RESEARCH ARTICLE

Comparison of Sizes of Zinc Oxide Nanoparticles Extracted from *Staphylococcus lugdunensis* and *Berberis vulgaris* Plant Extract Against Some Types of Bacteria and Yeast

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Received: 02nd December, 2021; Revised: 27th December, 2021; Accepted: 17th February, 2022; Available Online: 25th March, 2022

ABSTRACT

Staphylococcus lugdunensis, isolation between 12.5 to 1.8% routine works may be a possible peroral route of infective endocarditis and found in the oral cavity by examined using saliva. Similar supragingival plaque isolation was observed. The increased bacteria resistance to antibiotics multiple have led to novel methods for resistance bacteria; antimicrobial agents are well known (ZnO NPs) by biological method and are lower toxicity and biology safety ZnNOPs activity by plant extraction and less toxicity as well as bio-safe. The nanoparticle was synthesized by biological method (Green) by barberry (*Berberis vulgaris*) extract. In this study using (WAD) method using different concentrations between (128, 64, 32, and 16) mg/mL of ZnO NPs, The ZnO nanoparticles were synthesized by a biological method where pH = 10 and characterized by ultraviolet-visible spectroscopy, transmission electron microscopy and atomic force microscope.

Keywords: MIC, *Staphylococcus lugdunensis,* ZnO NPs.

International Journal of Drug Delivery Technology (2022); DOI: 10.25258/ijddt.12.1.20

How to cite this article: Abdel Majeed SM, Ahmed ME, Ali IA. Comparison of Sizes of Zinc Oxide Nanoparticles Extracted from *Staphylococcus lugdunensis* and *Berberis vulgaris* Plant Extract Against Some Types of Bacteria and Yeast. International Journal of Drug Delivery Technology. 2022;12(1):103-107.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

The oral cavity is a special microbial environment that allows for diverse ecological niches. Each of these ecological niches consists of soft tissue, non-shedding surfaces, and saliva. The properties of the microbiological niche play a role in the colonization of these places. Saliva is used as a medium by free-floating or planktonic microorganisms.[1] Because it reflects various symptoms and systemic illnesses, such as nutritional inadequacies and microbial infections, the mouth cavity important to the body's health.[2]

The most prevalent infectious disease, dental caries, is in the oral cavity, and if not treated, it can cause tooth decay, pulpitis, and periapical lesions.[3] Dental caries is a multifactorial illness influenced by various factors, microbial flora, and related environmental factors.[4]

Caries of the teeth are a global problem that affects millions of individuals. Dental caries varies between (49–83%), the prevalence of according to a recent survey by the worldwide oral health data bank. Dental caries affects people of all ages, the most children and then adults, according to data gathered from several studies.[5]

These bacteria are not pathogenic for healthy people such Streptococci and *Staphylococcus* species are common commensal residents of the oral microflora, as well as the gastrointestinal system and female vaginal tract.[6]

Because of its moisture, warmth, and nutrient content (lipid, carbohydrate, and protein), the human mouth cavity serves as a growing medium for pathogenic microbes;[7] therefore, staphylococcus species and oral streptococci, from dental plaque and saliva have generally been isolated.[8]

Staphylococcus lugdunensis is a staphylococcus that is gram-positive, catalase-positive, and coagulase-negative. It was once thought to be a cutaneous commensal or opportunistic pathogen.[9] Evidence published in the recent decade, however, confirms its life-threatening potential in both immunocompromised people. *S. lugdunensis* has now been linked to cardiovascular infections, acute oral infections, urinary tract infections, bone, and joint infections.[10] It's also been linked to soft tissue infections.[11] Occurrence of Staphylococcus species in saliva we examined dental plaque specimens from healthy adults.

Oral infections are treated with antibiotics and antiseptics, but the growth of resistant bacteria has become a major issue.[12,13] Vomiting, diarrhea, and tooth discolorations are frequent antibiotic side effects.[14] As a result, herbal medicines are more cost-effective and have fewer adverse effects.

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Barberry (*Berberis vulgaris* L., Berberidaceae) is a Berberidaceae plant that grows throughout Asia and Europe. *B. vulgaris* is a shrub with yellow blooms and oblong red berries.[15] The plant has all been utilized for medicinal purposes. Berberine, berbamine, and palmatine are the major alkaloids in this plant. Its primary ingredients have a variety of medicinal properties.[16] In the literature, it has been found to have potent antibacterial properties against *Staphylococcus aureus* (*S. aureus*) and *Candida spp*.[17]

MATERIAL AND METHODS

This study was conducted in 2021, with 44 samples taken from patients attending private dental clinics in Baghdad. All individuals in this study have signed a consent form to participate in this investigation. Swabs with specimens of deeply infected dentin were collected from deep carious lesion areas using a sterile dental explorer and small size bonding brushes with utmost caution to avoid exposure to mouth-contaminated materials.[18]

Identification Test Microorganism

The test microorganisms *S. aureus*, *E. coli*, *Pseudomonas aeruginosa*, and *Candida kefyer* were obtained from the Biology Department of the College of Science for Women, University of Baghdad. Microbes were isolated and identified using standard microbiological procedures.

Prepare a Suspension of *S. lugdunensis*

Suspension prepared on cultured MHB was incubated for one day at 37°C. The bacteria were centrifuged at 4° C for 10 minutes. Then compare with the turbidity of 0.5 McFarland.

Preparation Stock Solution of $Zn.2H_2O\ (CH_3CO_2)_2$

A stock solution of Zinc acetate $Zn.2H_2O\ (CH_3CO_2)_2$ was prepared by dissolving 0.01 g $Zn.2H_2O\ (CH_3CO_2)_2$ in 50 mL deionized water. (Figure 1)

MIC Determination

Doubling dilutions serial of ZnONPS were prepared using MHB, added to each test culture bacteria was tube incubator for 24 hours at 37°C.

Zn NPs Biosynthesis

For the synthesis of the Zn NPs, (7 mL) of the *S. lugdunensis* filtrate was added to 3 mL of 1-mM $Zn.2H_2O(CH_3CO_2)_2$ (final concentration) at room temperature 37°C.[19]

Preparation of Plant Stem Extraction

The plant was shifted and washed with distilled water and dried in the oven at 40°C. Then it was crushed into powder-fine, a powder (20.66 g) was soaked in 200 mL DW and boiled for 2 hours bath at 40°C after stirring for 12 hours, was filtered by filter paper prepared to extract and centrifuged to remove the remaining aggregates. For experimental analysis, the stock solution of BS was stored at 4°C. the extract doing different concentrations was used in the mixture reaction and diluted in 300 mL of double-distilled water-primarily and prepared ZnO NPs, 6 mL of barberry extract (Figure 2).

Antibacterial Assay

On an MHA media, the test bacterial species were inoculated activity antibacterial the effect by (WDA) examined the antibacterial activity. Barberry extract and ZnO NPs by *S. lugdunensis* and incubator at 37°C for 24 hours.

Figure 1: Synthesis of ZnO NPs by *S. lugdunensis*

Figure 2: Plant extraction

RESULT AND DISCUSSION

Isolation and Identification, *S. lugdunensis*

Spherical or ovoid in shape colonies are with raised or surface, as shown in Figure 3 under microscopic cells arranged in short non-spore-forming chains. Physiological properties of B-hemolytic, *S. lugdunensis* differ from nonhemolytic

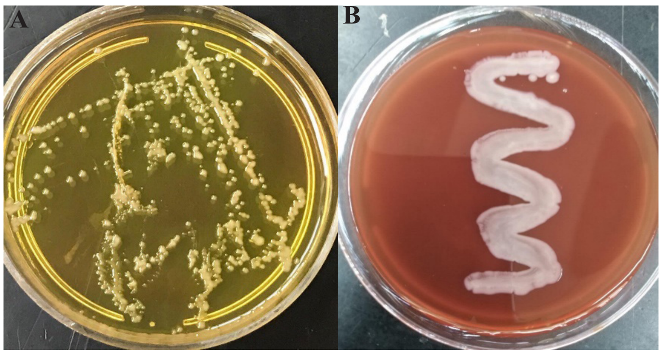

Figure 3: *S. lugdunensis* on (A) Blood agar (B) On mannitol salt agar 37°C for 24 hours (AST)

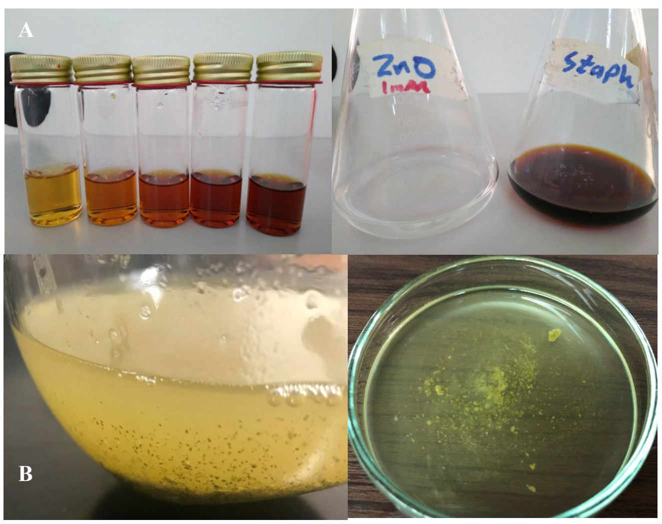

Figure 4: A) Synthesis ZnO nanoparticles by *S. lugdunensis*, B) Synthesis ZnO nanoparticles by *B. Vulgaris.*

Positive cases for the presence of *S. lugdunensis* in dental caries we found that most saliva was detected in female patients. Such a result was also achieved by Pannu *et al*.[20] In studies by Amoroso *et al*.[21] shown S. *mitis* and *S. salivarius* were commonly detected in saliva; we detected the presence *S. mitis* and *S. salivarius* and have found that *S. mutans* was more prevalent in our target patients than *S. mitis* and *S. salivarius*

Biosynthesis of ZnO Nanoparticles

The flasks indicate the biosynthesis process a white appeared at the bottom Figure 4A Isolate pathogenic *S. lugdunensis* was chosen for ZnO-NPs Biosynthesis. Both isolates gave positive results for ZnO-NPs synthesis. While synthesizing ZnO- NPs by plant extract Figure (4-B) Low–cost method to biosynthesis using bacteria. Change in color is due, which is a distinguishing property of the nanoparticles the two surface Plasmon resonance (SPR).[22]

Characterization Zn ONPs Compounds

Spectral ZnONPs

Figure (5 A-B) indicates the creation of ZnONPs. At 215 nm was selected, where absorption peak at 260 nm was obtained. Balogun *et al*.,[23] reported a corresponding absorption peak of ZnONPs at 207 nm due to the transmission of electrons between the ranges from the deep level of the valance range. Figure 1b represents

Atomic Force Electron Microscopy (AFM)

Figure (6 A-B) shows the surface roughness alterations, and the surface roughness change sample's grain size value was (37) nm synthesis by bacteria. Values were recognized and found section analysis the maximum crystal size of the sample plant extraction was found to be 31 nm. Zn NOPs have been synthesized and biological methods and physicochemical.[24]

TEM Analysis

For transmission electron microscopy, TEM micrographs of the ZnO NPs had a spherical shape, as shown in Figure (7 A-B). The diameter of the nanoparticles was measured to be nm For *S. lugdunensis* and *B. Vulgaris***.**

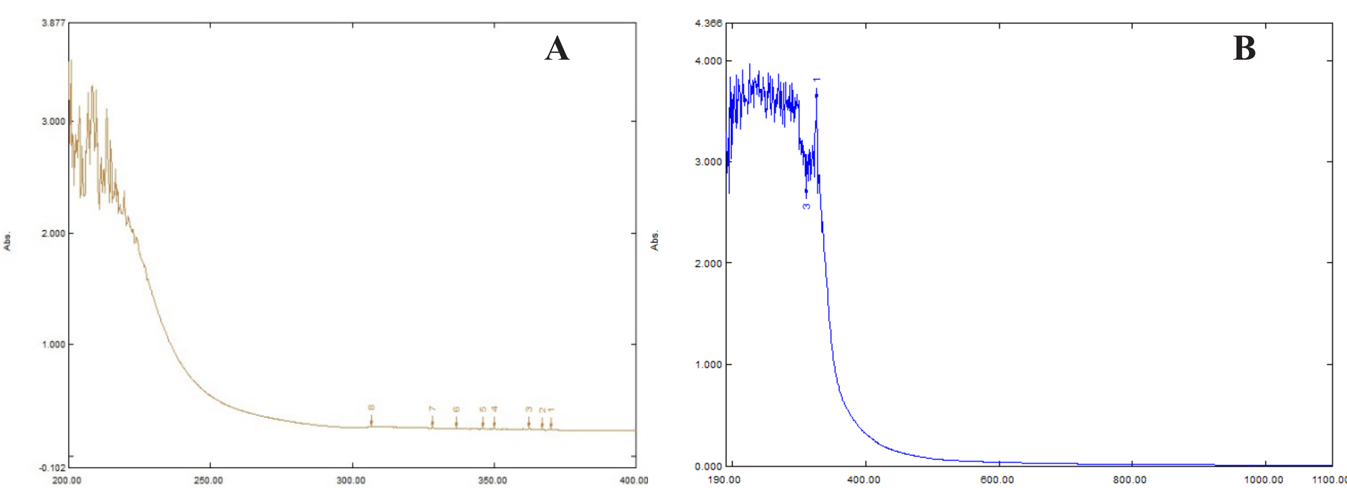

Figure 5: Absorption spectra of ZnO NPs nanoparticles A) *B. Vulgaris* B) *S. lugdunensis*

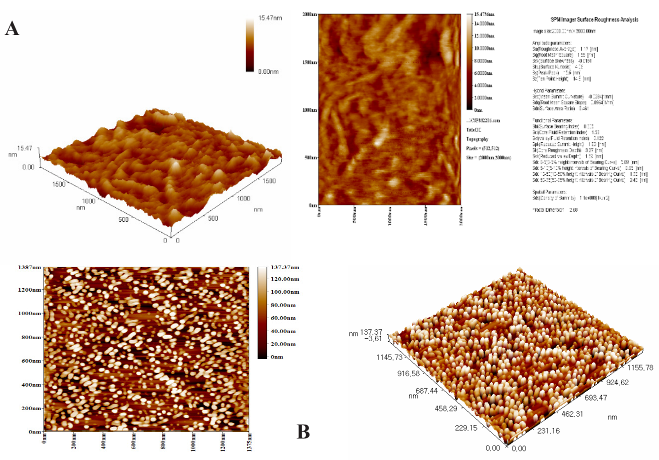

Figure 6: AFM for Zn NOPs nanoparticles A) *B. Vulgaris* B) *S. lugdunensis*

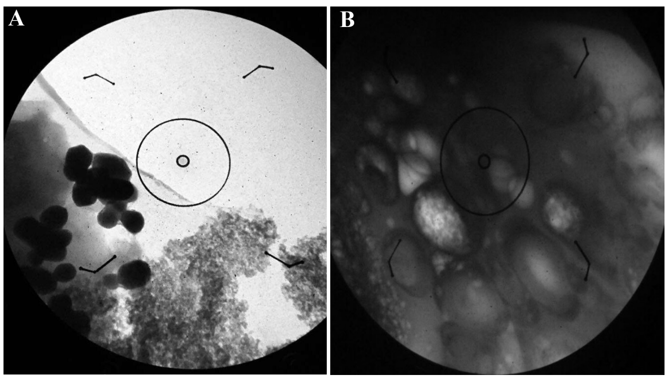

Figure 7: TEM for ZnO NPs A) *B. vulgaris* B) *S. lugdunensis*

Table 1: *In vivo* effects of Zn NOPs nanoparticles for *B. Vulgaris* and *S. lugdunensis* on the tested microorganisms' growth inhibition zone (mm).

Microbial test	*Inhibition zone (mm) Zn NPs. by S. lugdunensis*	*Inhibition zone (mm) Zn NPs. By B. vulgaris*
E. coli	12	14
S. aureus	9	11
Klebsiella spp	10	12
P. aeruginosa	11	9
S. lugdunensis	8	7
C. kefyer	12	11

The Figure (7) of the ZnO NPs it can be seen that crystalline ZnO particles have sphered shapes according to Jalal *et al.,*[25] Spherical structures synthesized NPs were well dispersed. The SEM successfully synthesized spherical NPs with an average particle size of 30 nm. Produced nanoparticles the shape and size by using the TEM microscopy silver nanoparticles spherical forms and the size ranges of 30 to 50 nm.[26]

ZnO NPs Assay

The strong antimicrobial effects in herbal products are very important because of the more resistance of many pathogens to antibiotics. More lethal and dangerous side effects when using antibiotics in treatment are hypersensitivity reactions (Anaphylactic shock).[27]

Evaluated at obtained optimum conditions showed Table 1. The antibacterial activity of plant ZnO NPs more effect than bacteria. Synthesized ZnO NPs more effects during incubation on gram-positive and negative bacteria growth.

Synthesized ZnO NPs using plant extract by Madan *et al.*[28] also indicated that toward *S. aureus* as compared to chemical nanostructure by study activity against *E. coli* had high antibacterial activity. ZnO NPs was assessed the antioxidant property of the formed at optimum synthesis conditions.

Antibacterial effects nanoparticles on the cell wall of microorganism's three-dimensional structure.[29] The aqueous extract antibacterial effect did not have a significance of *B. vulgaris*; however, biosynthetic nanoparticles were able to inhibit bacteria wide spectrum.[30] Method using leaf extract and plant their antibacterial activity, eco friendly is Green synthesis, and AgNPs were observed the biosynthesized AgNPs against *E. coli, P. aeruginosa, Mariniluteicoccus flavus, Bacillus subtilis,* and *K. pneumoniae*. The variation in the antimicrobial activates of ZnO NPs as manifested by MIC of nanoparticles may be result from the differences in the tested genes and species bacteria[31] ZnO NPs had antibacterial action against Gve+ *S. lugdunensis*).[32]

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