period was considered as the MBC value

Preparation of Ladvo Mouthwash:

Herbal mouthwash was prepared by suspension of prepared powder in polyethylene glycol and distilled water and was allowed to evaporate to get the final concentrate. The final concentrate was then diluted and as a flavoring agent, cardamom powder and aqueous mint extract was added.

Methods of data collection:

A double-blinded, parallel design, randomized controlled trial enrolled a total of 20 patients who met the inclusion criteria. The allocation of patients into two groups was performed by the examiner (SG) using a computer-generated randomization sequence. The patients were then assigned to the following interventions.

Group 1 (test group): Received Ladvo mouthwash = 10 patients

Group 2 (control group): Received Chlorhexidine mouthwash = 10 patients

Pre-clinical protocol:

All measurements were made with UNC 15 periodontal probe and recorded to the nearest millimeter by the same calibrated examiner.

Clinical protocol:

Clinical parameters assessed includes, Plaque index (PI) and Gingival index (GI) at baseline and at 6 weeks interval. All the patients received complete oral prophylaxis, including scaling and root planing (SRP), using ultrasonic scaler (Woodpecker, UDS-N1, Guilin, China)

Treatment protocol:

At the baseline, all patients underwent full mouth scaling and root planning. The patients were randomly divided into two Groups: Group A and Group B. Group A received treatment consisting of scaling and root planning (SRP) along with the adjunctive use of Ladvo mouthwash, while Group B received Chlorhexidine mouthwash.

Statistical analysis:

Data was coded and analysed in statistical software STATA, version 10.1, 2011 by STATA CORP, TEXAS, USA. Descriptive statistics included mean and standard deviation for quantitative parameters, frequency and percentage for qualitative parameters. Inferential statistics included test for significance and p values.

Within the group comparisons for change in mean at baseline and 6 weeks was performed with paired t-test in each treatment group. Between the group comparison in mean change in two treatment groups will be performed by two independent sample t-tests un-paired t-test. Person's chi-square test was also used to compare difference in proportion for qualitative parameters in two groups.

P-values less than 0.05 will be considered statistically significant in all the comparisons.

RESULT:

A total of 20 patients (10 test Group and 10 control Group) completed the study and there was no loss of sample in follow up.

The Comparison of minimum inhibitory concentration are presented in Table 1.

Table 2 shows comparison of Minimum bactericidal concentration.

The ratio of the MIC and MBC concentrations indicated that the extract is bacteriostatic at lower concentrations and bactericidal at relatively higher concentrations.

Table 3 shows comparison of mean score of different study variables at baseline between two groups done using paired t-test for all two groups. At baseline the mean value of the different study variables did not show any statistical significance.

Table 4 shows comparison of mean PI, GI scores between test and control groups at baseline and 6 weeks. The mean scores for group A and B from baseline to 6 weeks were statistically significant. The intragroup comparison showed statistically significant difference in all two groups at baseline and 6 weeks.

TABLE 1: Comparison of minimum inhibitory concentration

TEST MICROORGANISM	PERCENTAGE CONCENTRATION	TOTAL MIC VALUE (ug/ml)
STREPTOCOCCUS MUTANS	14.5%	50(ug/ml)
PORPHYROMONAS GINGIVALIS	7.25%	25(ug/ml)
AGGREGAGIBACTER ACETINOMYCETEMCOMITANS	4.15%	10(ug/ml)

TABLE 2: Comparison of MINIMUM BACTERIAL CONCENTRATION

TEST MICROORGANISM	PERCENTAGE CONCENTRATION	TOTAL MBC VALUE (ug/ml)
STREPTOCOCCUS MUTANS	25%	100(ug/ml)
PORPHYROMONAS GINGIVALIS	18.30%	50(ug/ml)
AGGREGAGIBACTER ACETINOMYCETEMCOMITANS	8.15%	25(ug/ml)

TABLE 3: Comparison of mean score of different study variables at base line between two groups.

Variables	Group	Value
PI	Group A	0.89± 0.13
	Group B	0.95 ± 0.08
GI	Group A	0.86 ± 0.11
	Group B	0.94 ± 0.05

TABLE 4: Comparison of mean score of different study variables at baseline and 6 weeks.

Variables	Group	Time	Mean value
PI	GROUP A	BASELINE	0.89± 0.13
		6 WEEKS	0.63 ± 0.14
	GROUP B	BASELINE	0.95 ± 0.08
		6 WEEKS	0.74 ± 0.16
GI	GROUP A	BASELINE	0.86 ± 0.11
		6 WEEKS	0.68 ± 0.13
	GROUP B	BASELINE	0.94 ± 0.05
		6 WEEKS	0.76 ± 0.07

DISCUSSION:

Chronic gingivitis, the early stage of periodontal disease, causes inflammation and damage to tooth-supporting structures. The primary objective of periodontal therapy is to achieve long-term improvement in clinically assessed parameters. Herbal antiseptic or antibiotic agents have been introduced very widely that are effective against plaque microorganisms for the healing of gingival tissue. In 2009 Carvadh.RR et al demonstrated that the oral administration of mangiferin significantly reduced alveolar bone loss by inhibiting COX-2 expression and the rolling and adhesion of leukocytes while maintaining normal lipoxin A(4) levels. ⁽⁷⁾

Mangiferin (1,3,6,7-tetrahydroxy-2- [3,4,5-trihydroxy-6-(hydroxymethyl) oxan-2-yl]xanthen-9-one) is a natural C-glucoside xanthone, which has multiple pharmacological activities, including inflammatory inhibition and immune modulation. H li et al in 2017 concluded that the mangiferin extracted from mango ameliorates Porphyromonas gingivalis induced experimental periodontitis. ⁽⁸⁾ Mangiferin in particular, detected at high concentrations in young leaves (Coite = 172 g/kg), in bark (Momika = 107 g/kg), and in old leaves (Itamaraka = 94 g/kg), shows an exceptionally strong antioxidant capacity. Bhavna Sharma 2017 demonstrated that the leaves of thirty mango varieties were interesting sources of the pharmacologically active C-glycosyl xanthone, mangiferin. From the percentage amount of isolated mangiferin, variety Ladvo was a good source of mangiferin which was similar to our studies. ⁽⁹⁾

The results obtained from the current study demonstrated that both therapeutic modalities, i.e., the use of mangiferin mouthwash as an adjunct to non-surgical periodontal therapy, led to statistically significant improvements in all clinical parameters examined after 6 weeks of initiating the treatment.

CONCLUSION:

The study confirms the antimicrobial effect of Mangiferin against the periodontal microbes and thus its potential usage as a therapeutic agent in chronic gingivitis.

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