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Synthesis of Silver Nanoparticles Using the Combined Extract of Guava (*Psidium guajava)* and Banyan (*Ficus benghalensis*) Leaves and Application in the Preparation of Nanobased Mouthwash

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Abstract: ***In this study the combined plant leaf extract of P.guajava and F.benghalensis were used for the production of silver nanoparticles and the green synthesized silver nanoparticles were used to prepare the nanobased mouthwash and its antimicrobial activity was evaluated against the oral infection causing microorganisms . The UV-Visible spectra of the synthesized nanoparticles were demonstrated the C-H bond of the plant extracts. The FTIR results explained the presence of biomolecules and the intense peaks value demonstrated the linkages of biomolecules in the synthesized nanoparticles.***
Keywords: Green synthesis, silver nanoparticles, FTIR spectra, UV-Visible spectrometer, antimicrobial activity.

I. INTRODUCTION

Nanotechnology or nanoscience refers to research and development of an applied science at the atomic or molecular Level (Kovvuru SK *et al.,* 2012) Nanomaterials can be well-defined as a material with sizes ranged between 1 and 100 nm, which influences the frontiers of nanomedicine starting from biosensors, microfluidics, drug delivery, and microarray tests to tissue engineering (Arayne MS *et al.,* 2007) Nanotechnology employs curative agents at the nanoscale level to develop nanomedicines (Mirza AZ *et al.,* 2014) It is shown to bridge the barrier of biological and physical sciences by applying nanostructures and nanophases at various fields of science (Liu Z *et al.,* 2009). Nanotechnology can be applied to various medical fields like Pharmacological research, clinical diagnosis, supplementing immune system, cryogenic storage of biological tissues, detection of proteins, probing of DNA structure, tissue engineering, tumor destruction via heating (hyperthermia) separation and purification of biological molecules and cells, magnetic resonance imaging (MRI) contrast enhancement, etc.(Freitas Jr RA *et al.,* 1999) Nowadays ecofriendly technologies for production of nanoparticles were given special attention due to the aforementioned facts. (Joerger *et al.,* 2000). Green nanotechnology is a branch of green technology that utilizes the concepts of green chemistry and green engineering, where the word "green" refers to the use of plant products. It reduces the use of energy and fuel by using less material and renewable inputs wherever possible (Hullmann A *et al.,* 2003) Introduction of plant extracts instead of hazardous chemicals made it eco-friendly and cost-effective (Sadia saif *et al.,* 2016). The advantage of green synthesis of nanoparticles over their chemical synthesis are due to the fact that the nanoparticles prepared are with diverse nature, greater stability and appropriate dimensions and they are synthesized using a one-step procedure ((Ingale *et al.,* 2013) Bio-inspired synthesis of nanoparticles provides advantages over chemical and physical methods because it is a cost effective and environment friendly and in this method there is no need to use high pressure, energy, temperature and toxic chemicals (Dama LB *et al.,* 2016). The oral cavity is colonized by wide range of microorganisms which may be harmless or harmful to respective individuals based on the level of immunity (Anitha M *et al.,* 2016). The oral cavity is a common colonization site for a number of multidrug resistant bacterial and fungal microorganisms that can cause infections. The oral cavity is a morpho-physiologically heterogeneous, dynamic environment with biotic and abiotic factors influencing with different intensity various specialized surfaces of its particular compounds (tongue, teeth, gums, etc.) (Abu-Elteen KH *et al.,* 1998) The oral cavity microbiome consists of a multi-species community with its complex relations to the human host (Filoche S *et al.,* 2010) Various species of the genus Streptococcus, Lactobacillus, Lactococcus, Enterococcus, Staphylococcus, Corynebacterium, Veillonella and Bacteroids are the prominent bacteria commonly found in the oral cavity (Rogers, A *et al.,* 2008).

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Mutants Streptococci, a group of cariogenic bacteria, is associated in the initiation of dental caries (Loesche (WJ *et al.,* 1986). *Streptococcus mutans* is commonly accepted as one of the most substantial etiologic agents in caries development and has been shown to directly cause caries in germ-free and specific pathogen-free rat models (Takahashi N *et al.,* 2008) Among the diseases caused by oral bacteria include dental caries, periodontitis, endocarditis, pharyngitis, pneumonia, meningitis etc (Cvitkovitch, D.G *et al.,* 2003)

Acoording to the statement of (Geetha V *et al.,* 2007), Nowadays Traditional medicine, which has compounds derived from medicinal plants are being used for the synthesis process and as well as were investigated for the therapeutic purposes. For example (Nakkala et al., 2014) studied that *Acorus calamus* extract can be used as capping agent for the synthesis of Ag-NPs to evaluate its oxidation state, anticancer, and antibacterial effect.

The formation of Ag-NPs has received significant interest because of their potential applications in catalysis, plasmonics, optoelectronics, biological sensors and antimicrobial activities (Savithramma *et al.,* 2011). (Gardea-Torresdey *et al.,* 2003) illustrated that the first approach of using plants for the synthesis of metallic NPs was done by using *Alfalfa sprouts*, which was the first description about the synthesis of Ag-NPs using living plant system. And it is proven that the silver NPs are synthesized by peanut shell extract has the characteristics of antifungal activity which was compared with commercial Ag-NPs. (Velmurugan *et al.,* 2015) In this present study the mixture plant extract of *P. guajava* and *Ficus religiosa* was studied for the synthesis of silver nanoparticles and the production of Nano-based mouth wash which was checked for the antibacterial activity against oral infection causing organisms.

II. MATERIALS & METHODS

A. Collection and Preparation of Leaf Extract

The leaves of *P. guajava* (guava) and *Ficus benghalensis* (banyan) were collected from the area of Kunnamkulam, Thrissur in Kerala.

Fresh leaves of *P. guajava* and *Ficus benghalensis* leaves were cut into small pieces and washed with double distilled water to remove dust particles (Sharmila *et al.,* 2018). 15g of freshly cut leaves were mixed with 300 ml of distilled water in a 500ml of Erlenmeyer flask and boiled at 60°C for 20 mins. The mixture was then filtered through using Whatman No.1 filter paper. The extract was kept in a refrigerator at 4°C for further use.

B. Green Synthesis of Silver Nanoparticles

Green synthesis of silver nanoparticles done by (Siddhant Jain *et al.,* 2017) dissolving 1mM solution of silver nitrate in 50mL of distilled water in a 100ml of Erlenmeyer flask. 10mL of silver nitrate solution was mixed with 5ml of leaf extract and control was also maintained. The colour change of dark brown denoted the synthesis of silver nanoparticles using *P. guajava* (guava) and *Ficus benghalensis* (banyan) leaf extract.

1) *Characterization of Silver Nanoparticles:* FTIR spectra of synthesized sample was recorded using FTIR spectrometer (SHIMADZU-Miracle 10) in the range number of 3750-500 cm-1 using KBr pellet method. The surface Plasmon resonances of synthesized nanoparticles were studied by a UV-Visible spectrometer (JASCO V-650) in the range of 250-700 nm.

C. Preparation of NANO-BASED Mouthwash

In the preparation of Nano-based mouthwash 2ml of hydrogen peroxide, 0.5grams of sodium fluoride, and 0.9ml of synthesised nanoparticles containing leaf extract were added into the 500ml of distilled water and thoroughly mixed. (Bharati Timmappa Naik *et al.,* 2018).

The free radicals produced from the hydrogen peroxide can break the alkene double bond responsible for discoloration and stain removal (Jingta *et al.,* 2013)

D. Collection Of Test Pathogens

The test pathogens used in this study were gram positive bacteria *Staphylococcus* sp and *Streptococcus sp* ,gram negative bacteria used was *Pseudomonas* sp. Test organisms used were clinical isolates.

1) *Biochemical Characterization of Test Pathogens:* The collected test pathogens were characterized by the biochemical tests. The isolates were subjected for Indole production, MR,VP, Citrate utilization, Urease production, catalase, Oxidase, H_2S production and sugars fermentation (glucose, maltose, and lactose) tests.

E. Antimicrobial Activity of NANO-BASED Mouthwash

The antimicrobial activity of the Nano-based mouthwash evaluated against the test pathogens. The antimicrobial activity was evaluated by well diffusion method. The test organisms were swabbed on the Mueller Hinton's Agar plates. The wells were made into the inoculated plates with the help of sterile cork borer. The different concentrations (40µl, 80µl and 120µl) of extracts were added to the wells. The plates were incubated at 37ºC for 24hours (Bharati Timmappa Naik *et al.*, 2018).

III. RESULTS

A. Synthesis of Silver Nanoparticles

The synthesis of silver nanoparticles were be observed based on the colour change of the solution. The particles exhibit different colour based on the concentration of leaf extract in the silver nitrate solution. Simple leaf extract reduction method has been developed for synthesizing silver nanoparticles. The appearance of a yellowish-brown colour in the reaction vessels after two hours of incubation at room temperature indicated the formation of silver nanoparticles.Result shown in Fig.1.



Fig.1 Synthesis of Silver Nanoparticles using the mixture of leaf extract of *P.guajava* and *F.benghalensis*

B. FTIR Analysis

Fourier Transform Infra-red spectroscopy analysis was performed for the studying the functional groups present in the synthesized silver nanoparticles. The FTIR spectrum is given in Fig.2. The spectra revealed the presence of different functional groups like C-N, CO-C, and amide linkages and -COO- between amino acid. Absorption peaks located at about,3348.42,1635.64,. 424.34, 455.20, 486.06, and 563.21 in the region 1,000-400 cm^{-1}. These peaks were confirmed that some of the organic compounds from leaf extract act as a reducing or capping agent of the nanoparticles. The spectrum demonstrated that the carbonyl group was involved in the reduction of Ag^{+} to Ag.

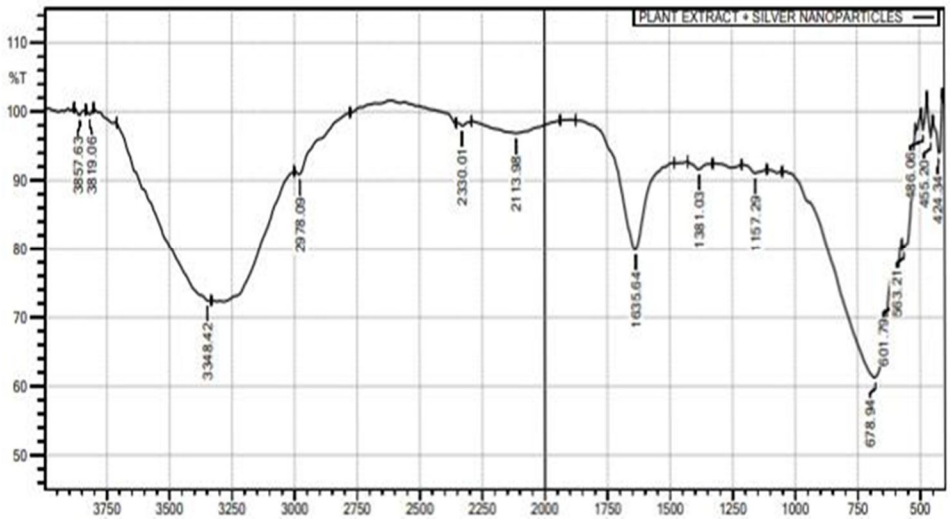


Fig.2 FTIR analysis of silver nanoparticles

C. UV-VISIBLE Spectrophotometric Analysis

The confirmation of formation and stability of the synthesized silver nanoparticles in the colloidal solution was monitored by using UV-Vis Spectra Analysis. The synthesized silver metal nanoparticles showed a well-defined absorption peak at the range of 410-430 nm and confirmed the reduction of silver by the colour change from colourless to brown colour. The change in colour was visually observed which indicates the presence of silver nanoparticles. The graphical representation of Fig.3 of UV-Vis spectroscopy analysis showed the absorption peak in the range of 410-430nm**.** The band at 393.57cm^{-1} represents stretch of C-H bond. The band at 292.78cm^{-1} corresponding to C-H bond. The band at 284.95cm^{-1} showed H-C=O bond.

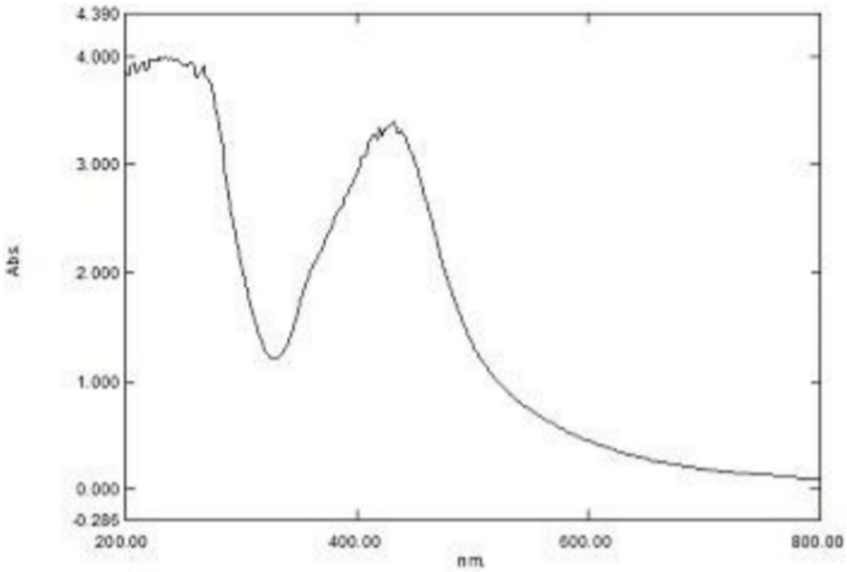


Fig.3 UV-Visible spectra of silver nanoparticles

D. Test for Biochemicals

The biochemicals of the isolated pathogens were listed and tabulated on Table.1 Most of the results were shown the positive results that confirmed the pathogen.

Table.1 Biochemicals of *S.aureus, S.pyogenes, P.aeruginosa*

Bio-chemicals	*S.aureus*	*S.pyogenes*	*P.aeruginosa*
Indole	Negative(-)	Negative(-)	Negative(-)
Methyl Red	Positive(+)	Positive(+)	Negative(-)
Voges-Proskauer	Positive(+)	Negative(-)	Negative(-)
Citrate	Positive(+)	Negative(-)	Positive(+)
Urease	Positive(+)	Negative(-)	Negative(-)
Oxidase	Negative(-)	Negative(-)	Positive(+)
Catalase	Positive(+)	Negative(-)	Positive(+)
H_2S	Negative(-)	Negative(-)	Negative(-)
Fermentation of Sugars			
Glucose	Positive(+)	Positive(+)	Positive(+)
Maltose	Positive(+)	Positive(+)	Negative(-)
Lactose	Positive(+)	Positive(+)	Negative(-)

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E. Antimicrobial Activity Of Nano-Based Mouthwash

The Fig.3 image of prepared Nano-based mouth wash was subjected for the antibacterial activity against oral infections. Fig.4, Fig.5, Fig.6 image demonstration of antibacterial activity of Nano based mouthwash results showed the positive result against oral and dental infections causing gram positive microorganisms *Staphylococcus sp* and *Streptococcus sp* and gram negative microorganism *Pseudomonas sp.* An amount of 20µl of Silver Nanoparticles incorporated mouthwash sample almost completely inhibited bacterial growth. The minimum inhibitory concentration of Silver Nanoparticles incorporated mouthwash against the pathogens were shown in the Table.2 which demonstrated the amount of the sample concentration and parameter of growth inhibition.



Fig.3 synthesized Nano-based Mouthwash



Fig.4 Antibacterial activity of Nano-based mouthwash *Staphylococcus sp*



Fig.5 Antibacterial activity of Nano-based mouthwash *Streptococcus sp*



Fig.4 Antibacterial activity of Nano-based mouthwash *Pseudomonas sp*

Table 2. Antibacterial activity zone size value of Nano-based mouthwash

Microorganisms /amount of Nano based mouthwash	20µL	40µL	60µL
Staphylococcus sp.	1.1cm	2cm	2.2cm
Streptococcus sp.	1.3cm	1.8cm	2cm
Pseudomonas sp.	1.3cm	2.8cm	3cm

IV. DISCUSSION

The present work demonstrated the ability of combined leaf extracts of Guava (Psidium guajava) and Banyan (Ficus benghalensis) leaves extract in synthesis of sliver nanoparticles and the incorporation of green synthesised Silver Nanoparticles in the preparation of Nano based mouth wash. Silver nanoparticle based moutwash found to be active against thee oral infection causing bacteria *Staphylococcus aureus*, *Streptococcus sp Pseudomonas sp*.on evaluating antimicrobial activity. The colour change of the plant extracts demonstrated the given plant extract mixture of *P.guajava* and *F.benghalensis* produce the silver nanoparticles. And the FTIR peak values demonstrated the presence of different functional groups like C-N, CO-C, and amide linkages and -COO- between amino acid. In the present work, FTIR spectral measurements were carried out to identify the potential biomolecules in the mixture of *P.guajava* and *F.benghalensis* leaf extract which acted as a capping agent and to produce sliver nanoparticles. The UV-visible spectra was showed the absorption peak at the range of 410-430 nm. This peak ranges between 410-430nm illustrated the C-N bonds. This result was matched with the statement of Krutyakov .Y. A *et al.*, 2008 the band absorption described by UV-Vis results confirm the presence of nanosized silver (408-410 nm wavelength), which depends on particle size due to the increment in the particle radius associated to the optical properties of Ag-NPs and their environmental conditions.

The antibacterial activity of synthesized Nano-based mouth wash showed the positive results against the Oral infection causing microorganisms. The results matched with the statement demonstration by Espinosa-Cristóbal.L.F *et al.*, 2009 that the synthesized Ag-NPs can promote good antimicrobial properties against *S. mutans* strain isolated from patients with dental caries (n = 30 patients), obtaining MIC values of 101.98 µg/mL for smaller Ag-NPs (8.4 nm) and 145.64 µg /mL for larger particles (16.1 nm).

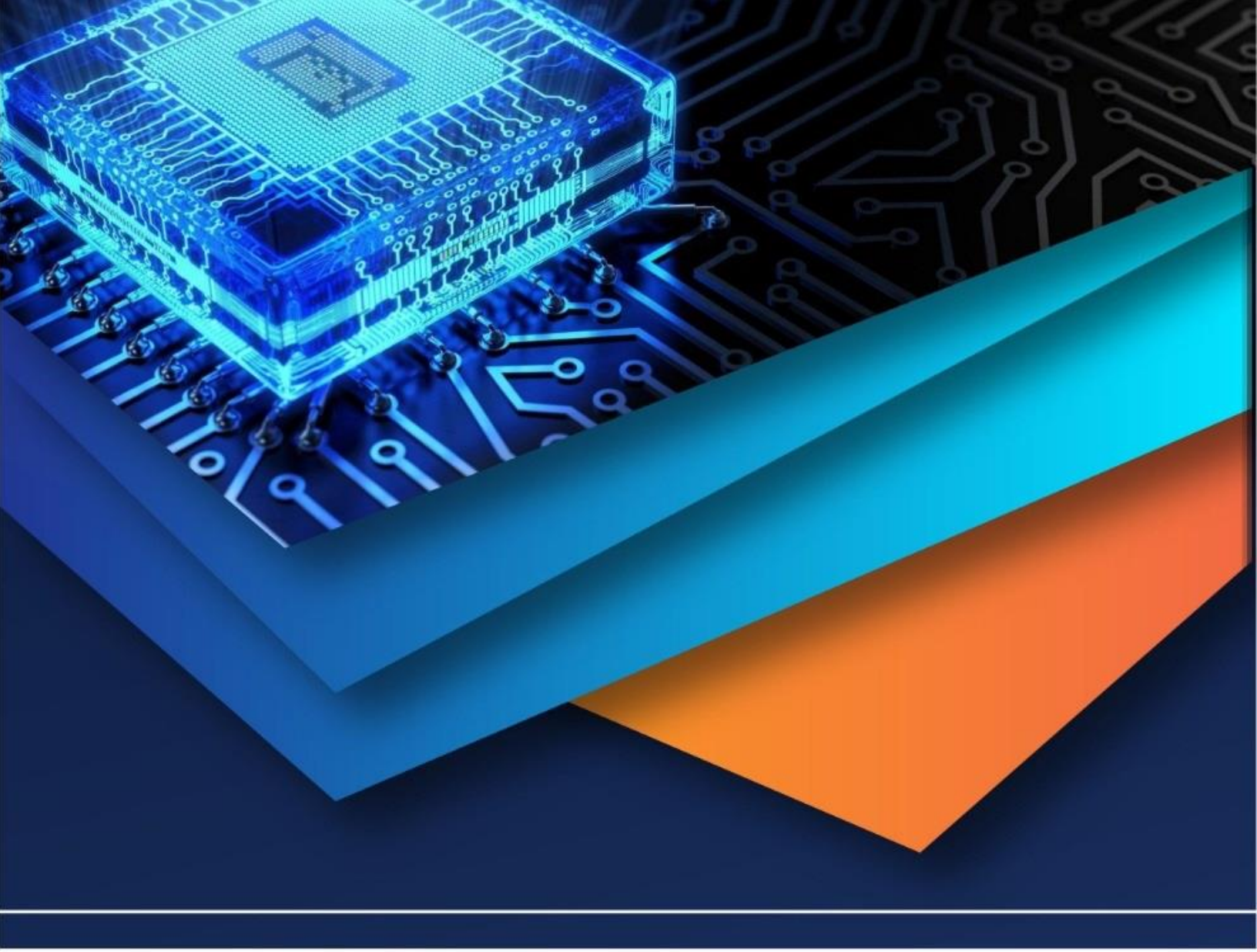
V. CONCLUSION

From this result it has been concluded that the Silver Nanoparticles synthesized using the combined plant leaves extracts of *P.guajava* and *F.benghalensis* have antibacterial activity against the Oral infection causing bacteria *S.aureus, S.pyogenes* and *P.aeruginosa sp.* The FTIR and UV – Visible spectra showed the C-H bonding of leaf extracts. The antibacterial activity of the Nano-based mouth wash showed the positive results at the different quantities. From this it has been concluded that the mixture of plant extracts have ability to act as a capping agent and reducing agent. The synthesized sliver nanoparticles may be used for the preparation of Nano-based mouthwash.

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