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Original Article

Phytochemical-Mediated Synthesis of Silver Nanoparticles from *Piper betle* and *Psidium guajava*: A Sustainable Approach to Oral Care

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Abstract

This study presents the development and characterization of a novel oral care formulation incorporating silver nanoparticles (AgNPs) synthesized using guava and betel leaf extracts. Oral infections caused by pathogenic microorganisms remain a significant global health challenge, necessitating innovative approaches that transcend conventional treatment modalities. The acetone extract (70:30) of betel and guava leaves was prepared and subjected to phytochemical analysis, revealing the presence of bioactive compounds including alkaloids, polyphenols, tannins, flavonoids, and saponins. GC-MS analysis identified 24 distinct compounds, with 2-pentanone,4-hydroxy-4-methyl- (37.26%), 1H-cyclopropa[a]naphthalene derivatives (19.26%), and phenolic compounds (8.27%) being predominant. The extract demonstrated significant antimicrobial activity against oral pathogens, with minimum inhibitory concentration (MIC) values of 500 µg/ml for Streptococcus spp. and 250 µg/ml for Candida albicans (NCIM 3628), outperforming conventional antimicrobials Streptomycin and Fluconazole (both 1000 µg/ml). Green synthesis of AgNPs was accomplished using the plant extracts as reducing and stabilizing agents, confirmed through visual colour change and FTIR spectroscopy. The FTIR spectrum revealed characteristic functional groups including hydroxyl (3438 cm^{-1}), carbonyl (1692 cm^{-1}), and aromatic compounds (1615 cm^{-1}, 1513 cm^{-1}), validating successful nanoparticle formation. The formulated gel exhibited optimal physical properties with a pH of 7.2, excellent homogeneity, spreadability, and stability. This innovative formulation represents a sustainable, eco-friendly, and economically viable alternative to conventional oral care products, effectively addressing the challenges of antimicrobial resistance while leveraging traditional ethnopharmacological knowledge through modern nanotechnological approaches.

Keywords: *Silver nanoparticles, Piper betle, Psidium guajava, green synthesis, antimicrobial activity, Oral pathogens, Sustainable oral care.*

Introduction

In the contemporary landscape of public health challenges, oral diseases persist as a significant global burden affecting an estimated 3.5 billion people worldwide. Despite advances in dental care accessibility, traditional oral hygiene products often demonstrate limited efficacy against complex oral biofilms and emerging antimicrobial resistance patterns. This discrepancy between preventive strategies and disease prevalence necessitates innovative approaches that transcend conventional paradigms of oral care (Lutfun and Sarker, 2022; Saxena et al., 2020). Nanotechnology—the manipulation of matter at dimensions between 1-100 nanometers—represents a transformative frontier in biomedicine, offering unprecedented opportunities to revolutionize oral healthcare modalities. At the nanoscale, materials exhibit unique physicochemical properties including enhanced surface area-to-volume ratios, increased reactivity, and superior penetration capabilities that dramatically alter their biological interactions (Wu et al., 2020). These characteristics enable targeted delivery of therapeutic agents to previously inaccessible microenvironments within the complex oral ecosystem (Dakhale et al., 2023; Damodharan 2021). Concurrent with technological advancement, there exists a resurgence of interest in ethnopharmacological approaches to oral health.

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Traditional medicinal systems across diverse cultures have documented the oral hygiene applications of botanical agents long before the advent of modern dentistry (Mahat et al., 2023). Among these botanical treasures, *Psidium guajava* L. and *Piper betle* L. stand as particularly promising candidates for integration into contemporary oral care formulations. Betel leaf, a cornerstone of traditional medicine in South and Southeast Asia, contains a rich phytochemical profile dominated by phenolics, alkaloids, and essential oils. Hydroxychavicol and eugenol, key constituents of betel leaf extract, have demonstrated remarkable antimicrobial activity against cariogenic and periodontopathic bacteria in vitro (F. Carrouel, et al., 2020). Similarly, guava leaf extracts contain flavonoids, tannins, and terpenoids that exhibit potent anti-inflammatory properties and inhibitory effects against oral pathogens, particularly *Streptococcus* mutants and *Porphyromonas gingivalis* (Shete, S., 2018). Despite the documented efficacy of these botanical agents, their integration into mainstream oral care products has been hindered by challenges related to stability, bioavailability, and controlled release kinetics. Traditional formulations often suffer from rapid clearance from oral surfaces, inconsistent dosing, and insufficient penetration into complex biofilm architectures—limitations that can potentially be overcome through nanoscale engineering approaches. (Glowacka-Sobotta et al., 2023; Mehdi et al., 2024).

The convergence of nanotechnology with botanical medicine represents an innovative intersection that remains largely unexplored in the context of oral healthcare. This research seeks to bridge this critical gap by developing and characterizing a novel nano formulated gel incorporating betel and guava leaf extracts designed for daily oral hygiene.

The significance of this research extends beyond academic inquiry into practical applications for addressing global oral health disparities. By leveraging indigenous botanical knowledge through modern nanotechnological approaches, this study may inform the development of next-generation oral care products that are efficacious, economically viable, and culturally relevant. Furthermore, successful integration of sustainable botanical resources into nanotechnology platforms could establish a blueprint for similar innovations across the broader spectrum of healthcare interventions.

As antimicrobial resistance continues to threaten conventional treatment modalities (Woo et al., 2023), and consumer preference shifts toward natural, sustainable healthcare products, this research addresses a timely and compelling need at the intersection of traditional wisdom and cutting-edge science—a convergence that may fundamentally transform our approach to oral disease prevention in the 21st century and beyond.

Material And Method

- **Plant Extract Preparation and Characterization**

1. **Collection of samples:** *Psidium guajava* L. and *Piper betle* L. leaves were collected from Solapur market and from Solapur University Campus, Solapur, Maharashtra. The Betel and Guava plant leaves were thoroughly washed and dried. Further they were powdered using pestle and mortar. The powder was stored in polythene bags at normal temperature before extraction.
2. **Plant extract preparation:** 50 gm of sample was weighed and added into the thimble then closed it. Solvent Acetone 70% was prepared Acetone ratio 70:30 (Acetone: water). The thimble placed in the Soxhlet apparatus for extraction. The solvent is poured in Soxhlet apparatus. That solvent is removed in half quantity then we add more solvent the temperature adjusted at 55-60°C near to their boiling point as well as the process of cycle is started. The cycle is done until extraction becomes light colour. The extract is subjected for evaporation of acetone. Then this extract is ready to use for further analysis (Tiwari et al., 2011; Zhang et al., 2018 and Abubakar & Haque, 2020).
3. **Phytochemical analysis:**
   Different phytochemicals like carbohydrates, alkaloids, proteins, tannins, flavonoids were preliminary detected in the laboratory using standard protocols (Mahat, *et al.*, 2023; Rao *et al.*, 2023 and Tiwari *et al.*, 2011). Then the extract was further analyzed by GCMS technique for presence of phytochemicals.

Antimicrobial Activity:

- **Agar Well Diffusion Method:**
  The antibacterial activity of Betel and Guava leaf extract was tested against *Streptococcus species* & *Candida albicans.* The antimicrobial activity was screened by agar well diffusion on nutrient agar (NA) for *Streptococcus* species and Potato dextrose agar for *Candida albicans* (NCIM 3628). 100 µl of the suspension of the respective organism is spread plated onto respective agar medium and incubated at respective temperatures, after incubation diameter of zone of inhibition was recorded (Mahat et al., 2023; Aboody and Mickymaray, 2020 and Thakur et al., 2025).
- **Broth Dilution Method- Turbidimetric assay (MIC):**
  The *Candida* and *Streptococcus* cultures were grown separately for 24 hr and the growth was adjusted to 0.5 McFarland standard (~1×10^8 CFU/mL). The extract was considered at a concentration of 1000 µg/ml used for determination of minimum inhibitory concentration by turbidimetric assay. Media used for *Candida albicans* (NCIM 3628) was potato dextrose broth and for *Streptococcus* spp Nutrient broth was preferred. While the incubation condition for *Candida* and *Streptococcus* was 28℃ for 48 hrs and 37℃ for 24hr respectively (Aboody and Mickymaray, 2020; Kowalska et al., 2021 and Aboody, 2021).

Synthesis and Characterisation of Silver Nanoparticles:

1. **Synthesis of Silver nanoparticles by using Betel and Guava extract**
   To synthesize silver nanoparticles, an extract of guava and betel leaves was prepared. Silver nitrate ($AgNO_3$)

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was then added to the extract, initiating a reduction process. The mixture was allowed to proceed for 3-4 days, during which the bioactive compounds in the plant extract facilitated the reduction of silver ions into silver nanoparticles. This green synthesis method is an eco-friendly and efficient approach to nanoparticle formation, leveraging the natural reducing and stabilizing agents found in the plant extract (Alzubaidi et al., 2023; Giri et al., 2022).

2. **Characterization of silver nanoparticles**
The bio-reduced silver nitrate solution was centrifuged at 10000 rpm for 10-15 min and dried pellet was used for FT-IR measurement. The spectra recorded in range of 4000-400 cm^1. The spectra were compared to the reference chart and the functional groups were identified (Kumar et al., 2022, Alzubaidi et al. 2023).

3. **Gel Formulation Development**
Varying proportions of Carbopol 934 and Methyl paraben was mixed in warm d/w with constant stirring. Let the solution cool down, then to that mixture add Propylene glycol 400. The necessary amount of prepared plant extract and produced silver nanoparticles have been combined, and the rest of the distilled water was added to get the volume up to 100 milliliters. After thoroughly mixing all the ingredients into the Carbopol 934 gel while stirring constantly, tri-ethanolamine was added drop by drop to the formulation to get the correct pH (6.8–7.2) and the necessary consistency for the gel (Jadhav et al., 2015; Shete, 2018; Das & Pallab, 2018 and Sari, et al., 2022).

1. **Results and Discussion:**

The extract so obtained appeared green in colour with sticky consistency and a phenolic (combination of spicy & sweet) odour.

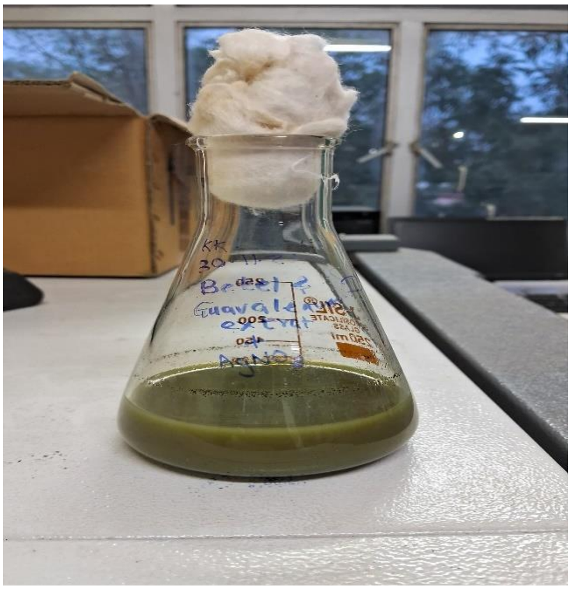

Figure1: Acetone extract of *Psidium guajava* L. and Piper *betle* L.

Characterization of plant extracts

Phytochemical Analysis:

In the preliminary phytochemical profiling of the extract alkaloids, polyphenols, tannins, flavonoid and saponins were detected as shown in tabe1. These phytochemicals exhibit various therapeutic properties as antimicrobial, anti-inflammatory, antioxidant, and insecticidal activities.

Table 1: Phytochemical analysis of the extract

Sr.no	TEST	RESULT
1	Alkaloids	+
2	Carbohydrate	-
3	Polyphenols	+
4	Tannins	+
5	Flavonoid	+
6	Saponins	+
7	Steroids	-
8	Glycosides	-

(+) indicates presence of phytochemical, (-) indicates absence of phytochemical

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GCMS Analysis:

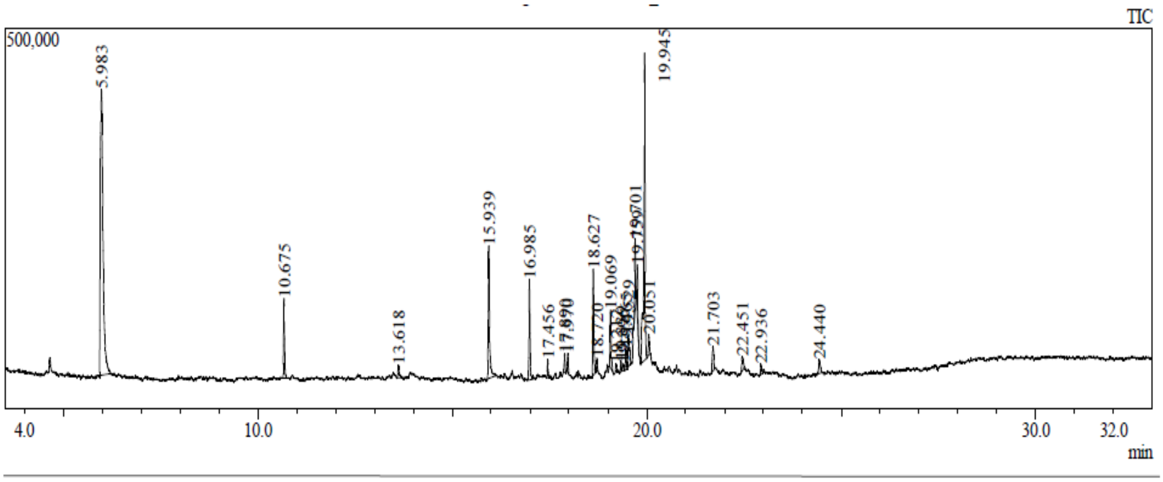

Figure 2: GCMS Analysis of the extract

The gas chromatography-mass spectrometry (GC-MS) analysis revealed a complex phytochemical profile with 24 distinct peaks identified with varying retention times (5.983-24.440 min). The total ion chromatogram (TIC) showed several major compounds with high percentage areas, indicating their predominance in the extract.

The most abundant compounds in the extract were:

1. **2-Pentanone, 4-hydroxy-4-methyl-** (RT 5.983 min, 37.26%): A ketone alcohol identified with high confidence (97% similarity). The mass spectrum shows characteristic fragments at m/z 43 (base peak), 58, 83, and 101, confirming the structure.
2. **1H-Cyclopropa[a]naphthalene derivative** (RT 19.945 min, 19.26%): A sesquiterpene with a tricyclic structure, identified as decahydro-1,1,3a-trimethyl-7-methylene- [1aS-(1a. alpha.,3a. alpha.,7a. beta.,7b. alpha.)]-1H-Cyclopropa[a]naphthalene with 91% similarity.
3. **Phenol, 2-methoxy-3-(2-propenyl)-** (RT 15.939 min, 8.27%): A phenolic compound with 92% similarity.
4. **Caryophyllene** (RT 16.985 min, 5.21%): A bicyclic sesquiterpene common in essential oils, identified with 91% similarity.
5. **Bicyclo [7.2.0] undec-4-ene derivative** (RT 18.627 min, 5.09%): A sesquiterpene identified as 4,11,11-trimethyl-8-methylene-[1R-(1R*,4Z,9S*)]-Bicyclo [7.2.0] undec-4-ene with 91% similarity.

Bioactive Compounds of Interest

Several bioactive compounds with known pharmacological properties were detected:

1. **Eucalyptol** (RT 10.675 min, 3.85%): A monoterpene oxide known for its anti-inflammatory and antioxidant properties.
2. **α-Terpineol** (RT 13.618 min, 0.88%): A monoterpene alcohol with reported antimicrobial activities.
3. **Andrographolide** (RT 19.069 min, 2.92% and RT 21.703 min, 1.72%): A major bioactive labdane diterpenoid known for its anti-inflammatory, antioxidant, and immunomodulatory properties. Its presence at two retention times might indicate isomers or fragmentation patterns.
4. **n-Hexadecanoic acid** (RT 22.936 min, 0.58%): A saturated fatty acid with antimicrobial and anti-inflammatory properties.
5. **3,7,11,15-Tetramethyl-2-hexadecen-1-ol** (RT 24.440 min, 1.05%): A diterpene alcohol (phytol) known for its antioxidant properties.

Terpene Profile

The extract shows a rich terpene profile with several mono- and sesquiterpenes including:

1. **Humulene** (RT 17.456 min, 0.80%): A monocyclic sesquiterpene.
2. **Aromandendrene** (RT 19.465 min, 0.97%): A tricyclic sesquiterpene.
3. **γ-Muurolene** (RT 19.759 min, 1.64%): A bicyclic sesquiterpene.

This GC-MS analysis reveals that the betel and guava leaves extract contains a diverse array of bioactive compounds, predominantly terpenes, phenolics, and diterpenoids. The major compound (2-Pentanone, 4-hydroxy-4-methyl-) constitutes over one-third of the total extract, followed by sesquiterpenes and phenolic compounds. The presence of compounds like andrographolide, eucalyptol, and caryophyllene suggests potential therapeutic applications due to their known bioactive properties. Further bioassay-guided fractionation would be beneficial to correlate the observed phytochemical profile with specific biological activities.

Antimicrobial efficacy:

The extract has potent inhibitory effect on both *Candida albicans* (NCIM 3628) as well as *Streptococcus* species. 50µl acetone was poured in the well as a control and after overnight incubation zone of inhibition were observed and are as figure 3 and table 2.

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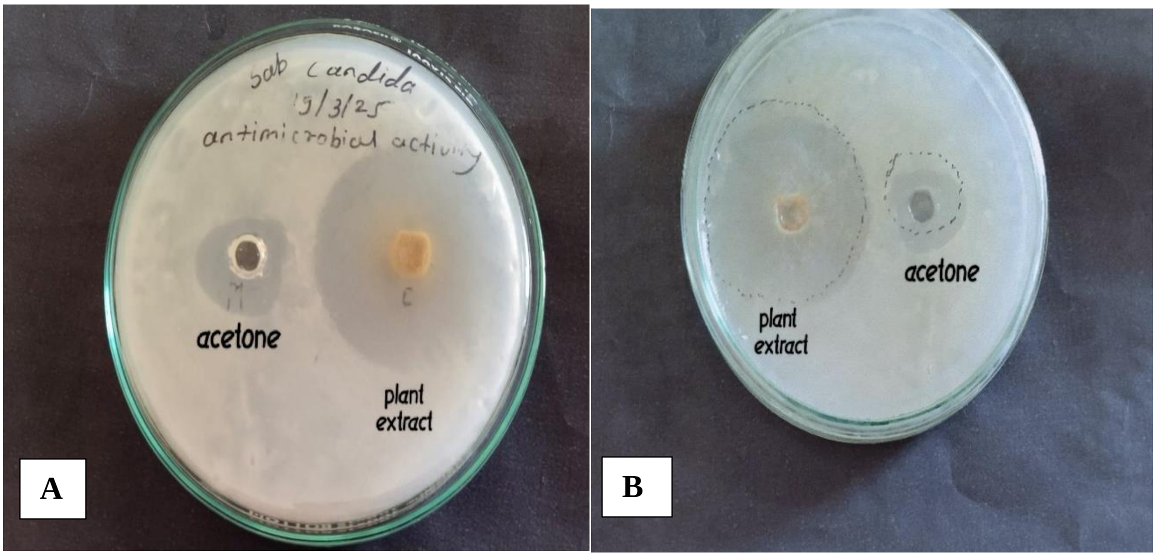


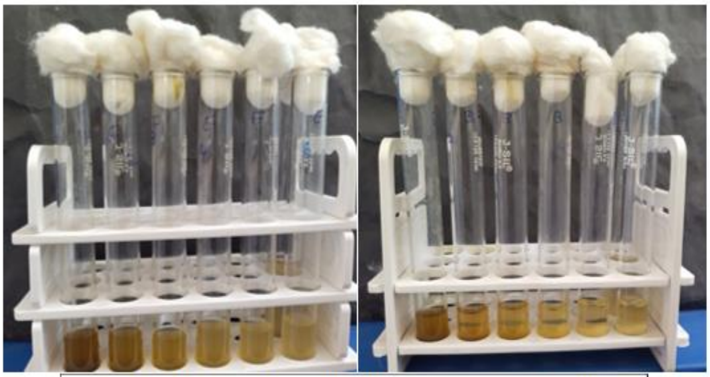
Figure 3: Antimicrobial Activity of (A) *Candida albicans* and (B) *Streptococcus spp*

Table 2: Diameter of Zone of inhibition against the respective organism

Sr. No.	Name of the organism	Diameter of zone of inhibition (cm) of Extract
1.	*Streptococcus spp*	4.2 cm
2.	*Candida albicans*	5.1 cm
3.	Acetone (control)	2.5 cm

MIC (Figure 4) is performed by tube methods and the results showed the extract possessed potent activity against both *Streptococcus* (MIC value 500 µg/ml) & *Candida albicans* (MIC value 250 µg/ml), as compared to standard Streptomycin (MIC Value 1000 µg/ml) and Fluconazole (MIC value 1000 µg/ml) respectively. Thus, opening different therapeutic avenues for the application of the extract.

A



B

Figure 4: MIC Assay (A) *Candida albicans* and (B) *Streptococcus spp*

Silver Nanoparticle Synthesis and Characterisation:

Physical properties of silver nanoparticles

After incubation for 3 days, the extract which was initially green in colour showed dark brown colour with silver sheen (Figure 5) indicated successful synthesis of silver nanoparticles using the leaves extract.

Characterization of synthesised silver nanoparticle by FTIR:

The FTIR spectrum displays distinct absorption bands, which can be attributed to the functional groups present in the synthesized nanoparticles. Following key peaks were observed:

1. **~3438 cm⁻¹**: This broad and strong peak corresponds to the **O–H stretching vibration**, indicative of hydroxyl groups typically associated with alcohols, phenols, or water molecules. This suggests the presence of polyphenolic or aqueous components, likely originating from plant extracts or moisture.
2. **~2926 cm⁻¹ and ~2855 cm⁻¹**: These peaks are attributed to **C–H stretching vibrations** of aliphatic –CH_2 and –CH_3 groups, indicating the presence of long-chain hydrocarbons or organic molecules.
3. **~1692 cm⁻¹**: This sharp peak can be ascribed to **C=O stretching vibrations** of carbonyl groups, such as

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those found in aldehydes, ketones, or carboxylic acids, pointing to oxidized organic compounds in the sample.

4. **~1615 cm^{-1} and ~1513 cm^{-1}**: These peaks are characteristic of **C=C stretching vibrations** in aromatic rings, suggesting the presence of aromatic compounds, possibly from phytochemicals in the plant extract.
5. **~1446 cm^{-1} and ~1382 cm^{-1}**: These may correspond to **bending vibrations of $–CH_3$ and $–CH_2$ groups**, commonly seen in organic molecules.
6. **~1181 cm^{-1} and ~1034 cm^{-1}**: These peaks are associated with **C–O stretching vibrations**, indicative of alcohols, ethers, or esters.
7. **~862 cm^{-1}, ~699 cm^{-1}, and ~535 cm^{-1}**: These lower frequency peaks suggest **out-of-plane bending vibrations** of aromatic C–H or possibly **metal–oxygen (M–O) bonds**, indicating the formation of metal nanoparticles capped with organic ligands from plant metabolites.

The FTIR spectrum (Figure 6) confirms the presence of various functional groups, including hydroxyl, carbonyl, aromatic, and aliphatic chains, likely originating from plant-derived capping agents or stabilizers. The presence of metal–oxygen-related vibrations support the successful synthesis of metal nanoparticles, where bioactive compounds have played a role in reduction and stabilization.

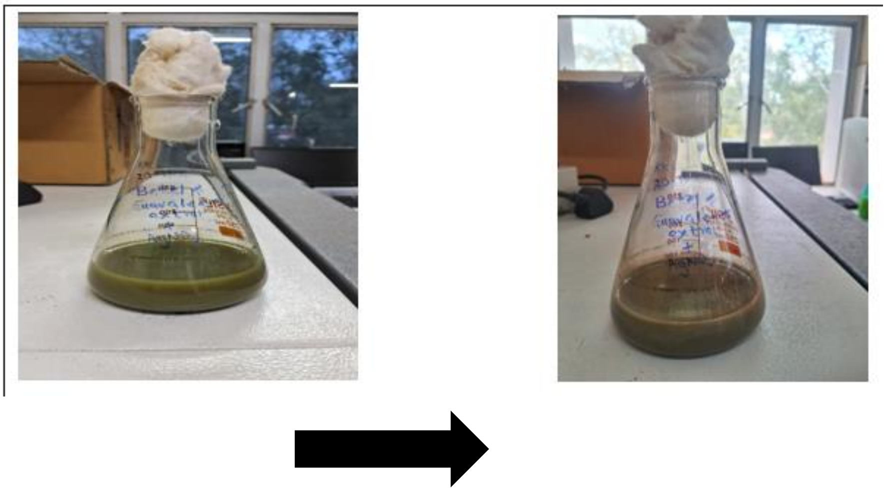

Figure 5: (A) Plant Extract before addition of silver nitrate
(B) Synthesized Silver Nanoparticle

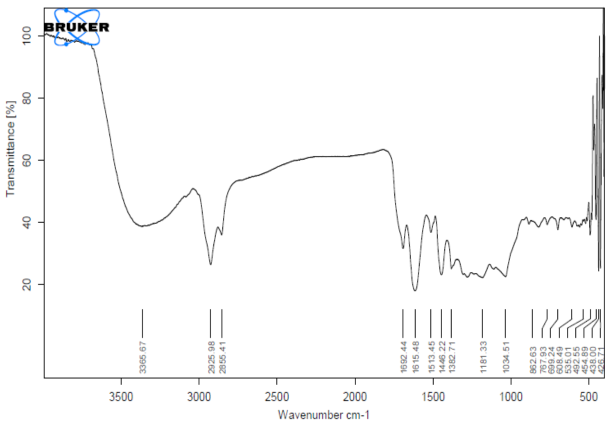

Figure6: FTIR results of synthesised silver nanoparticle using the prepared extract.

Gel formulation properties

Our synthesised gel possesses a yellowish colour with a pure Odor, ensuring a natural and pleasant experience. It has a soft consistency with no greasiness, making it comfortable for application. The gel maintains an ideal pH of 7.2, ensuring oral hygiene & compatibility. Its washability is effortless, while its homogeneity and extrudability are both excellent. The gel spreads smoothly and evenly, providing a consistent user experience (Table 3). Furthermore, its stability (Table 4) is remarkable, as there are no changes in colour, pH, Odor, or viscosity over time. This ensures long-term effectiveness and reliability, making it a superior choice in herbal formulations for oral care.

Table 3: Physical properties of the synthesised gel

Sr. No	Physical appearance	Synthesised Gel
1	Colour	Yellowish
2	Odor	Pungent
3	Consistency	Soft
4	Greasiness	No greasiness
5	PH	7.2
6	Wash ability	Easily washed
7	Homogeneity	Good
8	Extrudability	Good
9	Spreadability	good

Table 4: Stability of Formulated gel

Sr.no	Formulation code	Gel
1	Colour	Yellowish
2	Odor	Pungent
3	PH	No change
4	Spreadability	Good
5	Viscosity	No change

Conclusion:

This study demonstrates the successful development of a novel oral care formulation combining traditional ethnopharmacological knowledge with modern nanotechnology. The obtained plants leaf extract demonstrated significant antimicrobial efficacy against common oral pathogens, with minimum inhibitory concentration (MIC) values of 500 μg/ml for *Streptococcus spp*. and 250 μg/ml for *Candida albicans*, outperforming conventional antimicrobials such as Streptomycin and Fluconazole (both with MIC values of 1000 μg/ml).

Phytochemical profiling through preliminary screening and GC-MS analysis revealed a rich composition of bioactive compounds, particularly terpenes, phenolics, and diterpenoids. The predominant compounds identified included 2-Pentanone, 4-hydroxy-4-methyl- (37.26%), 1H-Cyclopropa[a]naphthalene derivatives (19.26%), phenolic compounds (8.27%), caryophyllene (5.21%), and several other therapeutically relevant molecules including eucalyptol, α-terpineol, andrographolide, and phytol. These compounds likely contribute to the observed antimicrobial properties through synergistic mechanisms.

The green synthesis of silver nanoparticles using the plant extracts was confirmed through colour change observations and FTIR spectroscopy. The FTIR spectrum revealed characteristic peaks corresponding to hydroxyl (3438 cm^{-1}), carbonyl (1692 cm^{-1}), aromatic (1615 cm^{-1}, 1513 cm^{-1}), and metal-oxygen bonds (862 cm^{-1}, 699 cm^{-1}, 535 cm^{-1}), validating the successful formation of nanoparticles with plant-derived capping agents serving as stabilizers. The formulated gel demonstrated excellent physical properties, including appropriate consistency, pH (7.2), homogeneity, spreadability, and stability over time. These characteristics make it suitable for daily oral hygiene applications. Furthermore, the economic analysis suggests that this formulation represents a cost-effective alternative to commercially available products while maintaining therapeutic efficacy.

This research addresses the growing global burden of oral diseases by providing a sustainable, eco-friendly, and economically viable oral care solution that leverages indigenous botanical knowledge through modern nanotechnological approaches. The convergence of traditional wisdom with cutting-edge science represents a promising avenue for developing next-generation oral care products that can overcome limitations associated with conventional formulations, particularly regarding antimicrobial resistance patterns and biofilm penetration. Further investigations are warranted to elucidate the precise mechanisms of antimicrobial action, optimize the nanoparticle synthesis process for scaled production, and conduct comprehensive in vivo studies to establish safety profiles and clinical efficacy. Nevertheless, this study lays a robust foundation for integrating nanotechnology with ethnopharmacology in addressing contemporary oral health challenges in a sustainable and accessible manner.

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Conflicts of interest
The authors declare that there are no conflicts of interest regarding the publication of this paper.

Declarations: Competing interests: The authors declare no competing interests.

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