

A Comparative Clinical Study on Oral Hygiene with Conventional Brushing and *Madhuca* Twig (*Madhuca longifolia*): Phytochemical Characterisation and Clinical Evidence

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Abstract:

➤ Background:

Oral hygiene is a critical component of general health. Ayurvedic tradition has long advocated the use of medicinal twigs (*Dantadhavana*) for oral care. *Madhuca longifolia* (*Mahua*), classified as the best among *Madhura rasa dravyas* by Acharya Sushruta, possesses documented antiseptic, antibacterial, and spasmolytic properties.

➤ Objectives:

To compare the effectiveness of *Madhuca* twig with conventional toothbrushing in maintaining oral hygiene, measured by the Oral Hygiene Index (OHI), and to characterise the phytochemical profile of *Madhuca* twig extract via HPLC-DAD analysis.

➤ Methods:

A prospective, non-randomised clinical study was conducted at Shri Khudadaad Dunga ji Government Ayurved College and Hospital, Raipur, enrolling 60 participants (aged 18–50 years) in two groups of 30. Group B used *Madhuca* twig

twice daily; Group A used conventional toothbrush and toothpaste twice daily for 60 days. OHI scores (Debris Index + Calculus Index) were recorded at baseline, Day 15, 30, 45, and 60. HPLC-DAD phytochemical analysis of dried *Madhuca* twig powder was performed by UltraChrom Innovatives Pvt. Ltd. (ISO 17025 accredited), Wardha.

➤ Results:

Both interventions significantly reduced OHI scores (paired t-test $p < 0.001$). Group B (*Madhuca*) achieved a 41.6% reduction (0.961 to 0.561) vs 35.6% reduction (0.983 to 0.633) in Group A. The difference between groups at Day 60 was not statistically significant ($p = 0.509$), indicating comparable efficacy. HPLC-DAD analysis confirmed nine phenolic/polyphenolic acid components (30%), saponin glycosides (6%), esterified triterpinoids (13%), and phytosterols (0.5%). Alkaloids and flavonoids were absent.

➤ Conclusion:

Madhuca twig demonstrated oral hygiene efficacy comparable to conventional brushing, with a marginally superior reduction in OHI. Its rich phenolic, saponin, and triterpinoid content provides a pharmacological basis for its traditional use. *Madhuca* twig offers a cost-effective, chemical-free, and culturally relevant alternative for oral care.

Keywords: *Madhuca Longifolia*, *Mahua Datun*, *Dantadhavana*, Oral Hygiene Index, HPLC-DAD, Ayurveda, Conventional Brushing, Phytochemical Analysis.

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I. INTRODUCTION

Oral health is an integral component of overall systemic wellbeing. According to the World Health Organization (WHO), oral health is essential to general health and quality of life, with an estimated 3.5 billion people worldwide affected by oral diseases. Oral cavity diseases cause pain, discomfort, disfigurement and can even contribute to mortality when left unaddressed.

Ayurveda, one of the oldest systems of holistic medicine, emphasises prevention over cure — encapsulated in the twin goals of '*Swasthasya Swasthya Rakshanam*' (preservation of health in the healthy) and '*Aturasya Vikar Prashamanam*' (alleviation of disease in the diseased). *Dinacharya* (daily regimen) forms the cornerstone of Ayurvedic preventive medicine, within which *Dantadhavana* (oral cleansing with herbal twigs) occupies an important position.

Acharya Charaka advocates the use of herbal twigs including *Neem* (*Azadirachta indica*), *Madhuca* (*Madhuca longifolia*), *Khadir* (*Acacia catechu*), and *Karanja* (*Pongamia pinnata*) — for oral hygiene, noting that twig use removes *mukha durgandha* (oral malodour), cleanses debris from teeth and tongue, and stimulates taste buds. Acharya Sushruta specifically identifies *Madhuca* as the best herb within the *Madhura rasa gana* (sweet-tasting group) and recommends *Madhura rasa dantadhavani* for *Pittaja* oral conditions such as *mukhapaka* (oral ulcers) and *mukhadaha* (burning sensation in the mouth).

Madhuca longifolia (Lam.) J.F. Macbr., commonly known as *Mahua*, is a large deciduous tree of the Sapotaceae family distributed widely across central and peninsular India. Its twigs have been used traditionally for oral hygiene by

tribal and rural populations. The plant is documented to possess antiseptic, antibacterial, spasmolytic, and insecticidal properties. Despite its extensive traditional use, rigorous clinical evidence comparing *Madhuca* twig to modern conventional brushing methods is limited.

This study was designed to bridge that gap, combining a prospective clinical comparison of Oral Hygiene Index (OHI) outcomes with an independent HPLC-DAD phytochemical characterisation of *Madhuca* twig extract, providing both clinical and mechanistic evidence for its oral health benefits.

➤ Aims and Objectives

• Aim

- ✓ To compare the effectiveness of conventional toothbrushing with the use of *Madhuca* (*Madhuca longifolia*) twigs in maintaining oral hygiene among healthy individuals aged 18–50 years.

• Objectives

- ✓ To measure and compare gingival health and overall oral cleanliness between individuals using conventional brushing (Group A) and those using *Madhuca* twigs (Group B) using the Oral Hygiene Index.
- ✓ To determine the effectiveness of *Madhuca* twigs in maintaining or improving oral hygiene in comparison to conventional toothbrushing over a 60-day intervention period.
- ✓ To evaluate the change in OHI scores at multiple time points (Day 0, 15, 30, 45, and 60) within and between both groups.

- ✓ To characterise the phytochemical constituents of *Madhuca* twig extract using HPLC-DAD analysis and correlate identified compounds with observed oral health effects.
- ✓ To identify additional benefits or limitations of *Madhuca* twig use, including cost-effectiveness, accessibility, and cultural relevance.

II. MATERIALS AND METHODS

➤ Study Design

A prospective, non-randomised, comparative clinical study was conducted over a duration of 60 days, with follow-up assessments every 15 days.

➤ Study Centre

Shri Khudadaad Dunga ji Government Ayurved College and Hospital, Raipur, Chhattisgarh.

➤ Sample Size and Grouping

A total of 60 individuals meeting inclusion criteria were enrolled and divided into two groups of 30:

- Group B (*Madhuca* Twig): Participants were instructed to use freshly prepared *Madhuca* twig twice daily (morning and before bedtime). One end of the twig was chewed to form a brush-like bristle surface, which was used to clean teeth in horizontal and vertical strokes.
- Group A (Conventional Brushing): Participants used a commercial manual toothbrush with toothpaste twice daily following standard technique.

➤ Inclusion Criteria

- Adults aged 18–50 years of any gender, religion, or socioeconomic background
- Generally healthy individuals without active dental caries, severe gingivitis, or periodontitis
- No major dental procedures within the preceding 3 months
- Non-smokers and non-tobacco users
- Willingness to provide written informed consent

➤ Exclusion Criteria

- Active dental caries, severe gingivitis, periodontal disease, or oral cancer
- Chronic systemic illnesses or current use of antibiotics, anti-inflammatory drugs, or medications affecting oral flora
- Users of natural twigs or non-conventional oral hygiene methods at enrolment
- Presence of dental caps or fillings
- Age below 18 or above 50 years

➤ Assessment Criteria — Oral Hygiene Index (OHI)

The simplified Oral Hygiene Index (OHI-S) of Greene and Vermillion was used, comprising the Debris Index (DI) and the Calculus Index (CI): $OHI = DI + CI$. Six teeth surfaces (upper right buccal, upper anterior buccal, upper left buccal, lower left lingual, lower anterior lingual, lower right lingual) were examined and scored on a 0–3 scale for each component. Assessments were performed at baseline (Day 0), Day 15, Day 30, Day 45, and Day 60.

➤ Phytochemical Analysis (HPLC-DAD)

200 mg of dried fine powder of *Madhuca* twig was extracted in 5 ml of acetonitrile–methanol–water (2:2:1, v/v), kept overnight at room temperature, placed in a water bath at 55°C for 10 minutes, and ultrasonicated for 10 minutes. The extract was filtered through a 0.45 µm nylon filter. Analysis was performed on a Shimadzu HPLC-UV system using an ACE-AR C18 column (5 µm, 150 × 4.6 mm) with a water–acetonitrile gradient (30:70 v/v), 1.0 ml/min flow rate, 20 µl injection volume, 75-minute run time, and detection at 210 and 254 nm. The analysis was performed by UltraChrom Innovatives Pvt. Ltd., Wardha (ISO/IEC 17025:2017 accredited).

➤ Statistical Analysis

Paired samples t-test was applied for objective parameters (OHI scores within groups), and the Mann–Whitney U test was used for subjective parameters. An independent samples t-test was applied to compare OHI scores between groups at each time point. Statistical analysis was certified by Dr. Pradip Chourasia, Lecturer (Statistics), Department of Samhita Siddhanta, Shri NPA Govt. Ayurved College, Raipur.

III. DEMOGRAPHIC PROFILE OF PARTICIPANTS

➤ The Demographic Characteristics of Participants in Both Groups are Summarised Below:

Table 1 Demographic Characteristics of Participants in Both Groups are Summarised

Parameter	Group B: <i>Madhuca</i> Twig (n=30)	Group A: Conventional Brushing (n=30)
Age Range	18–50 years	18–50 years
Mean Age	28.4 years	32.9 years
Males	10 (33.3%)	6 (20%)
Females	20 (66.7%)	24 (80%)
Socioeconomic Status	Middle class (all)	Middle class (all)
Study Duration	60 days	60 days
Follow-up Intervals	0, 15, 30, 45, 60 days	0, 15, 30, 45, 60 days

Both groups were drawn from predominantly middle-class, urban participants. Group B had a higher proportion of students (reflecting the younger mean age of 28.4 years), while Group B was more gender-balanced. All participants across both groups were of middle socioeconomic status and resided in urban or peri-urban areas of Chhattisgarh.

Table 2 Oral Hygiene Index — Temporal Progression

Time Point	Group B: <i>Madhuca</i> Twig (Mean $\pm$ SD)	Group A: Conventional Brushing (Mean $\pm$ SD)
Day 0 (Baseline)	0.961 $\pm$ 0.461	0.983 $\pm$ 0.468
Day 15	0.950 $\pm$ 0.457	0.939 $\pm$ 0.443
Day 30	0.856 $\pm$ 0.450	0.811 $\pm$ 0.450
Day 45	0.583 $\pm$ 0.396	0.678 $\pm$ 0.438
Day 60	0.561 $\pm$ 0.395	0.633 $\pm$ 0.445
% Reduction	41.6%	35.6%

Group B (*Madhuca* Twig) exhibited a progressive and steeper decline in OHI, particularly between Day 30 and Day 45, suggesting a consolidation of the twig's cleansing effect over consistent use. Group A showed a comparably steady decline throughout the intervention.

➤ Statistical Analysis of OHI

Within-group analysis using paired t-test revealed that both groups achieved highly significant reductions in OHI from baseline to Day 60:

- Group B (*Madhuca* Twig): $t = 16.16$, $p < 0.0001$ — highly significant reduction of 41.6%
- Group A (Conventional Brushing): $t = 17.38$, $p < 0.0001$ — highly significant reduction of 35.6%

Table 3 HPLC-DAD Phytochemical Characterisation

Phytoconstituent Class	Components Identified	No. of Components	Contribution (%)
Aliphatic/Phenolic/Polyphenolic Acids	Tannic Acid and related phenolics	9	30%
Phytoamines/Alkaloids	Not Detected	—	—
Polyphenols/Flavonoids/Polar Antioxidants	Not Detected	—	—
Acid/Sugar/Saponin Glycosides	Mi-Saponins Glycosides, Madlongisides, Madhucoside A & B	2	6%
Sesquiterpenoids/Non-Polar Antioxidants	3-(27-Carboxyoleanyl)-octanoate esterified triterpinoid	2	13%
Phytosterols/Steroids/Triterpinoids	β -Sitosterol, Betulinic Acid, Oleanolic Acid	1	0.5%

• Key Findings of the Chromatographic Analysis Included:

- ✓ Nine phenolic/polyphenolic acid components were identified, predominantly Tannic acid, constituting approximately 30% of all detected compounds. Notably, several of these acid derivatives had not been previously reported in published literature for this species.
- ✓ Two saponin glycosides — Mi-Saponin Glycosides, Madlongisides, and Madhucoside A and B — were identified, contributing 6% of the total detected compounds.
- ✓ Two esterified triterpinoids of the 3-(27-Carboxyoleanyl)-octanoate class were detected (13% contribution), alongside minor amounts of β -Sitosterol, Betulinic acid, and Oleanolic acid (0.5%).

IV. OBSERVATIONS AND RESULTS

➤ Oral Hygiene Index — Temporal Progression

OHI scores in both groups declined progressively over the 60-day study period. The mean OHI values at each time point are presented below:

The between-group comparison at Day 60 using an independent t-test yielded $t = -0.665$, $p = 0.509$, indicating no statistically significant difference between the two groups' final OHI scores. This finding supports the null hypothesis in terms of statistical equivalence, while the numerically superior reduction in Group B suggests a clinical trend favouring *Madhuca* twig.

➤ HPLC-DAD Phytochemical Characterisation

The HPLC-DAD analysis of *Madhuca longifolia* twig extract (dried fine powder) confirmed the presence of multiple bioactive phytoconstituents:

- ✓ No alkaloids or polar flavonoids were detected in the RP-HPLC analysis.
- ✓ The IE-HPLC/NP-HPLC class separation was not performed as it was not specifically requested.

V. DISCUSSION

The results of this study demonstrate that *Madhuca* twig is a clinically effective oral hygiene tool, achieving OHI reduction comparable to conventional toothbrushing over a 60-day period. Both interventions produced statistically significant improvements, and the 41.6% OHI reduction observed in the *Madhuca* group slightly exceeded the 35.6% reduction in the conventional brushing group, though the difference did not reach statistical significance.

➤ Mechanistic Basis from Phytochemistry

The HPLC-DAD findings provide a compelling pharmacological basis for the observed clinical effects. The dominant phenolic acid fraction (30%), predominantly tannic acid, is well-established in the literature for its astringent, antibacterial, and anti-inflammatory properties. Tannic acid forms complexes with proteins on bacterial cell surfaces, disrupting membrane integrity and inhibiting the adhesion of cariogenic bacteria such as *Streptococcus mutans* to tooth surfaces — a mechanism directly relevant to debris and calculus reduction.

The saponin glycosides (Madhucoiside A & B, Madlongisides) identified at 6% concentration are known surfactants that reduce surface tension and aid in the mechanical displacement of dental plaque. They also exhibit documented antimicrobial activity. The esterified triterpinoids (13%), particularly oleanolic acid and betulinic acid, have demonstrated anti-gingivitis, anti-inflammatory, and wound-healing properties in experimental models, which may account for the gingival health benefits associated with twig use.

The absence of alkaloids and flavonoids is clinically reassuring from a safety perspective, as high alkaloid content is associated with systemic toxicity.

➤ Ayurvedic Rationale

From the Ayurvedic pharmacological perspective, *Madhuca longifolia* possesses *Madhura* (sweet) *rasa*, *Sita* (cool) *vipaka*, and *Snigdha* (unctuous) *guna*, rendering it *Vata-Pittahara* and *Raktapittahara*. Acharya Sushruta's prescription of *Madhura rasa dantadhavani* for *Pittaja* oral conditions such as *mukhapaka* and *mukhadaha* aligns well with the documented anti-inflammatory and soothing properties of the identified phytoconstituents. The cooling and astringent properties of tannins in particular correspond to the Ayurvedic concept of Pitta pacification at mucosal surfaces.

➤ Practical Advantages

Madhuca twig offers several practical benefits over conventional toothbrushing in the Indian context:

- Cost-effectiveness: *Madhuca* trees are abundant across central India. Twigs are freely available or inexpensive, making this intervention economically accessible for rural and tribal populations.
- Chemical-free oral care: Conventional toothpastes contain fluoride, sodium lauryl sulfate, and preservatives whose cumulative effects have drawn scrutiny. *Madhuca* twig offers a natural, additive-free alternative.
- Dual action: The chewing action at the twig end produces natural bristles that simultaneously deliver phytochemicals to oral tissues, combining mechanical cleaning with chemotherapeutic benefit.

- Cultural acceptability: Twig-based oral hygiene is a familiar practice in many communities across Chhattisgarh and adjoining states, facilitating compliance.

➤ Limitations

The study was non-randomised, which may introduce selection bias. The sample size of 60 participants, while adequate for this preliminary comparison, limits generalisability. Long-term outcomes beyond 60 days, effects on specific microbiological parameters, and patient-reported outcomes such as bleeding on probing or gingival scoring were not included. Future randomised controlled trials with microbiological endpoints and longer follow-up periods are warranted.

VI. CONCLUSION

This comparative clinical study demonstrates that *Madhuca* twig (*Madhuca longifolia*) achieves clinically significant improvement in oral hygiene, with a 41.6% reduction in OHI score over 60 days — marginally superior to, and statistically comparable with, conventional toothbrushing (35.6% reduction). The HPLC-DAD phytochemical analysis confirms the presence of bioactive phenolic acids (predominantly tannic acid, 30%), saponin glycosides (6%), and esterified triterpinoids (13%), providing a robust pharmacological basis for the observed clinical efficacy.

Madhuca twig thus represents a scientifically validated, traditionally grounded, and practically accessible alternative for daily oral hygiene. These findings support its integration into public health oral care recommendations, particularly for communities in central India where the tree is endemic and twig use is culturally embedded. Larger randomised trials with microbiological and long-term safety endpoints are recommended to confirm and extend these findings.

DECLARATION

The authors declare no conflicts of interest. This study was conducted as part of the MD (Ayurveda) dissertation research at Shri Narayan Prasad Awasthi Government Ayurved College, Raipur (C.G.), under the Pt. Deendayal Upadhyay Memorial Health Sciences and AYUSH University of Chhattisgarh. Informed consent was obtained from all participants prior to enrolment. Statistical methods were certified by an independent statistician.

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