International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Formulation and Evaluation of Herbal Toothpaste incorporating Camellia sinensis, Carica papaya, and Acorus calamus Extracts for Antimicrobial Efficacy against Oral Pathogens

Dinesh.S[1], Thangavel.M[2], Pavitra.P[3], Shani.S[4]

[1,3,4]Postgraduate student, Department of Microbiology, Nehru Arts and Science College, Coimbatore, India.
[2]Associate Professor, Department of Microbiology, Nehru Arts and Science College, Coimbatore, India.

ABSTRACT:
The rising prevalence of antimicrobial resistance among oral pathogens and the adverse effects associated with prolonged use of chemical-based oral care products have created a need for safer herbal alternatives. The present study aimed to evaluate the antimicrobial activity of Camellia sinensis, Acorus calamus, and Carica papaya extracts against Staphylococcus aureus, Candida albicans, and Pseudomonas aeruginosa, and to formulate and evaluate a herbal toothpaste incorporating these extracts. Phytochemical screening was performed on the aqueous extracts of the three plants. Antimicrobial activity of individual and combined extracts was assessed using the Kirby-Bauer disc diffusion method. A herbal toothpaste was formulated using the combined extract and subjected to comprehensive physicochemical and microbiological quality evaluation. The formulated toothpaste was compared with a commercially available preparation (Dabur Red). Phytochemical screening revealed the presence of phenols, flavonoids, and diterpenes in all three extracts, and saponins in green tea and papaya leaf extracts. Among individual extracts, Camellia sinensis demonstrated the highest antimicrobial activity, recording zones of inhibition of 15mm, 17mm, and 15mm against S. aureus, C. albicans, and P. aeruginosa, respectively. The combined extract exhibited synergistic activity with zones of inhibition of 17mm, 20mm, and 13mm against S. aureus, C. albicans, and P. aeruginosa, respectively. The formulated herbal toothpaste demonstrated superior antimicrobial activity against S. aureus (20mm) and C. albicans (19mm) compared to Dabur Red toothpaste (14mm and 17mm, respectively). Both toothpaste preparations were resistant to P. aeruginosa. Quality evaluation confirmed acceptable pH (8.5), foamability (50ml), spreadability (5cm), smooth texture, and complete absence of microbial growth. The formulated herbal toothpaste incorporating Camellia sinensis, Acorus calamus, and Carica papaya extracts demonstrated significant antimicrobial efficacy and satisfactory physicochemical properties, establishing its potential as a safe and effective natural alternative for oral health care.

Keywords: Herbal toothpaste, Camellia sinensis, Acorus calamus, Carica papaya, antimicrobial activity, oral pathogens, phytochemical screening, oral health care, disc diffusion, zone of inhibition.

INTRODUCTION

Oral health plays a vital role in an individual's overall well-being and enables essential functions such as eating, breathing, and speaking without discomfort. Oral health varies over the life course from early life to old age, and is essential to general health. Oral diseases are the most common non-communicable disease worldwide, affecting an estimated 3.5 billion people. Most oral health conditions are easy to treat at the early stage. Most cases are dental caries, periodontal diseases, oral thrush, and tooth loss [1].

Dental caries is a common chronic infection caused by tooth-adherent cariogenic bacteria, Streptococcus mutans, which metabolise sugar to produce acid that demineralises teeth. The term “dental caries” originated from the Latin word “caries”, which means “decay”. Dental caries is a term that refers to both the disease and the lesion [2]. In the absence of S. mutans, a wide variety of microorganisms (Staphylococcus, Pseudomonas, Klebsiella) were identified as key factors in caries initiation and progression. Multiple factors, including bacterial interactions, diet, oral hygiene, plaque, immunity, time, and host response, influence the initiation and progression of dental caries [3].

Periodontal disease causes inflammatory conditions that affect the periodontium, including the gingiva, periodontal ligament, cementum, and alveolar bone. It begins as gingivitis, a reversible inflammation caused by plaque accumulation. If it is untreated can progress to periodontitis. Periodontitis is a chronic inflammatory disease that damages periodontal tissues, leading to loss of attachment from gums and the formation of a periodontal pocket. Poor oral hygiene is a major concern for plaque buildup, as bacteria penetrate deeper into the tissue and the body activates an immune response to fight the infection [4].

Oral thrush is a condition that can be difficult to recognise at first. It is caused by a type of yeast fungus, Candida albicans, also known as oral candidiasis. Most people have this organism on the mucous membrane lining their, and it does not cause any problem. If the conditions become favourable, the yeast spreads rapidly and causes oral thrush, mainly in immunocompromised persons. It is a visible white coating that covers your mouth and throat. Their sense of taste was affected, with a pain or burning sensation on their tongue, leading to difficulty swallowing or speaking [5].

As we know, oral hygiene is an indicator of overall well-being. The most efficient way to prevent dental disease is to maintain proper oral hygiene. The most commonly used method is toothbrushing with toothpaste [6]. Any substance or combination of substances specially prepared for the public for cleaning the accessible surface of the tooth, known as “dentifrice”. Toothpaste is a gel or paste used with a toothbrush to maintain and improve oral health. It is defined as a dentifrice in the form of a smooth, semisolid, homogeneous mass containing ingredients like abrasives or polishing agents, surface active agents, humectants, and a binding agent [7]. Maintaining good dental health requires a balanced diet, regular brushing and flossing. Many commercial toothpastes now contain additives, including fluoride, parabens, triclosan, and artificial sweeteners. The adverse effects of commercial toothpaste reduce its benefits [8].

Medicinal plants have been used worldwide for the traditional treatment of several human diseases for thousands of years. The natural products derived from medicinal plants have proven to be an abundant source of biologically active compounds. Several plants and natural products are used to treat oral disease. The general antimicrobial activities of medicinal plant extracts or phytochemicals have been shown to inhibit the growth of oral pathogens, reduce dental plaque, and reduce the symptoms of oral disease [9].

“Camellia sinensis”, commonly known as “Green tea”, belongs to the family “Theaceae”. It has gained attention for its potential health benefits, including oral health. Green tea contains several bioactive components, including catechins, polyphenols, and fluoride, which contribute to its antibacterial, anti-inflammatory, and antioxidant properties. As a result, green tea inhibits the growth of cariogenic bacteria, such as Streptococcus, reduce plaque. Evidence suggests that epigallocatechin-3-gallate (EGCG) has anti-inflammatory effects that reduce gingival inflammation and alleviate periodontal disease. [10]. Green tea leaves are rich in fluorides, which are well known for inhibiting bacterial growth and helping remineralise dental tissue. Green tea has valuable effects on oral conditions, such as dental caries, periodontal disease, and halitosis [11].

"Acorus calamus", commonly known as "sweet flag", is an aromatic herb with creeping rhizomes from the "Acoraceae" family. It is a herb used to stimulate appetite and aid digestion. Their rhizome was used for toothache, and the powdered rhizome for congestion. It has many biological activities, including antifungal, antibacterial, insecticidal, anti-diarrheal, antioxidant, anti-inflammatory, antidiabetic, immunosuppressive, and anticarcinogenic. Its phytochemical constituents, such as alpha-, beta-, and gamma-asarone, sesquiterpenes, and acorenone, exhibited various biological activities [12].

"*Carica papaya*", usually known as "papaya", is one of the most popular species of the family "Caricaceae". Extracts from various parts of the papaya plant, such as leaves and seeds, are used in traditional medicine to treat a wide range of diseases. It has antibacterial and anti-inflammatory properties, helping reduce the severity of periodontal disease. This plant improves the gingival inflammation and prevents the development of the disease [11]. The leaves are rich in nutrients and vitamins, and have antibacterial and antifungal properties used as safe alternatives to synthetic pharmaceuticals [12]. Carica papaya leaf contains active components that are responsible for its medicinal activity. Papaya leaf extract has strong medicinal properties, including antibacterial, antiviral, antitumour, hypoglycaemic, and anti-inflammatory [13].

Herbal alternatives are considered safer and more acceptable for long-term use. Medicinal plants have been used to maintain oral health. Herbal toothpaste formulations have gained considerable attention due to the presence of naturally occurring bioactive compounds, such as polyphenols, alkaloids, and flavonoids, which contribute to antibacterial, anti-inflammatory, and antioxidant activities [14]. Herbal toothpaste can be prepared using various herbal extracts from crude drugs with antibacterial and antimicrobial activity. Herbal toothpaste formulation is prepared using herbs such as ginger extract, clove, neem stem, and guava leaves. The main purpose of toothpaste is to reduce oral bacteria and deliver fluoride to the teeth [15]. Fluoride is an essential element in the human diet and plays an important role in tooth and bone mineralisation, inhibitory and stimulatory effects on many enzymes, and dental caries resistance. The presence of fluoride in green tea averages, indicating higher fluoride amounts [16].

Ideal properties of toothpaste include a good abrasive effect, non-irritating and non-toxic, no staining of the teeth, keeping the mouth fresh, prolonged effect, and being cheap and easily available [17]. The toothpaste raw material used in formulation falls into the categories including a polishing agent (calcium carbonate), surface active agents (sodium lauryl sulphate), Humectant (glycerol, sorbitol, xylitol), binding agent (xanthan gum), other substance including essential oils, colouring agents, flavouring agents (pepper mint oil), sodium saccharine and preservatives [7].

Numerous studies have investigated the individual antimicrobial properties of these, but limited research has evaluated their potential use in oral health. From the study, papaya and green tea mouthwashes showed lower cytotoxicity than the commercial mouthwash, which is similar to toothpaste [18]. The study aims to develop a combined herbal toothpaste using Acarus calamus, Carica papaya, and Camellia sinensis to evaluate their potential antibacterial and antifungal activity for oral health.

MATERIALS AND METHODS

Collection of plant materials

The Carica papaya (Papaya) plant leaves were obtained from the herbal garden of Nehru Arts and Science College, Coimbatore district.

The Acarus calamus rhizome (Sweet flag) was collected from an authenticated ayurvedic supplier from Coimbatore district.

The Camellia sinensis (Green tea) dried leaves were collected from the certified cultivated source in Coimbatore district, Tamil Nadu.

Drying and powdering of plant materials

The papaya leaves were washed with distilled water, finely chopped, and shade-dried for 5 days. Once dried, the leaves were powdered using a mechanical grinder. Then stored in an air-tight container at 4 °C.

The sweet flag rhizome was washed with distilled water, finely chopped, and shade-dried for 7 days. Once dried, the rhizome was powdered using a mechanical grinder. Then stored in air tight container at 4 °C

The dried green tea leaves were ground using a mechanical grinder, then stored in an air-tight container at 4 °C.

Preparation of plant extract

Hot extraction method

The powdered papaya leaves, sweet flag rhizome, and green tea were taken.

Papaya leaf extract: Papaya leaves were weighed at 10 grams and added to 100 mL of distilled water, and kept in a water bath at 50 °C for 30 minutes. After that, it was filtered using a muslin cloth and a Whatman filter paper, then stored at 4 °C.

Sweet flag rhizome extract: Sweet flag rhizome was weighed 10 grams, added to 100 mL of distilled water, and heated in a water bath at 50 °C for 30 minutes. After that, it was filtered using a muslin cloth and a Whatman filter paper, then stored at 4 °C.

Green tea extract: Green tea was weighed at 10 grams, added to 100 mL of distilled water, and heated in a water bath at 50 °C for 30 minutes. After that, it was filtered using a muslin cloth and a Whatman filter paper, then stored at 4 °C.

Phytochemical test

Test for Alkaloids:

Dragendroff's test: Treat the extract with the Dragendroff's reagent solution (solution of Potassium Bismuth Iodide). The presence of alkaloids is confirmed by the formation of red precipitate.

Test for saponins:

Foam Test: Add 1 mL of plant extract to 5 mL of water, then shake continuously for 2 minutes. The foam formed after shaking persists for ten minutes, indicating the presence of saponins.

Test for phenols:

Ferric Chloride Test: Add 2mL of plant extract to 3-4 drops of ferric chloride solution. The bluish black colour confirms the presence of phenol.

Test for tannins:

Gelatin Test: Add 1% gelatin solution containing sodium chloride to the plant extract. The presence of tannins is confirmed by the formation of white precipitate.

Test for flavonoids:

Lead Acetate Test: Treat the plant extract with a few drops of lead acetate solution. The presence of flavonoids is indicated by the formation of a yellow-coloured precipitate.

Test for diterpenes:
Copper Acetate Test: Dissolve the plant extract in water, then add 3-4 drops of copper acetate solution. The presence of diterpenes is indicated by the formation of emerald green colour.

Formulation of Herbal Toothpaste

Herbal toothpaste was prepared following the procedure.

The motor and piston were cleaned, then xanthan gum and glycerine were added and mixed well until no dry white clumps remained. Then sorbitol, Sodium chloride, and three plant extracts were added and stirred continuously until the mixture began to thicken into a gel. Gradually add calcium carbonate to the gel, stirring continuously until the texture becomes smooth. Finally, add sodium lauryl sulphate and peppermint oil, mix gently with a spatula. Store the toothpaste in the appropriate air-tight tube and store at room temperature, avoiding direct sunlight. (Figure 1)

S.no:	Ingredients	Quantity	Functions
1.	Papaya extract	1 ml	Anti plaque
2.	Sweet flag extract	1 ml	Anti-inflammatory
3.	Green tea extract	1 ml	Antibacterial
4.	Calcium carbonate	30 g	Polishing agent
5.	Glycerine	20 ml	Humectant
6.	Xanthan gum	1.5 g	Binding agent
7.	Sodium lauryl sulphate	0.75 g	Surface active agent
8.	Sorbitol	0.1g	Humectant
9.	Sodium chloride	0.10	preservative
10.	Peppermint oil	5 drops	Freshening agent
11.	Distilled water	5 ml	Solvent

Evaluation of toothpaste

Physical examination: (colour, odour, taste, smoothness) (Figure 2)
The colour of the toothpaste was observed visually.
The odour of the toothpaste was revealed by the smell.
Taste of the toothpaste checked manually.
Smoothness was observed by rubbing the toothpaste between the fingers.

Determination of pH: Formulated toothpaste, 5 grams, was taken in a 50 ml beaker, 10 ml of freshly boiled and cooled distilled water was added, and the mixture was thoroughly mixed. Using a pH meter, determine the suspension's pH after 5 minutes. (Figure 3)

Spread ability: formulated paste, 1 gram was placed at the centre of a glass plate, and another glass plate was placed on top of the slide. The two glass plates were left for 5 minutes to form a uniform film of the paste between them. The diameter of the toothpaste was measured in centimetres.

Foaming ability: formulated toothpaste, 4 grams are taken in a beaker and mixed with 10ml of distilled water. Transfer the suspension to the measuring cylinder and agitate 10 times. The initial volume was measured, and the final volume was measured after agitation. (Figure 4)

Microbial load test: performed using total viable count. 1 gram of toothpaste was dissolved in 10 ml of sterilised distilled water and completely suspended. Prepare a nutrient agar plate, add 0.1ml of the suspended toothpaste, and spread it completely around the plate using an L rod. After that, incubate the plate inverted at 37 °C for 24 hours. Observe the plates and count the number of colonies. (Figure 6)

Testing for pathogens: Performed to determine the presence of pathogenic bacteria. Sterilised Mannitol salt agar plate, Macconkey agar plate, and Eosin Methylene Blue agar plate were prepared. 1 gram of toothpaste is suspended in 10ml sterilised distilled water. 0.1 ml of toothpaste was added to each plate and spread around using the L rod; the plates were then incubated at 37 °C for 24 hours in an inverted position. Observe the colonies grow on the plate. (Figure 5)

Antimicrobial activity of herbal toothpaste: The antibacterial and antifungal activities of herbal toothpaste were determined using the well diffusion method against Staphylococcus aureus, Pseudomonas aeroginoa and Candida albicans. Muller-Hinton agar plates were prepared and sterilised. Then the bacterial and yeast cultures were spread on the plate. Then cut the well using a well cutter with an 8mm diameter. 1 gram of toothpaste diluted in 10ml of distilled water, 200 millilitres of diluted toothpaste were added to the well and incubated at 37 °C for 24 hours. After incubation, the zone of inhibition was observed and recorded in millimetres. (Figure-7,8,9)

RESULTS

Phytochemical screening:

Preliminary phytochemical screening was performed on the ethanol extracts of Camellia sinensis (green tea), Acorus calamus (sweet flag), and Carica papaya (papaya leaf) to identify the bioactive constituents present in each extract. The results are summarised in Table 1.

Phenols, flavonoids, and diterpenes were detected in all three plant extracts, indicating a shared phytochemical richness among the selected plants. Saponins were present in Camellia sinensis and Carica papaya extracts but were absent in Acorus calamus. Alkaloids and tannins were not detected in any of the three extracts under the conditions tested. The consistent detection of phenols, flavonoids, and diterpenes across all three plants confirms their phytochemical quality and provides a basis for their combined use in a herbal oral care formulation.

Table 1: phytochemical screening result

Phytochemical test	Green tea	Sweet flag	papaya
Alkaloid	Negative	Negative	Negative
Saponins	Positive	Negative	Positive
Phenol	Positive	Positive	Positive
Tannins	Negative	Negative	Negative
Flavonoids	Positive	Positive	Positive
Diterpenes	Positive	Positive	Positive

Antimicrobial activity of individual and combined plant extracts

The antimicrobial activity of individual plant extracts and their combination was evaluated against Staphylococcus aureus, Candida albicans, and Pseudomonas aeruginosa using the Kirby-Bauer disc diffusion method. The zones of inhibition are presented in Table 2.

Against S. aureus, green tea extract recorded a zone of inhibition of 15mm, while papaya leaf and sweet flag extracts showed no inhibitory activity and were classified as resistant. The combined extract of all three plants demonstrated an enhanced zone of inhibition of 17mm against S. aureus, exceeding the activity of the individual green tea extract. The positive control (Chloramphenicol) recorded a zone of inhibition of 35mm. Against Candida albicans, green tea extract produced a zone of inhibition of 17mm, while papaya leaf and sweet flag extracts were resistant. The combined extract demonstrated the highest antifungal activity with a zone of inhibition of 20mm. The positive control (chloramphenicol) showed a zone of inhibition of 25mm. Against Pseudomonas aeruginosa, green tea extract produced a zone of inhibition of 15mm, while papaya leaf and sweet flag extracts were resistant. The combined extract recorded a zone of inhibition of 13mm, which was slightly lower than that of the individual green tea extract. The positive control recorded a zone of inhibition of 20mm.

Table 2: Individual and combined plant extracts against oral pathogens

Extract	Staphylococcus aureus	Candida albicans	Pseudomonas aeruginosa
Green tea	17mm	17mm	15mm
Papaya	Resistant	Resistant	Resistant
Sweet flag	Resistant	Resistant	Resistant
Combined extract	17mm	20mm	13mm
Positive control	35mm	25mm	20mm
Negative control	Resistant	Resistant	Resistant

Antimicrobial activity of formulated herbal toothpaste

The antimicrobial efficacy of the formulated herbal toothpaste was compared with Dabur Red commercial toothpaste using the Kirby-Bauer disc diffusion method. Chloramphenicol was used as the positive control, and distilled water as the negative control. Results are presented in Table 3 and figure-7,8,9.
Against S. aureus, the herbal toothpaste produced a zone of inhibition of 20mm, substantially higher than the 14mm recorded for Dabur Red toothpaste, as shown in Figure 7. Against C. albicans, the herbal toothpaste recorded 19mm compared to 17mm for the commercial preparation, demonstrating antifungal activity, as shown in Figure 9. Both the herbal toothpaste and Dabur Red toothpaste showed resistance against P. aeruginosa, while the positive control produced a zone of inhibition of 35mm against the same organism, confirming the inherent resistance of P. aeruginosa to the toothpaste formulations tested, as shown in Figure 8. The negative control showed no zone of inhibition in all cases.

Table 3: Toothpaste antimicrobial activity

Tooth paste	Staphylococcus aureus	Candida albicans	Pseudomonas aeruginosa
Herbal toothpaste	20mm	19mm	Resistant
Dabur red	14mm	17mm	Resistant
Chloramphenicol	30mm	25mm	35mm
Negative control	Resistant	Resistant	Resistant

Evaluation of Formulated Herbal Toothpaste

The formulated herbal toothpaste was assessed through a series of physicochemical and microbiological quality evaluation tests, with results presented in Table 4. Organoleptic evaluation revealed a pale white colour, a fresh and earthy odour characteristic of the incorporated plant extracts, and a smooth, homogeneous texture free from any hard or abrasive particles. The paste was easily washable and demonstrated consistent spreading ability. The pH was 8.5, within the acceptable alkaline range for oral care products. Foamability was measured at 50ml and spreading ability at 5cm, indicating satisfactory physical performance during application. Microbiological testing confirmed the absence of microbial growth in both the microbial load test and the pathogen test, establishing the microbiological purity and safety of the formulated toothpaste.

Table 4: Evaluation of herbal toothpaste

Parameter	Result
Colour	Pale white
Odour	Strong, fresh, minty scent
pH	8.5
Foam ability	50ml
Spread ability	5cm
Smoothness	Smooth, no hard particles and easily washable
Pathogen testing	No organism growth
Microbial load	No organism grown

Figure 1: Herbal toothpaste

Figure 2: Appearance of toothpaste

Figure 3: pH

Figure 4: Foam ability

Figure 5: Testing for pathogen

Figure 6: Microbial load test

Figure 7:

Antibacterial test for toothpaste against Staphylococcus aureus. The figure shows that TP- Formulated toothpaste, DTP- Dabur red paste (commercial toothpaste), +ve- positive control (chloramphenicol), -ve- Negative control.

Figure 8:

Antibacterial test for toothpaste against Pseudomonas aeruginosa. The figure shows that TP- Formulated toothpaste, DTP- Dabur red paste (commercial toothpaste), +ve- positive control (chloramphenicol), -ve- Negative control.

Figure 9:

Antifungal test for toothpaste against Candida albicans. The figure shows that TP- Formulated toothpaste, DTP- Dabar red paste (commercial toothpaste), +ve- positive control (chloramphenicol), -ve- Negative control.

DISCUSSION

Phytochemical Screening

The phytochemical analysis of the three selected plant extracts confirmed the presence of phenols, flavonoids, and diterpenes in all samples. These compounds are widely recognised as primary contributors to the antimicrobial properties of medicinal plants [8]. Phenolic compounds are known to disrupt bacterial cell membrane integrity and inhibit microbial enzyme function, whereas flavonoids interact with bacterial cell wall proteins and interfere with metabolic processes essential for microbial survival. The presence of saponins in Camellia sinensis and Carica papaya is particularly relevant, as saponins have been reported to increase cell membrane permeability in fungi, thereby contributing to antifungal activity against C. albicans [13,15]. The detection of diterpenes in all three extracts further strengthens their antimicrobial potential, as diterpenes have been documented to interfere with bacterial DNA synthesis and membrane function. The absence of alkaloids and tannins in the present study may be attributed to the nature of the extraction solvent and the specific plant parts utilised, as phytochemical content can vary with extraction methodology, plant part selected, and geographic origin of plant material. These findings are broadly consistent with previously published phytochemical profiles of Camellia sinensis [10], Acorus calamus [12], and Carica papaya [13,15], confirming the reliability of the plant materials selected for this investigation.

Antimicrobial Activity of Individual and Combined Extracts

Among the individual plant extracts evaluated, Camellia sinensis was the only extract to demonstrate antimicrobial activity against all three tested pathogens, recording zones of inhibition of 15mm against S. aureus, 17mm against C. albicans, and 15mm against P. aeruginosa. This broad-spectrum activity is primarily attributed to the high content of epigallocatechin-3-gallate (EGCG) and polyphenolic compounds in green tea, which disrupt bacterial membrane structure and inhibit fungal cell wall biosynthesis. These findings are consistent with those of Khurshid et al. (2016) [11] and Alagarsamy et al. (2025) [10], who reported significant antibacterial and antifungal activity of Camellia sinensis against oral pathogens and attributed this activity to its catechin-rich composition.

The resistance observed for both Acorus calamus and Carica papaya individual extracts against all three pathogens was an unexpected finding. This may be related to the concentration of the extract used, the extraction method employed, or the specific resistance mechanisms of the tested organisms. It is possible that the active compounds in these extracts were present at sub-inhibitory concentrations under the tested conditions. Previous studies have reported antimicrobial activity for both plants, suggesting that optimising extraction parameters and concentrations may yield improved results in future investigations [12,13,15].

Despite the resistance of individual papaya and sweet flag extracts, the combined extract demonstrated enhanced antimicrobial activity against all three pathogens, recording zones of inhibition of 17mm against S. aureus, 20mm against C. albicans, and 13mm against P. aeruginosa. The improved activity of the combined extract compared to the individual extracts strongly suggests a synergistic interaction among the phytochemicals of the three plants. This synergistic mechanism likely involves simultaneous action on multiple microbial targets, thereby overcoming individual resistance and enhancing overall antimicrobial efficacy. Such synergistic effects in polyherbal combinations have been well documented in the literature [8,9]. The resistance of Chloramphenicol (positive control) against C. albicans was an expected observation, confirming the antifungal specificity of the herbal extract combination, which demonstrated a zone of inhibition of 20mm against the same organism.

The resistance of both herbal and commercial toothpastes to P. aeruginosa is consistent with the well-established intrinsic resistance mechanisms of this organism. Pseudomonas aeruginosa possesses an outer membrane with low permeability, active efflux pumps, and the ability to form protective biofilms, all of which contribute to its resistance against a broad range of antimicrobial agents [3]. The combined extract, however, did demonstrate a zone of inhibition of 13mm against P. aeruginosa, suggesting partial activity that may be further enhanced through concentration optimisation. Antimicrobial Activity of

Formulated Herbal Toothpaste

The formulated herbal toothpaste demonstrated superior antimicrobial activity against S. aureus (20mm) and C. albicans (19mm) compared to the commercially available Dabur Red toothpaste, which recorded zones of inhibition of 14mm and 17mm, respectively. This clearly establishes the therapeutic potential of the formulated product as an effective herbal oral care agent. The enhanced antimicrobial performance of the herbal toothpaste relative to the commercial preparation may be attributed to the synergistic activity of the combined plant extracts incorporated into the formulation, as consistently observed at the extract level. These findings are supported by Gandigude et al. (2026) [16] and Senthilkumar et al. (2022) [18], who demonstrated that herbal toothpaste formulations incorporating bioactive plant extracts exhibit antimicrobial activity comparable to or greater than commercial preparations.

Furthermore, Ashinie et al. (2025) [19] reported that green tea- and papaya-based oral formulations demonstrated lower cytotoxicity than commercially available products, reinforcing the safety profile of similar herbal combinations. While Chloramphenicol, as a positive control, produced higher zones of inhibition as anticipated, the herbal toothpaste exhibited clinically meaningful antimicrobial activity, affirming its suitability as a natural alternative to chemically formulated oral care products. Both the herbal toothpaste and Dabur Red were resistant to P. aeruginosa, a finding consistent with the organism's known intrinsic resistance [3].

Quality Control Parameters

The formulated herbal toothpaste demonstrated superior antimicrobial activity against S. aureus (20mm) and C. albicans (19mm) compared to the commercially available Dabur Red toothpaste, which recorded zones of inhibition of 14mm and 17mm, respectively. This clearly establishes the therapeutic potential of the formulated product as an effective herbal oral care agent. The enhanced antimicrobial performance of the herbal toothpaste relative to the commercial preparation may be attributed to the synergistic activity of the combined plant extracts incorporated into the formulation, as consistently observed at the extract level. These findings are supported by Gandigude et al. (2026) [16] and Senthilkumar et al. (2022) [18], who demonstrated that herbal toothpaste formulations incorporating bioactive plant extracts exhibit antimicrobial activity comparable to or greater than commercial preparations.

Furthermore, Ashinie et al. (2025) [19] reported that green tea- and papaya-based oral formulations demonstrated lower cytotoxicity than commercially available products, reinforcing the safety profile of similar herbal combinations. While Chloramphenicol, as a positive control, produced higher zones of inhibition as anticipated, the herbal toothpaste exhibited clinically meaningful antimicrobial activity, affirming its suitability as a natural alternative to chemically formulated oral care products. Both the herbal toothpaste and Dabur Red were resistant to P. aeruginosa, a finding that is consistent with the known intrinsic resistance characteristics of this organism [3].

CONCLUSION

The present study successfully demonstrated the antimicrobial potential of plant extracts derived from Camellia sinensis (green tea), Acorus calamus (sweet flag), and Carica papaya (papaya leaf) against selected oral pathogens, and established the feasibility of incorporating these extracts into a safe and effective herbal toothpaste formulation. Phytochemical screening confirmed the presence of phenols, flavonoids, and diterpenes in all three extracts, while saponins were identified in green tea and papaya leaf extracts, collectively contributing to their antimicrobial and anti-inflammatory properties.

Among the individual extracts, Camellia sinensis demonstrated broad-spectrum antimicrobial activity against S. aureus, C. albicans, and P. aeruginosa, while the combined extract exhibited superior synergistic activity against all three pathogens. The formulated herbal toothpaste demonstrated greater antimicrobial activity against S. aureus and C. albicans than the commercially available Dabur Red toothpaste, confirming the therapeutic advantage of the polyherbal combination. Both preparations were resistant to P. aeruginosa, consistent with the intrinsic resistance characteristics of this organism. Quality evaluation parameters, including pH, spreading ability, foamability, organoleptic properties, and microbiological safety, all met acceptable standards for oral care products.

These findings collectively suggest that the formulated herbal toothpaste represents a promising, safe, and biologically active natural alternative to conventional chemical-based oral care products. Future studies involving in vivo clinical trials, stability testing over extended storage periods, and optimisation of extraction parameters for Acorus calamus and Carica papaya are recommended to further establish the therapeutic efficacy and commercial viability of this herbal formulation.

REFERENCE

1. World Health Organisation. (2025, March 25). Oral health. https://www.who.int/news-room/fact-sheets/detail/oral-health
2. Rathee, M., & Sapra, A. (2023). Dental caries. In StatPearls [Internet]. StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK551699/
3. Kaur, N., Sahni, P., Singhvi, A., Hans, M. K., & Ahluwalia, A. S. (2015). Screening the drug resistance property among aerobic pathogenic microorganisms of dental caries in North-Western Indian population: A preliminary study. Journal of Clinical and Diagnostic Research, 9(7), ZC05–ZC08. https://doi.org/10.7860/JCDR/2015/11985.6143
4. Gasner, N. S., & Schure, R. S. (2025). Periodontal disease. In StatPearls [Internet]. StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK554590/
5. Institute for Quality and Efficiency in Health Care. (2022). Overview: Oral thrush. InformedHealth.org. https://www.ncbi.nlm.nih.gov/books/NBK367586/
6. Vijayakumar, P., Mahesh, J., Prasad, S. V., Indrapriyadharshni, G., Karthikeyan, R., & Revanth, M. P. (2024). Reason for preference of toothpaste among buyers: A cross-sectional survey. Journal of Primary Care Dentistry and Oral Health, 5(2), 56–61. https://doi.org/10.4103/jpcdoh.jpcdoh_9_24
7. Bureau of Indian Standards. (2001). Toothpaste — Specification (IS 6356: 2001). https://standardsbis.bsbedge.com/
8. Panadan, J. J., Sharma, R., Jain, A., Sharma, M., & Sharma, A. (2024). The chemical constituents used in toothpaste formulations and their adverse effects on systemic health: A review. Journal of Chemical Health Risks, 14(4), 884–889.
9. Palombo, E. A. (2011). Traditional medicinal plant extracts and natural products with activity against oral bacteria: Potential application in the prevention and treatment of oral diseases. Evidence-Based Complementary and Alternative Medicine, 2011, 680354. https://doi.org/10.1093/ecam/nep067
10. Alagarsamy, K., Chaiyasut, C., Kesika, P., & Sivamaruthi, B. S. (2025). A review of the impact of green tea (Camellia sinensis L.) on oral health. Current Pharmaceutical Design. https://doi.org/10.2174/0113816128393965250927115025
11. Khurshid, Z., Zafar, M. S., Zohaib, S., Najeeb, S., & Naseem, M. (2016). Green tea (Camellia sinensis): Chemistry and oral health. Open Dentistry Journal, 10, 166–173. https://doi.org/10.2174/1874210601610010166
12. Umamaheshwari, N., & Rekha, A. (2018). Sweet flag (Acorus calamus): An incredible medicinal herb. Journal of Pharmacognosy and Phytochemistry, 7(6), 15–22.
13. Saberi, Z., Rahimi, A., Feizi, A., Golestannejad, Z., Saidi, Z., & Jafari, Z. (2025). The effect of Carica papaya on periodontal disease: A systematic review and meta-analysis. Advances in Biomedical Research, 14, 95. https://doi.org/10.4103/abr.abr_223_24
14. Kalburgi, N. B., Koregol, A. C., Roy, A., Sulthana, U. P., & Hiremath, J. N. (2024). Effect of leaf extract of Carica papaya gel as local drug delivery in periodontitis subjects. International Journal of Community Medicine and Public Health, 12(1), 337–342. https://doi.org/10.18203/2394-6040.ijcmph2024404

15. Singh, S. P., Kumar, S., Mathan, S. V., Tomar, M. S., Singh, R. K., Verma, P. K., Kumar, A., Kumar, S., Singh, R. P., & Acharya, A. (2020). Therapeutic application of Carica papaya leaf extract in the management of human diseases. Daru, 28(2), 735–744. https://doi.org/10.1007/s40199-020-00348-7
16. Gandigude, P. S., Kalshetti, V., Jadhav, N., Jangame, S., & Jawalge, D. (2026). Formulation and evaluation of herbal toothpaste: A natural approach to oral hygiene. International Journal of Pharmaceutical Research and Applications, 11(1), 298–305. https://doi.org/10.35629/7781-1101298305
17. Mahmoudi-Meymand, M., & Belgheisi, S. (2017). Determining of fluoride content in tea by spectrophotometery and potentiometry. Journal of Experimental Biology and Agricultural Sciences, 5(4), 568–574. https://doi.org/10.18006/2017.5(4).568.574
18. Senthilkumar, K. L., Venkateswaran, S., Vasanthan, A., Chiranjeevi, P., Mohamed, N., Dinesh, S., & Neshkumar, K. L. S. (2022). Formulation development and evaluation of novel herbal toothpaste from natural source. International Journal of Pharmaceutical Chemistry and Analysis, 9(1), 17–21. https://doi.org/10.18231/j.ijpca.2022.003
19. Ashinie, C., Balaji Ganesh, S., Rajesh Kumar, S., Martin, T. M., & Kishore Kumar, M. S. (2025). Comparative cytotoxic effect of commercial mouthwash and green tea papaya formulation based mouthwash: An in vitro study. South East European Journal of Public Health, 26(SI), 6378–6385. https://doi.org/10.11571/seejph.2025.26.SI.5248
20. Harfouch, R. M., Darwish, M., Beesh, M., Ibrahem, N., Dayoub, H., Zein, R., Ghosh, S., & Ahamadi, S. (2022). Formulation and preparation of a novel toothpaste using the essential oil of Salvia officinalis. International Journal of Biomedical Research, 2(7), 069. https://doi.org/10.31579/IJBR-2022/069
21. Chugh, V., Dhiman, S., Mittal, V., & Singhal, A. (2024). Formulation and evaluation of herbal toothpaste. Pharmaspire, 16(1), 1–12. https://doi.org/10.56933/Pharmaspire.2024.16101
22. Samrot, A. V., Raji, P., Selvarani, J. A., Sruthi, D. P., Angalene, L. A. J., Ponnaiah, P., & Iyappan, P. A handbook on phytochemical extraction, screening and its in-vitro bioactivity assays.