AN INVITRO EVALUATION OF ANTIBACTERIAL EFFICACY OF BROMELAIN MEDIATED SILVER NANOPARTICLES AGAINST E-FAECALIS

Dr. Sathya Kumaresan[1], Dr. Jayashri Prabakar[2*], Dr. S.Rajeshkumar[3], Dr. MeignanaArumughamIndiran[4]

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Abstract

Introduction: Nanotechnology is a rapidly manifesting scientific field with various biomedical and pharmaceutical applications. Among the various metal nanoparticles, silver has gained popularity because of its potent antibacterial effects. In this investigation, silver nanoparticles were synthesized by bromelain, which is an elementary extract from pineapple employing a nontoxic, cost efficient and environmental friendly process.
The aim of the present study was to synthesize bromelain mediated silver nanoparticles and to evaluate its antibacterial efficacy against E-faecalis.
Materials and Methods: Aqueous extract of bromelain was formulated. An ecofriendly green synthesis was employed to fabricate bromelain mediated silver nanoparticles. The synthesized nanoparticles were characterized by UV- visible absorption spectra. The structure of the nanoparticles was analyzed by TEM. The antibacterial activity of silver nanoparticles was determined by agar well diffusion method against E.faecalis.
Results: Antibacterial activity demonstrates that the zone of inhibition of E-faecalis varies with an increase in concentration of Ag and remains constant at 50µl and 100µl respectively. The characterization of Ag+ ions by UV- visible absorption spectra confirms the reduction of silver ions to silver nanoparticles. TEM affirms that the synthesized nanoparticles were of spherical shape and the size of the nanoparticles ranges from 3-20nm.
Conclusion: This study states that bromelain mediated silver nanoparticles possess good antibacterial properties.

Keywords: Bromelain, bromelain mediated nanoparticles, silver nanoparticles, E.faecalis

[1]Post graduate, Department Public Health Dentistry, Saveetha Dental college, Saveetha Institute of Medical and Technical Sciences, Saveetha University No.162, Poonamallee high road, Chennai 600077, Tamil Nadu, India.
[2*]Reader, Department of Public Health Dentistry, Saveetha Dental college, Saveetha Institute of Medical and Technical Sciences, Saveetha University No.162, Poonamallee high road, Chennai 600077, Tamil Nadu, India.
[3]Professor Department of Pharmacology Saveetha Dental college, Saveetha Institute of Medical and Technical Sciences, Saveetha University No.162, Poonamallee high road, Chennai 600077, Tamil Nadu, India.
[4]Professor and Head, Department of Public Health Dentistry, Saveetha Dental college, Saveetha Institute of Medical and Technical Sciences, Saveetha University No.162, Poonamallee high road, Chennai 600077, Tamil Nadu, India.

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1. Introduction

The green synthesis of nanoparticles is an innovative ecofriendly process that is gaining popularity across the world[1,2].

Nanobiotechnology is an expeditiously growing scientific field that operates nanosized particles of about nanometer scale level(1 to 100nm).Nanoparticles have promising outcomes in human health care applications. Amidst the nanoparticles, silver nanoparticles contribute an important role in the field of nanomedicine[3-4].

Silver nanoparticles possess an inherent antimicrobial effect against bacteria,fungi,viruses and yeast[5]. Recently,silver nanoparticles are constructed using plant extracts.The biosynthesized nanoparticle is known to exhibit antibacterial activity against various microorganisms[6,7-8]. This green chemistry has several advantages like cost effectiveness and compatibility for pharmaceutical and biomedical applications[9].

Plant mediated biosynthesis of nanoparticles is considered as an acclaimed technology for the rapid synthesis of nanoparticles[10].

Plants possess various cellular structures and physiological processes to combat the toxicity of metals[10,11]. They play a vital role in maintaining homeostasis. They also hold dynamic solutions to bring about metal detoxifications .In plants or plant derived materials, a wide range of metabolites with redox potentials is determined, which are playing a principal role as a reducing agent in the biogenic synthesis of nanoparticles[12]. Highly stable nanoparticles are synthesized by plant or plant extracts with the higher rate of production[13].Furthermore, plant mediated biosynthesis of nanoparticles require minimal maintenance and are cost efficient[14].

Ananas comosus L., also known as, pineapple belongs to the Bromeliaceae family. Pineapple is native to Thailand, China, Brazil and India. Pineapples comprise phytochemicals that are advocated to be the crucial bioactive compounds for health[15].

Bromelain is a cysteine protease found in pineapples[16].It has been proved in various therapeutic areas because of its anti-inflammatory and anti-cancer activities[17-18].Several studies have also summarized potential applications of bromelain[19-20].

It has been well known that Enterococcus faecalis, gram positive bacterium is predominantly isolated from failed root canals[21].It has the potential to invade dentinal tubules and is the principal cause for persistent failure of root canal treatment[22]. According to literature, sodium hypochlorite is one of the successfully used chemical root canal irrigants[23-24].However, it is analogous to several pitfalls like tissue toxicity, allergic reactions irritation to periapical tissues, inability to remove smear layer. In addition, it also has an undesirable taste and smell.

Constant increase in antibiotic resistant strains and side effects of these chemical root canal irrigants have paved way for alternative herbal medicaments[20,25]. According to studies, various herbal extracts like neem, tulsi, aloe vera and turmeric have been employed as promising root canal irrigants[26].However, there is a lack of evidence reporting bromelain mediated silver nanoparticles as root canal irrigant and investigating their antibacterial actions. Our team has extensive knowledge and research experience that has translate into high quality publications [27–36].

Hence, the present study was undertaken to evaluate the antibacterial efficacy of bromelain, bromelain mediated silver nanoparticles against E-faecalis and comparing it with 5% sodium hypochlorite.

2. Materials and Methods

This in vitro study was carried out in Nanomedicine Lab,Saveetha Dental College,Chennai.

Aqueous extract of bromelain was formulated. An eco friendly green synthesis was employed to fabricate bromelain mediated silver nanoparticles. The synthesized nanoparticles were characterized by UV- visible absorption spectra. The structure of the nanoparticles was analyzed by TEM. The antibacterial activity of silver nanoparticles was determined by agar well diffusion method against E.faecalis.

Preparation of bromelain extract

Bromelain Powder was procured from an online retailer of Southern India. The fresh extract of bromelain powder was prepared in aqueous form. The obtained extract mixture was filtered by Whatman filter paper no.1 and stored in airtight container for further synthesis process. bromelain extract was prepared by adding 0.05g in 50ml of distilled water.

Formulation of silver nanoparticles

0.019g of aqueous silver nitrate solution in 50ml was prepared and the formulated bromelain extract was added into aqueous silver nitrate solution drop by drop. After the addition, the reaction mixture colour turned slowly as yellowish brown colloidal suspension(Fig.1).

The colloidal suspension was centrifuged at 2000rpm for 20 min and then Ag NPs were collected from reaction mixture by Whatman filter. The collected Ag NPs were washed with ethanol and then dried at 100degree celsius for 2 hours.

Structural analysis of nanoparticles

The optical absorption spectra were recorded by UV-vis spectrophotometer. The surface morphology was studied using transmission electron micrograph(TEM).

Antibacterial assay

By well diffusion method, the antibacterial activity of synthesized Ag NPs were studied against E.faecalis. Three wells were loaded with bromelain, bromelain mediated silver nanoparticles and 5% sodium hypochlorite and the agar plates were incubated at 36degree celsius for 24 hours. After the incubation, the zone of inhibition was around the well was measured.

This indicates the antibacterial activity of synthesized Ag NPs.

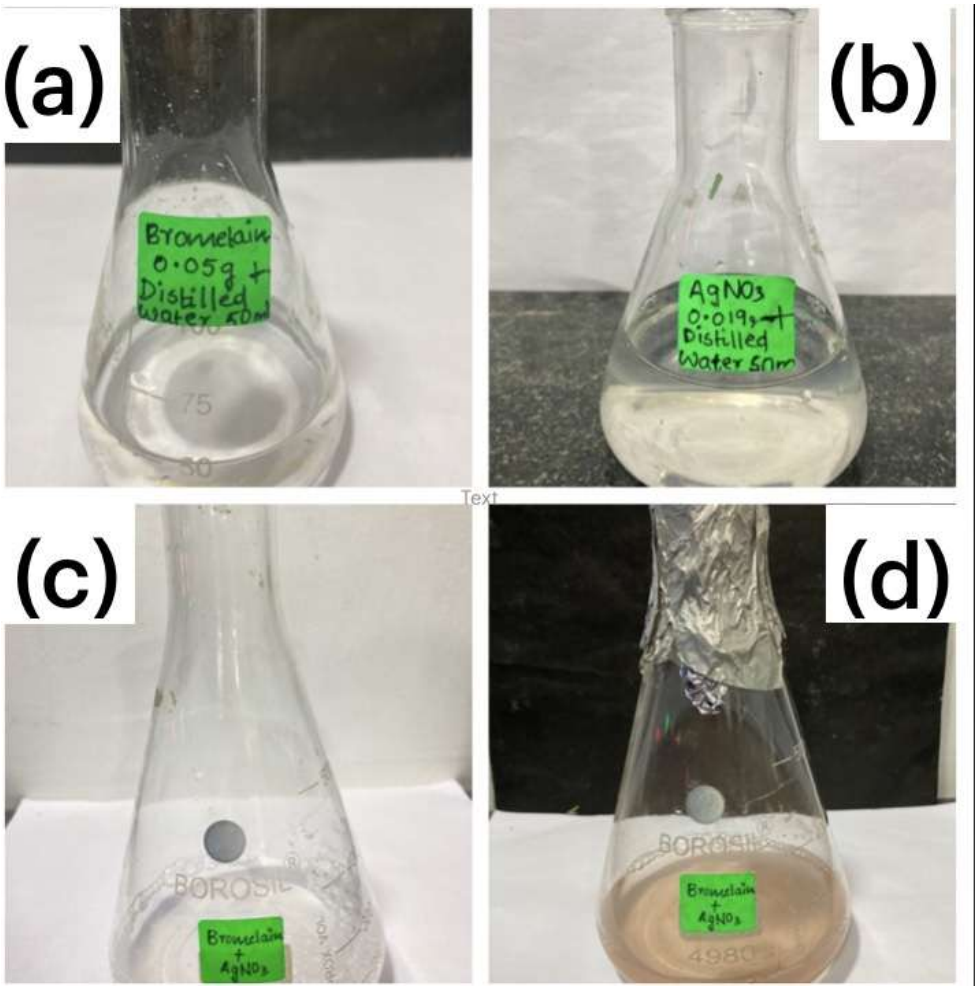

Fig.1. Visual observation showing (a) formulation of bromelain extract, (b) formulation of silver nitrate solution,(c) reaction mixture of bromelain extract and silver nitrate (d) colour change of reaction mixture after 24h.

3. Results

Fig.2. displays the UV-vis absorption spectra of aqueous silver nitrate and bromelain solution. Initially the silver nitrate solution was colourless and it turned as light brown after the addition of bromelain extract into the reaction mixture. The brown colour was much more evident because of the constant rotations of the shaker for 3 consecutive days. This change in colour of the silver solution strongly affirms the development of Ag NPs in the aqueous medium and is also the naked eye confirmation. The colour of the reaction mixture varied from yellowish brown, brown and deep brown with respect to the addition of extract volume 5ml,10ml and 15ml in the reaction solution respectively.

The increase of extract volume increases the reduction rate of Ag+ ions to Ag, this gives higher concentration of Ag NPs a deep brown solution. The resonance of incident photons energy to surface electrons on the Ag NPs gives a strong and single surface Plasmon resonance(SPR) peak at 460nm in the absorption spectra; this is the characteristic peak of Ag NPs. No any addition resonance peaks in the spectra were observed.

Hence, the synthesized samples have uniform size distribution and spherical shape nanoparticles.

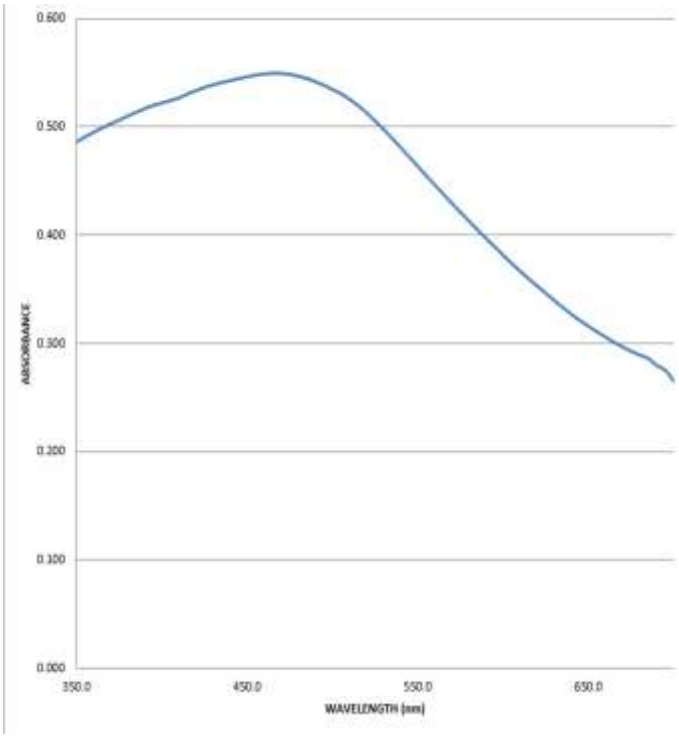

Fig.2. UV-vis spectra of silver nanoparticles synthesized by bromelain extract show the SPR band at 460nm confirming the formation of silver nanoparticles in the reaction mixture.

TEM Analysis

The TEM image of bromelain mediated silver nanoparticles is shown in Fig.3.

The image displayed that the synthesized nanoparticles were uniformly dispersed and mostly having spherical shape with an average size of 3-20nm.The well dispersion of the nanoparticles may be ascribed to the stabilizing phytochemicals in the bromelain extract.

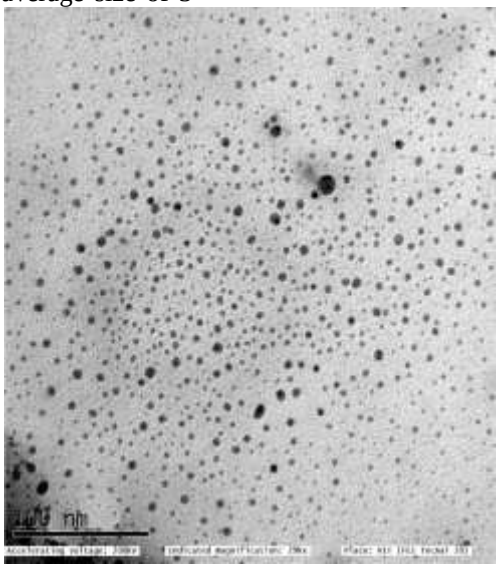

Fig.3. Transmission Electron Microscopic image of silver nanoparticles synthesized by bromelain extracts.

Antibacterial Study

Fig.4. signifies a graphical representation of the antibacterial activity of bromelain and bromelain mediated silver nanoparticles against E-faecalis in comparison with sodium hypochlorite.

The graph demonstrates that the zone of inhibition of E-faecalis varies with an increase in concentration of Ag and remains constant at 50µl and 100µl respectively.

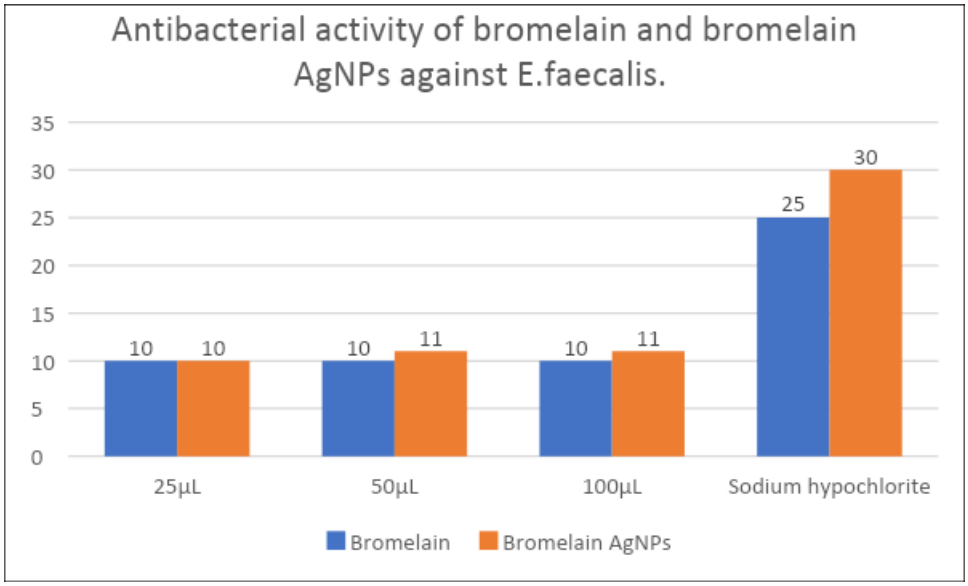

Fig.4. Antibacterial activity of bromelain and bromelain mediated silver nanoparticles against E-faecalis.

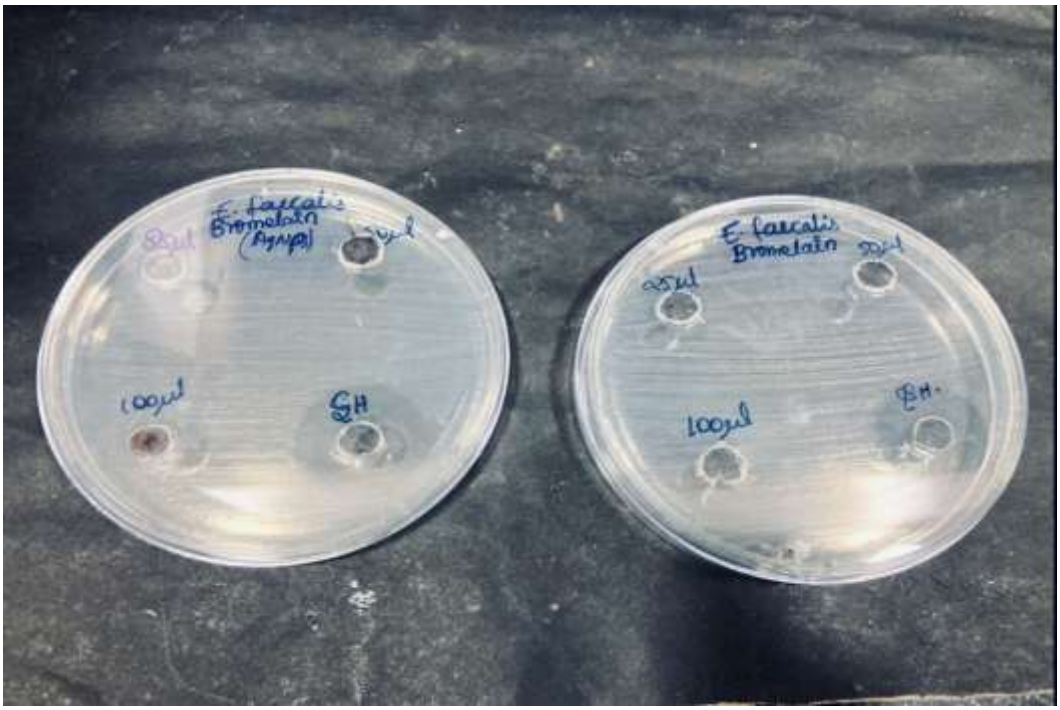

Fig.5.Antibacterial activity of Bromelain and Bromelain AgNPs against E-faecalis.

Antibacterial activity of Bromelain extract and green synthesized Ag NPs was observed by well diffusion method against E.faecalis(Fig.5).Both the plant extract and synthesized Ag NPs show a significant inhibition against the selected bacteria. Predominantly, the maximum zone of inhibition was observed for silver nanoparticles. Indeed, the antibacterial activity was higher in silver nitrate solution as compared to the plant extract.

4. Discussion

The pineapple stem enzyme, Bromelain appears to be a potential source for hydrocarbons. It has been reported that the enzyme contains various organic compounds such as alkaloids, flavonoids, tannins, terpenoids, steroids, glycosides and benzenoids. Bromelain also exerts an antibacterial effect against potent periodontal pathogens[37].In this study, we considered the fresh extract of bromelain for reduction of Ag ions. The bromelain extract was transparent but after addition of AgNO3 solution and stirring at room temperature, gradually the colour changed into brown. In other words, by passing the incubation time, the colour intensity increased, which confirmed Ag ion reduction and the formation of Ag NPs.

In the current study, UV-Vis absorption spectrum of Ag NPs is shown at 460 nm. While it was observed that the AgNP's surface plasmon resonance peak occurs at 440nm with high absorbance[38].

Vilchis-Nestor et al.revealed the reduction of silver ion and formation of stable nanoparticles within 4hours of reaction with Camellia sinensis extract[39].Chandran et al synthesized silver nanoparticles within 24hours of reaction using aloe vera[40].In the current study the stable Ag NPs were formed in 48hours.

Researchers reported that small sizes Ag NPs are having excellent antibacterial activity, because of its larger surface area. The synthesized Ag NPs are small in size ranging from 3-20nm and are attached to the cell wall membrane thereby entering the bacteria. Thus, it disturbs respiration process of the bacteria finally leading to death.

In a study conducted by Rajeshkumar et al,that used marine bacteria,Vibrioalginolyticus for the biosynthesis of gold nanoparticles reports the successful colour change from light yellow to final reddish brown incubated at 24hours[41].Similar results were observed with Stenotrophomonas maltophilia[42].In the present study, the visual observation of light brown to dark brown confirms the fabrication of silver nanoparticles incubated for 48hours.

In a similar study carried on marine brown seaweed Padina tetrasromatica, the TEM examination reveals spherical, rounded, rectangle and pentagon shaped gold nanoparticles ranging with a size of 10-70 nm. These findings may be attributed to the improper dispersion of the solution[43].Similar findings were reported with studies done on Pongamia pinnata[44].

However, in our study, the synthesized nanoparticles were well dispersed, spherical shaped with the size of the particles ranging from 3-20 nm.

In a report that assessed the antibacterial activity of silver nanoparticles against E-coli and S-aureus, the findings confirm that the synthesized nanoparticles were more effective against E-coli as compared to S-aureus[45].These results are in accordance with the current study. This rapid structural change leading to cell death may be due to fast internalization of nanoparticles from the bacterial cell wall.

5. Conclusion

The plant mediated biosynthesis of silver nanoparticles is comparatively a cost efficient,non toxic method. The current study signifies a dark brown colour of the synthesized nanoparticles, a significant characteristic of visual observation. The UV-vis spectra exhibited maximum absorbance peak at 460 nm. The TEM analysis proved the synthesis of well dispersed, spherical shaped nanoparticles with the size ranging from 3-20 nm. The synthesized bromelain mediated silver nanoparticles showed higher antibacterial activity against E -faecalis in comparison with sodium hypochlorite. Bromelain mediated silver nanoparticles can be used as an alternative therapeutic agent in the field of dentistry for effective management of resistant microorganisms

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