

# Investigation and Comparison of Antimicrobial Effects of Synthetic Zinc Nanoparticles in *Nannorrhops ritchiana* L against on Gram-negative and Gram-positive Bacteria

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## ABSTRACT

Due to their unique physicochemical properties, the production and application of nanomaterials have significantly increased across biomedical, environmental, and pharmaceutical fields. Ethanoic extract of *Nannorrhops ritchiana* was prepared by the maceration method. For the synthesis of zinc nanoparticles, the aqueous extract of *Nannorrhops ritchiana* is combined with 0.1 M zinc sulfate solution to form zinc nanoparticles. The average diameter of the nanoparticles is measured by X-ray diffraction and scanning electron microscopy (SEM). The antimicrobial activity of the synthesized zinc nanoparticles was assessed against standard bacterial strains using the microdilution method, and the diameter of the inhibitory zone and the minimum inhibitory concentration against the bacterial strains were determined. The results revealed that at a concentration of nanoparticles *Nannorrhops ritchiana* 1024 µg/mL, the largest inhibition zones were observed against *Proteus mirabilis* (25 mm) and *Streptococcus mutans* (15 mm). At 256 µg/mL, no inhibition zone was detected against *Streptococcus pyogenes*, while *Proteus mirabilis* showed a 17 mm zone of inhibition, and ethanol extract showed that the largest diameter of the inhibitory zone was at a concentration of 1024 µg/mL against *Proteus mirabilis* bacteria (10 mm), while the lowest inhibition was against *Bacillus cereus* and *Streptococcus pyogenes* bacteria. These findings suggest that zinc nanoparticles synthesized from *Nannorrhops ritchiana* exhibit strong antibacterial activity against human pathogens, even under particulate matter-contaminated environmental conditions.

**Keywords:** Zinc nanoparticles, *Nannorrhops ritchiana*, Gram positive bacteria, Gram negative, Ethanol extract

## INTRODUCTION

*Nannorrhops ritchiana*, locally known as DAAZ, is a plant of the Arecaceae family. This plant has a very high economic and cultural value in indigenous communities, and all parts of this plant are used for traditional treatments. This plant has a very high resistance to bad weather conditions [1]. *Nannorrhops ritchiana* is used for roofing and supporting. The leaves of this plant are used to make brooms, slippers, and bracelets [2]. This plant is distributed in tropical and subtropical regions. It has berry-like fruits that contain oil and are used in industry and medicine [3]. This plant has a very high adaptability to dry environments [4]. In recent decades, researchers have used extracts of medicinal plants to synthesize metal nanoparticles, and zinc oxide has been the most widely used metal [4]. Zinc oxide nanoparticles are used in the cosmetic, agricultural, and food packaging industries due to their antimicrobial properties and chemical stability [5]. Zinc oxide nanoparticles have the ability to produce active oxygen, which disrupts fungal cell structures, food safety, and shelf life [6]. Zinc nanoparticles are used for the biological removal of seaweed [7]. The adoption of biomimetic strategies in nanoparticle synthesis fosters enhanced catalytic efficiency while circumventing the need for conventional synthetic reagents that are often costly and hazardous [8]. Plant-derived phytochemicals such as flavonoids, phenolics, and alkaloids act as natural reducing and stabilizing agents in nanoparticle synthesis, offering a sustainable and eco-friendly alternative to conventional chemical methods [9-11].

## MATERIALS AND METHODS

Bacterial strains were procured from the reference microbiology laboratory of the Veterinary Department, University of Zabol (Table1).

**Table 1** Gram-positive and Gram-negative bacterial strains used

Strain bacteria	Gram+	Gram-
<i>Streptococcus pyogenes</i>	+	
<i>Staphylococcus aureus</i>	+	
<i>Enterococcus faecalis</i>	+	
<i>Hafnia Alei</i>		-
<i>Proteus Mirabilis</i>		-
<i>Streptococcus mutans</i>	+	
<i>Streptococcus pneumoniae</i>	+	

### Preparation of Ethanolic Extract

The maceration method was used to prepare the ethanolic extract. After crushing the sample, 50 grams of each sample were soaked in 96% ethanol for 48 hours, and after this period of time, the sample was filtered with filter paper. The ethanolic extract was concentrated using a rotary evaporator and stored in the refrigerator until use.

### Synthesis of Zinc Nanoparticles using Medicinal Plants

In this study, we conducted the biosynthesis of oxide nanoparticles using a Fruit extract of *Nannorrhops ritchiana*, deposited in the Herbarium of the University of Zabol, under herbarium code: 1501 ... [14].

The determination of bacterial susceptibility to zinc (Zn) and the ethanol extract of *Nannorrhops ritchiana* was carried out using the well-dilution method. The diameter of the inhibition zone at concentrations of 1024-512-256 µg/ml was determined by the well method on the bacteria studied [15].

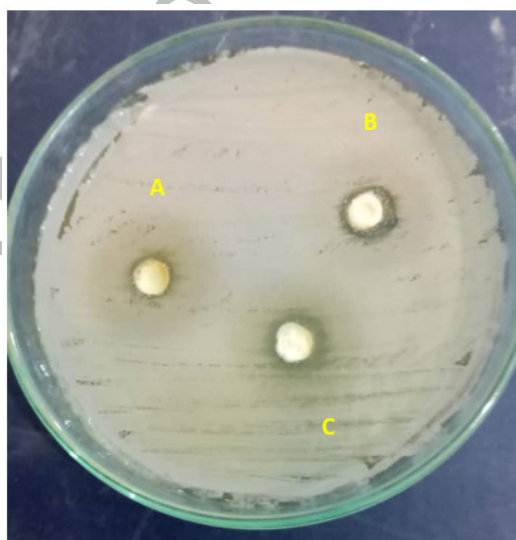
### RESULTS

the maximum inhibition zone diameter was recorded at a concentration of nanoparticles 1024 µg/mL, showing 25 mm against *Proteus mirabilis* and 15 mm against *Streptococcus mutans* (Table 2). At a lower concentration of 256 µg/mL, no inhibitory activity was observed against *Streptococcus pyogenes*, whereas *Proteus mirabilis* exhibited an inhibition zone of 17 mm at the same concentration (Table 2- Fig. 1).

**Table 2** Inhibitory Corona Diameter at Different Concentrations of nanoparticles on gram+ and gram - Bacteria (mm)

Strain bacteria	1024 µg/mL	512 µg/mL	256 µg/mL
<i>Streptococcus pyogenes</i>	2	1	0
<i>Staphylococcus aureus</i>	15	13	11
<i>Enterococcus faecalis</i>	13	10	5
<i>Hafnia Aloy</i>	8	5	2
<i>Proteus Mirabilis</i>	25	20	17
<i>Streptococcus mutans</i>	15	13	8
<i>Streptococcus pneumoniae</i>	8	6	5
<i>Pseudomonas aeruginosa</i>	5	3	1
<i>Bacillus cereus</i>	2	1	0

**Fig. 1** The diameter of the Zone of synthesized nanoparticles at different concentrations on standard bacteria



The results of the ethanol extract showed that the largest diameter of the inhibitory zone was at a concentration of 1024 micrograms on *Proteus mirabilis* bacteria (10 mm), while the lowest inhibition was against *Bacillus cereus* and *Streptococcus pyogenes* bacteria (Table 3).

**Table 3** Inhibitory Corona Diameter at Different Concentrations of Ethanol extract of *Nannorrhops ritchiana* against gram+ and gram - Bacteria (mm)

Strain bacteria	1024 µg/mL	512 µg/mL	256 µg/mL
<i>Streptococcus pyogenes</i>	1	0	0
<i>Staphylococcus aureus</i>	7	5	3
<i>Enterococcus faecalis</i>	6	3	1
<i>Hafnia Aloy</i>	4	3	2
<i>Proteus Mirabilis</i>	10	7	5
<i>Streptococcus mutans</i>	8	5	2

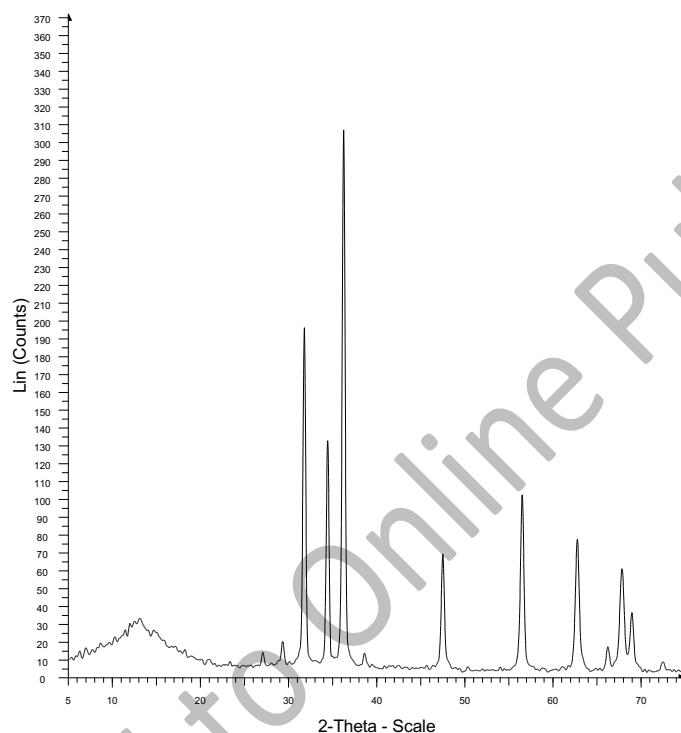
<i>Streptococcus pneumoniae</i>	5	3	2
<i>Pseudomonas aeruginosa</i>	2	1	0
<i>Bacillus cereus</i>	1	0	0

### FTIR Results Analysis of Zinc Nanoparticles Synthesized in *Nannorrhops ritchiana*

FTIR spectroscopy was utilized to identify the predominant functional groups present in the *Nannorrhops ritchiana* extract and to elucidate their role in the reduction of zinc ions ( $Zn^{2+}$ ) to elemental zinc ( $Zn^0$ ) and subsequent nanoparticle stabilization.

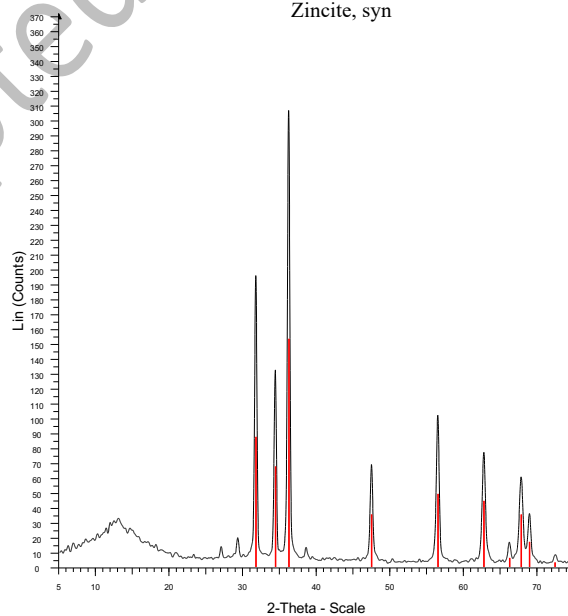
### X-ray Diffraction Pattern Analysis

(XRD) A profile of the zinc-based nanoparticles synthesized using *Nannorrhops ritchiana* extract is presented in Figure 2. The absence of extraneous peaks confirms the phase purity of the synthesized material. Moreover, the sharpness and well-defined nature of the diffraction peaks indicate a high degree of crystallinity and suggest the formation of a single-phase structure.



File: saeedi 2.raw - Type: 2Th/Th locked - Start: 5.000 ° - End: 75.000 ° - Step: 0.020 ° - Step time: 1. s - Temp.: 25 °C (Room) - Tim  
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Zincite, syn

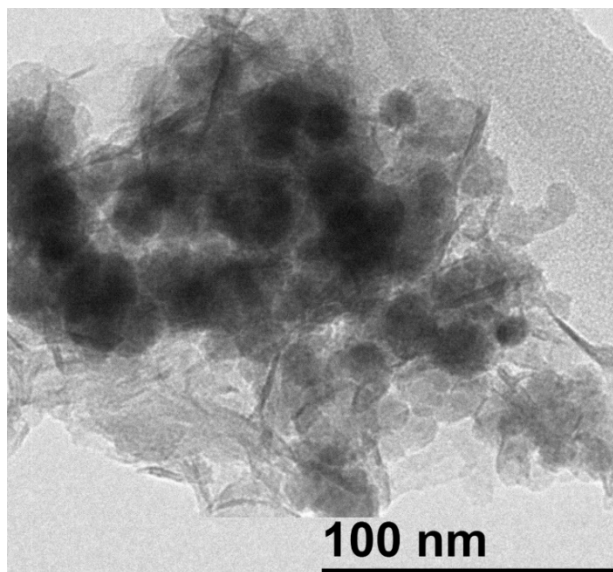


File: saeedi 2.raw - Type: 2Th/Th locked - Start: 5.000 ° - End: 75.000 ° - Step: 0.020 ° - Step time: 1. s - Temp.: 25 °C (Room) - Tim  
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36-1451 (\*) - Zincite, syn - ZnO - Y: 50.00 % - d x by: 1. - WL: 1.5406 - Hexagonal - a 3.24982 - b 3.24982 - c 5.20561 - alpha 90.00

Fig. 2 XRE for nano partical

### SEM Analysis

The SEM micrographs revealed that the nanoparticles were uniformly spherical in shape, with an average diameter of less than 20 nm, indicating a consistent nanoscale architecture and effective size control during biosynthesis (Fig. 3).



**Fig. 3** Electron microscopic image of green zinc nanoparticles synthesized in *Nannorrhops ritchiana* L extract

## DISCUSSION

The results of this study showed that synthetic nanoparticles have much higher antimicrobial properties than ethanol extract and can be used to treat bacterial diseases. In another study conducted on this plant, the results showed that at initial concentrations, ethanol extracts showed inhibitory effects against various strains of pathogenic bacteria and fungi [12]. In a study that investigated the antifungal and cytotoxic activities of *Nannorrhops ritchiana* root extracts, the results of the study showed antifungal properties on *Aspergillus flavus*, *Trichophyton longifusus*, *Trichophyton mentagrophytes*, and *Microsporium canis* [13].

The study of Javadian, green Synthesis of Silver Nanoparticles and Antibacterial Properties of Extracts of *Capparis spinosa* Leaves. The results show that the greatest effect of the ME of the medicinal plant *Capparis* was 0.2315 on the inactivity of *E. coli*, and the greatest effect of green AgNPs synthesized from the AE of the medicinal plant *Capparis* with, an ELISA of 0.3740, was on the inactivity of *S. mutanis*. The maximum diameter of the inhibitor zone (MDIZ) was 5.5 mm due to the inactivity of *H. alvi* bacteria [16]. In another study that investigated the antimicrobial properties of ethanolic extracts of several medicinal plants against gram-positive and gram-negative bacteria, the results showed that the largest diffusion disk diameter (25 mm) was observed in a medium containing rosemary extract against *Streptococcus pyogenes* [17]. The study of Saeidi, Investigating the Antimicrobial Properties of Synthetic Zinc Nanoparticles in the Medicinal Plant on *Escherichia Coli* causative of Bacteriuria in Pregnant Women. The results of the study showed that the size of the obtained nanoparticles was 21 nm, and the lowest inhibitory concentration was 1500 µg/ml [18].

## CONCLUSION

The results of this study demonstrated that the nanoparticles synthesized using *Nannorrhops ritchiana* medicinal plant exhibited strong antibacterial activity against human pathogenic bacteria, even under particulate matter (PM)-contaminated environmental conditions.

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## Footnotes

Authors contribution: All authors read and approved the final manuscript.

## Conflict of Interests

The authors declare no conflict of interest.

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