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Research Article

Preparation of Silver Nano Particles Using Aqueous Solution of *Ocimum sanctum* and *Piper betle* and Evaluation of Its Antimicrobial Activity Against *Enterococcus faecalis*

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ABSTRACT

Enterococcus faecalis is commonly found in a high percentage of root canal failures and it is able to survive in the root canal as a single organism. Silver nano particles (AgNPs) are nano particles of silver between 1 nm to 100nm and are known to have excellent antimicrobial properties thus used as disinfectants. An attempt has been made in this study to evaluate the antibacterial activity of AgNPs prepared by the aqueous extracts of *Ocimum sanctum* (Thulasi) and *Piper betle* (betel leaf) against *E. faecalis*. 9ml of 5mM Silver nitrate was added to 1ml of aqueous extract of plants and incubated at 37ºC for 24 hrs. The change in colour from yellow to brown indicates the production of Silver nano particles. UV–Vis spectral analysis was done by UV–visible spectrophotometer. UV–Visible absorption between 200 and 800 nm was used. The antibacterial study was done by well diffusion method. The silver nano particles were successfully synthesised from the aqueous extracts of *O. sanctum* (Thulasi) and *P. betle* (betel) leaves). The formation of AgNPs was indicated by the peak absorption between 410-450nm in UV-Vis spectrophotometer. The present study showed that the AgNPs prepared from the two plant extracts have anti-bacterial activity against *E. faecalis*. From the present study it has been concluded that the green synthesized AgNPs has antibacterial activity against *E. faecalis* and hence can be used for endodontic treatment.

Keywords: Endodontic treatment, AgNPs, antibacterial activity, *Ocimum sanctum*, *Piper betle*

INTRODUCTION

Current concept in endodontic microbiology emphasizes endodontic disease is a biofilm mediated infection[1]. Elimination or significant reduction of bacterial biofilm is an essential element for successful outcome of endodontic treatment[2]. However clinical studies have shown that even after chemo-mechanical disinfection and obturation of root canals, bacterial biofilm may still persist in root canal system[3]. Thus, it is imperative to develop an endodontic disinfection strategy that is effective in eliminating bacteria within root canals. *Enterococcus faecalis* is formerly classified as Group D Streptococcus. It is commonly found in a high percentage of root canal failures and it is able to survive in the root canal as a single organism. In dentinal tubules it can resist intracanal dressing of Calcium hydroxide for over 10 days[4]. The pH needed to kill *E. faecalis* is 10.5 to 11.0. The pH of $Ca(OH)_2$ can only reach upto 10.3 because of buffering effect of dentin. Thus *E. faecalis* is resistant to Calcium hydroxide intracanal treatments[5]. Silver nano particles (AgNPs) are nano particles of silver between 1 nm to 100nm in size. They are known to have excellent antimicrobial properties thus used as disinfectants[6,7]. AgNPs are synthesized by different methods viz., physical, chemical and biological. The biological or green synthesis of AgNPs is most preferred method than the chemical and physical methods as they are eco friendly, economic and easy for large scale synthesis[8]. Hence an attempt has been in this study to evaluate the antibacterial activity of AgNPs prepared by the aqueous extracts of *Ocimum sanctum* (Thulasi) and *Piper betle* (bettle leaf) against *E. faecalis*.

MATERIALS AND METHODS

Preparation of plant extracts

The leaves of *Ocimum sanctum* (Thulasi) and *Piper betle* (betel leaf) were collected and cleaned with sterile distilled water. The leaves were ground in the sterile mortar and pestle with sterile distilled water. The material was then filtered through whatman filter paper No.1 and the filtrate was stored at 4ºC until use.

Preparation of AgNPs

9ml of 5mM Silver nitrate was added to 1ml of aqueous extract of plants and incubated at 37ºC for 24 hrs.

Characterization of synthesized AgNPs

The change in colour from yellow to brown indicates the production of Silver nano particles. UV–Vis spectral analysis was done by UV–visible spectrophotometer.

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Figure 1: Silver nano particles prepared by the plant extracts

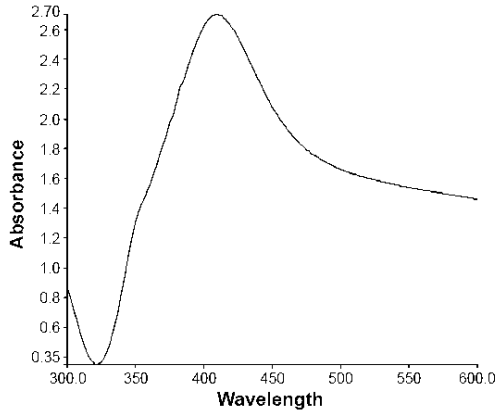

Figure 2: UV-Vis spectrum of silver nano particles

Figure 3: The antibacterial activity of AgNPs. *A- Betel extract, B- Thulasi extract*.

UV–Visible absorption between 200 and 800 nm was used.

Antibacterial study

The antibacterial study was done by well diffusion method. The Brain heart infusion agar plates were prepared. A lawn culture of *E. faecalis* (ATCC 51299) was made. Then wells were made and 50 microlitres of silver nano particles prepared with thulasi and betel leaf extracts were filled in the wells. 1% $Ca(OH)_2$ was also included in the plate. The plates were incubated for 48 hours at 37ºC. The antibacterial activity was indicated by the zone of inhibition around the well.

RESULTS AND DISCUSSION

The silver nano particles were successfully synthesised from the aqueous extracts of *O. sanctum* (Thulasi) and *P. betle* (betel) leaves. The reaction mixture changed to dark brownish suspension (Figure 1) after 24 hours of incubation. The colour change is taken as the indicator of conversion of silver nitrate solution to metallic silver as reported in many other similar studies[9,10]. Further the UV-Vis spectroscopy was used to detect the production of AgNPs in green synthesis. The absorbance in the range of 420–450 nm has been used as an indicator to confirm the production of AgNPs[11,12]. In the present study, the formation of AgNPs was indicated by the peak absorption between 410-450nm (Figure 2). The antibacterial activity of silver compounds and their ions are recognized long before. Their antibacterial activity is attributed to strong interaction of silver with thiol groups of respiratory enzymes in bacteria[13]. The Table 1 shows the results of well diffusion method to study the anti-bacterial study. The present study showed that the AgNPs prepared from the two plant extracts have anti-bacterial activity against *E. faecalis* (Figure 3). The antibacterial activity of *Ocimum sanctum* and *Piper betle* is well recorded[14,15]. When these plants are used to prepare AgNPs, they can combine with the AgNPs to exert an enhanced anti-bacterial activity. It has been established that the green synthesis of AgNPs shown significant increase in their activity than that of plant extract alone[16]. Further this study has shown that there is no anti-bacterial activity for calcium hydroxide against *E. faecalis*. Thus it is imperative to find an alternative endodontic treatment agent. The green AgNPs can be a solution to treat the root canal as it has an excellent activity against important root canal pathogen, *E. faecalis*. The use of AgNPs is new to the field of dentistry. They have been tried in different areas of dentistry like endodontics, dental prostheses, implantology, and restorative dentistry[17-19]. However the use of green synthesized AgNPs is new to the field of dentistry. The green synthesized AgNPs has the advantage of having the antibacterial activity of the plant extracts used for their synthesis along with their individual antibacterial activity.

Table 1: Results of anti-bacterial activity

S. No.	Material used	Diameter of zone of inhibition
1.	Silver nano particles prepared from aqueous extract of *Ocimum sanctum.*	10mm
2.	Silver nano particles prepared from aqueous extract of *Piper betle.*	14mm
3.	Calcium hydroxide	No zone

CONCLUSION

In the present study, an attempt has been made to study the antibacterial activity of AgNPs synthesised against *E. faecalis* by the aqueous extracts of *Ocimum sanctum* and *Piper betle.* From the present study it has been concluded that the green synthesized AgNPs has antibacterial activity against *E. faecalis* and hence can be used for endodontic treatment.

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