Research Article

Effect of nanoparticles incorporated into herbal substitutes against endodontic microorganisms

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ABSTRACT

Introduction: This study aims to evaluate the antimicrobial effect of nanoparticles incorporated into herbal substitutes against endodontic infections. **Materials and Methods:** The samples (30) were screened for antibacterial activity against *Enterococcus faecalis* using agar well diffusion method. The extract was mixed with ethanol and vortexed for maximum dissolution. Luria-Bertani agar plates were inoculated with test organism. The plates were evenly spread out. Then, wells were prepared in the plates with a cork borer. Each well was loaded with 50 µl. The plates were incubated for 24 h at 37°C. The development of the inhibition zone around the well was measured (diameter) and recorded. Tetracycline was used as a positive control. **Results:** In this *in vitro* study, we assessed the antimicrobial efficacy of sliver nanoparticles with *Calotropis gingantea* extract and tetracycline against *E. faecalis*. The antimicrobial activity of nanosilver particles with *C. gingantea* extract was moderate compared to that of conventional tetracycline. Tetracycline rapidly killed the bacteria in the Petri dish, only partial disinfection can be achieved in the surface wall of the root canal and tetracycline was relatively effective. More the concentration of the sample, more is the antibacterial effect of the sample. **Conclusion:** Silver nanoparticles incorporated into the extract *C. gingantea* against *E. faecalis* are less effective than tetracycline. Therefore, increasing concentration of the extract *C. gingantea* in nanoparticles can be assessed in the future.

KEY WORDS: Antibacterial pattern, *Calotropis gingantea*, *Enterococcus faecalis*, Silver nanoparticles

INTRODUCTION

Incomplete root canal of the advanced passage system could end in treatment failures and cause various diseases like periodontitis.[1] The microorganism penetration occurs into the depths of 300–1500 µm in dentinal tubules.[2] The microorganism in these depths would stay inaccessible for typical irrigants, medicaments, and sealers.[3,4] *Enterococcus faecalis* may be a facultative Gram-positive bacteria, isolated largely from the root-filled teeth with chronic top disease. *E. faecalis* has been found in 38th of the unsuccessful root canal-treated teeth. The ability to tolerate the rough environmental changes is believed to result to its high alkali tolerance,[5] and hollow invasion ability of this coccus that protects it from intracanal dentistry medicaments has made *E. faecalis* treatment-resistant bacteria. *E. faecalis* has the power to invade the dentinal tubules and cling to scleroprotein and form a type of biofilm on dentin. *E. faecalis* can even survive high pH levels varied from 9–11.[6]

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The scope of such strategies includes a wide variety of oral health-related issues such as treatment of biofilm elimination, diagnosis dentin hypersensitivity, and oral cancer. In the field of endodontics, the nanomaterials are focused on steps that would improve mechanical integrity, antimicrobial of previously diseased tissue regeneration, and dentin matrix. At present, it is a new technology tested in endodontics to have challenges over microorganisms.[7,8]

Nanodentistry implies the appliance of nanomaterials and dental nanorobots toward identification and treatment, with the goal of rising comprehensive oral health. Nanomaterial denotes an incidental, natural, or manufactured materials containing particles in an unbound state.[9] Nanomaterials as

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unique physicochemical properties, such as large surface, ultra-small size, and increased chemical reactivity, compared with their bulk counterpart.[10,11] The surface increased to volume ratio and the number is increased of atoms that are present near the surface compared with micro-/macro-structures which are suggested to contribute to the distinctly different properties of nanomaterial. Nanotechnology has formed rapidly as science and technology creating a myriad of biomedical applications such as tissue regeneration, drug delivery system, antimicrobial application, gene transformation, and imaging.[10-13] The term nanodentistry implies the application of nanomaterials and dental nanorobots toward diagnosis and treatment, with the goal of improving comprehensive oral health.

MATERIALS AND METHODS

Preparation of Extract

Calotropis gingantea extract was extracted with ethanol at room temperature for 72 h using a shaker. After filtration with Whitman filter paper No. 1 using a vacuum pump, the residue was re-extracted again with ethanol solvent. The solvent was completely removed using a rotary vacuum evaporator at 40°C. The concentrated extract was then kept in dark bottles at 4°C until used.

Preparation of Silver Nitrate (Ag NO_3) and Synthesis of Silver Nanoparticles (Ag-NPs)

The ethanolic extract of *C. gingantea* (1 g) was added to distilled deionized water (100 mL) with vigorous stirring for 1 h. A 100 mL of Ag NO_3 (1×10^{-2} M) was then added and mixed at room temperature (25°C) for 48 h. Ag-NPs were gradually obtained during the incubation period.

Ultraviolet (UV)–Visible Spectroscopy Analysis

UV–visible spectroscopy analysis was carried out using UV–visible absorption spectrophotometer between 200 nm and 700 nm. The reduction of silver ions into metallic Ag-NPs was monitored by UV–visible spectra of Ag-NPs in aqueous solution. The interactions of these particles with light occur as electrons on the metal surface undergo oscillations when excited by light at specific wavelengths. Ag-NPs obtained exhibit a unique peak in the range of 470 nm.

Antibacterial Activity using Agar Well Diffusion Method

The samples were screened for antibacterial activity against *E. faecalis* using agar well diffusion method.[14]

Agar Well Diffusion Method

Sample preparation

The extract was mixed with ethanol and vortexed for maximum dissolution.

Procedure

Luria-Bertani agar plates were inoculated with test organism. The plates were evenly spread out. Then, wells were prepared in the plates with a cork borer. Each well was loaded with 50 µl. The plates were incubated for 24 h at 37°C. The development of the inhibition zone around the well was measured (diameter) and recorded. Tetracycline was used as a positive control [Figure 1].

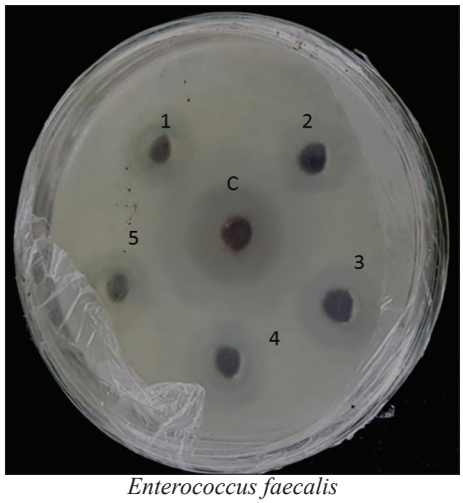
RESULTS

Ag-NPs with *C. gingantea* extract were prepared, which were evidenced by UV absorption spectra [Figure 2 and Table 1].

The antimicrobial activity of Ag-NPs with *C. gingantea* extract was moderate compared to that of conventional tetracycline. Tetracycline which was the positive control group showed an antibacterial activity of 30 µg.

DISCUSSION

In this *in vitro* study, we assessed the antimicrobial efficacy of Ag-NPs with *C. gingantea* extract and tetracycline against *E. faecalis*. *C. gingantea* extract was used because it has good allelopathic effect. The antimicrobial activity of Ag-NPs with *C. gingantea* extract was moderate compared to that of conventional tetracycline. Tetracycline rapidly killed the bacteria in the Petri dish, only partial disinfection can be achieved



Enterococcus faecalis

Figure 1: Antibacterial activity of the sample

Table 1: Antibacterial activity of samples

Sample	Concentration (µg)	Zone of inhibition (mm)	
		Sample	Control (400 µg)
Extract	200	11	30
	400	11	
	600	13	
	800	14	
	1000	16	

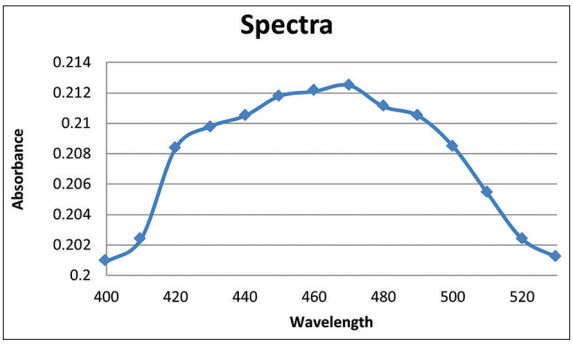


Figure 2: The ultraviolet–visible spectroscopy of silver nanoparticles synthesized from *Calotropis gigantea* extract after 48 h of incubation

in the surface wall of the root canal and tetracycline was relatively effective. More the concentration of the sample, more is the antibacterial effect of the sample.

Silver compounds and nanoparticles are employed in biomedicine, mainly as a result of their antibacterial drug property.[15] In case of its dental application, silver and its nanoparticles are tested for application as dentistry retrograde filling material, dental restorative material, dental implants, and in decay.[16] Silver is understood to provide an antibacterial drug impact by performing on multiple targets ranging from interaction with the sulfhydryl teams of proteins and DNA, and they alter the chemical element bonding/respiratory chain, unwind DNA, and interfere with plasma membrane synthesis/cell division.[17] Ag-NPs additionally destabilize the microorganism membrane and increase the permeability of the cellular constituents.[18] Ag-NPs with vital antibacterial drug activity can be used for root canal medical aid. However, the prolonged interaction time needed by Ag-NPs for effective microorganism killing has to be thought of, and its use ideally ought to be restricted to medicinal drug instead of as AN irrigant. Most of the nanoparticles were tested for passage medical aid depends on time-dependent and contact-mediated antibacterial drug activity. Adding of varied nanoparticles into root filling material sealers has considerably improved the antibacterial drug effectivity by inhibition of biofilm formation on the surface moreover because the resin-dentin interface. The buffering capability of dentin was evidenced by another study which assesses the effectiveness of CH paste against *E. faecalis* combined with totally different concentrations of dentin.[19] *E. faecalis* resists high hydrogen ion concentration levels. It maintains a hydrogen ion concentration level by the buffering capability of its living substance. It additionally encompasses a nucleon pump within which it provides further physiological state. However, studies have rumored that this organism cannot resist hydrogen ion concentration levels over 11.[20] Therefore, CH must penetrate dentinal tubules in enough concentrations to succeed a hydrogen ion concentration of ≥11.5.[21] Ag NO_3 is employed as reducer as silver has distinctive properties such as sensible conduction, chemical action, and chemical stability.[22] The liquid silver ions once exposed, its extracts were reduced in resolution, thereby resulting in the formation of silver hydrosol. Silver has a lot of microbic effectivity and more effective with the presence of macromolecule material (proteinaceous metal).[23]

CONCLUSION

Ag-NPs incorporated into the extract *C. gingantea* against *E. faecalis* are less effective than tetracycline. Therefore, increasing the concentration of the extract *C. gingantea* in nanoparticles can be assessed for better antimicrobial efficacy against *E. faecalis*.

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