Synthesis and Characterization of Zinc Oxide Nanoparticles Using Rosa Damascena Petals and their Bactericidal Effects on S. Mutans And E. Faecalis

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**Abstract:**Nanoparticle application is highly suitable as biological scaffolds. Various studies have suggested that plants are suitable for large scale biosynthesis of nanoparticles. An Ornamental plant *Rosa damascena* has been used in the present study for the synthesis of ZnO nanoparticles. The present study focuses on the green synthesis and characterization of zinc oxide nanoparticles using Rosa damascena petals and their bactericidal effects on *S. mutans* and *E. faecalis*.Preparation of extract:- Rosa indica leaves were washed thoroughly and dried at room temperature. The leaves were then ground finely and used for experimental studies. Antimicrobial activity studies were carried out by agar disc diffusion method and MIC assay. Antibacterial activity was compared with the standard amoxicillin.It was observed that Zinc Oxide nanoparticles synthesised from Rosa damascena petal extract showed promising antibacterial activity.Petal extract is used as a capping agent for the stability of nanoparticles. Spherical ZnO nanoparticles showed excellent antibacterial activity against *S. mutans* and *E. faecalis* bacteria. It can be concluded that the green synthesis of ZnO nanoparticles is an eco-friendly, non-toxic and a very conventional method.Chemical methods used for the synthesis of nanoparticles are expensive and involve the use of toxic and hazardous chemicals that are responsible for various biological risks. In the case of biological methods, synthesis using plant extracts is the most adopted method, since it is eco-friendly. *Rosa damascena, well-known* as Rose, king of flowers has numerous pharmacological properties. Extracts from different parts of the rose plant have also been reported to show antibacterial and antifungal activity.

**Keywords:** Antibacterial activity, *Rosa damascena* petals, Zinc oxide nanoparticles, *S. mutants* and *E. faecalis*

# Introduction

Industrialization and urbanization have led to the discharge of multiple volatile chemical substances into the atmosphere leading to environmental damage [(Hamdy et al., 2023)](https://paperpile.com/c/3YEuvW/CHlu). Utilising natural yields have gained importance in recent years leading to the development and advancement of a new interdisciplinary scientific field, nanoscience [(Aparna et al., 2021; Ganapathy 2023)](https://paperpile.com/c/3YEuvW/CHlu+8VpX+J9oe+3JU8).Nanotechnology is a proven state-of-the-art technology with numerous branches embedded in multiple industrial fields and food processing industries [(Chokkattu et al., 2022; Nahhas, 2019)](https://paperpile.com/c/3YEuvW/IFwt+9yF1). In the advent of nanotechnology, many nanoscale devices have been developed using numerous (physical, chemical, and green) approaches. Yet, green nanoparticle synthesis is preferred since it can be easily prepared and engineered [(Awwad, 2013; Verma & Muthuswamy Pandian, 2021)](https://paperpile.com/c/3YEuvW/oQTx+VeyX). ZnO-NPs can be used in various sectors, such as energy conservation, electronics, healthcare and chemical sensing. The NPs are nontoxic, biocompatible and display excellent biomedical applications in targeted drug delivery, wound healing, and bioimaging [(Pandiyan et al., 2022; Poornima et al., 2021; Sharma, 2011)](https://paperpile.com/c/3YEuvW/h9FA+xM6H+qGRn).

*Rosa damascena* plants are of considerable interest and a well-known compound because of their antioxidant, antidiabetic, anti-inflammatory and antimicrobial activities [(Seyed Hajizadeh et al., 2023)](https://paperpile.com/c/3YEuvW/y1BH). Considering the side effects of antibiotics and the increased resistance of microorganisms, the use of medicinal plants is more popular against bacterial infections [(Önder, 2023)](https://paperpile.com/c/3YEuvW/pTxS). This plant has antidepressant effects, sedative, anti-itch effects, reduces sympathetic and strengthens the parasympathetic system. Various products and isolated constituents from flowers, petals and hips (seed-pot) of this plant have been studied in a variety of *in vivo* and *in vitro* studies. However, there are not any reviews to evaluate the pharmacological effects of *R. damascena* in the present time [(Marya et al., 2022; Önder, 2023; Ramamurthy et al., 2022; Rostami et al., 2023)](https://paperpile.com/c/3YEuvW/pTxS+zYJ2+L7HA+TKUj). Therefore, The present study focuses on the Green synthesis and characterization of zinc oxide nanoparticles using *Rosa damascena* petals and their bactericidal effects on *S. mutans* and *E. faecalis* [(Bashir et al., 2023)](https://paperpile.com/c/3YEuvW/cyD3).

# Materials and Methods

## Collection of Rosa damascena

The petals were collected, thoroughly washed under running water, dried at room temperature, and then cut into small pieces to remove adhering dust particles.

## Petal extract preparation

In a 500-mL Erlenmeyer flask, 10 g of chopped petals was combined with 100 mL of double distilled water to produce an aqueous extract. The mixture was allowed to stand at 60 °C for 20 min before being filtered through a Whatman no. 1 filter paper. Extra petal solution was stored at − 20 °C.

## Biosynthesis of Zinc Nanoparticles

Ten millilitres was mixed with 90 mL of a 1.0mM in distilled water solution. Within 24h, a colour change was observed. This indicated the formation of colloidal ZnO NPs. After 15 min of centrifugation at 10,000 rpm, ZnO NPs were obtained. Before the pellet was characterised, it was spun in a centrifuge three times, freeze- dried, ground into a powder and then put in deionized water.

## Green Synthesis of ZnO NPs

The supplier of the zinc nitrate hexahydrate (Zn (NO3)2. 6H2O) was Sigma-Aldrich Chemicals in India. Fresh petals were washed three times in the presence of distilled water to remove dust, chopped, and added to water (1:10) at 60oC while being continuously stirred for 30 minutes. After filtering, the mixture was cooled and kept at 40oC for additional use. 24h were spent in mixing the petal extract with 0.2M zinc nitrate (1: 9). The colour change of the liquid indicated the formation of ZnO NPs. The phytochemicals found in biomaterials can function as reducing agents, transforming the metal precursors into metal nanoparticles (NPs).

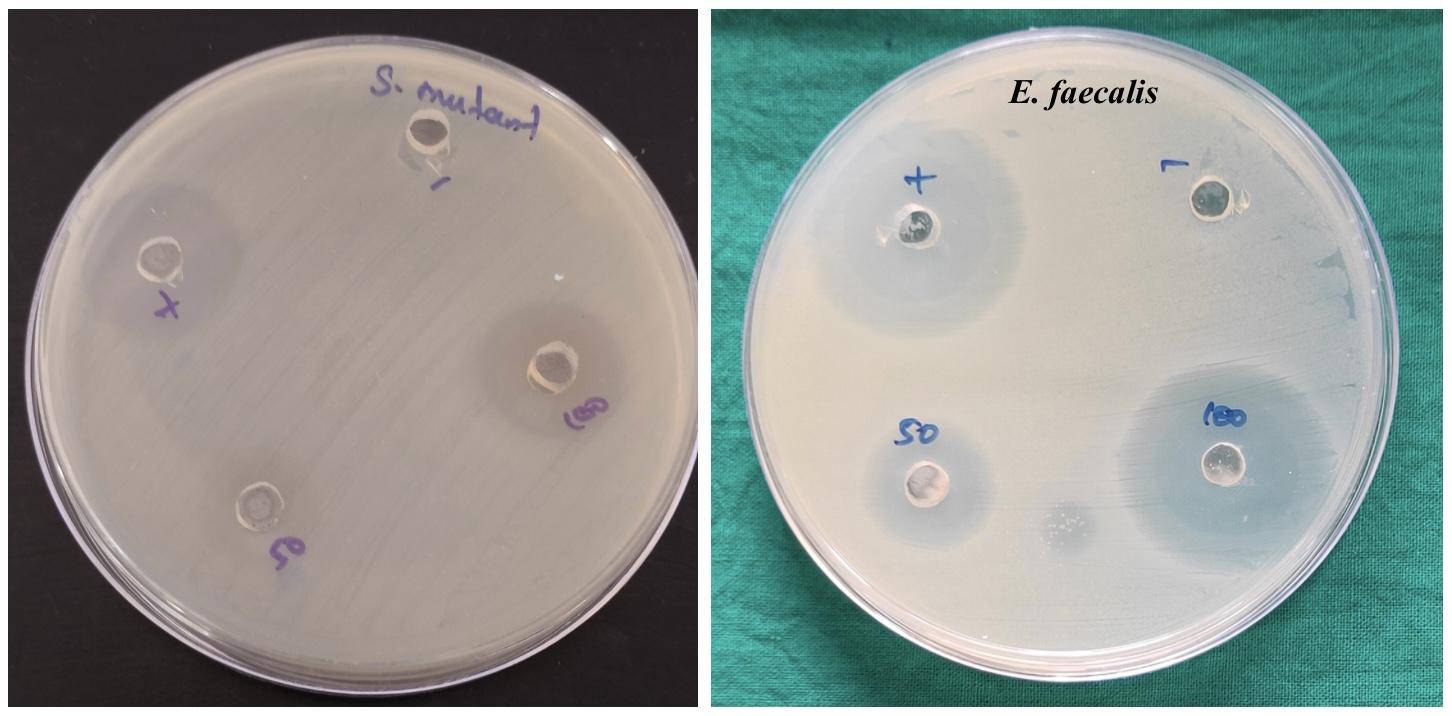
## Antibacterial activity

The S. mutants and E. faecalis mediated Zinc nanoparticles were tested for antibacterial activity. Clinical pathogenic strains of S. mutans and C. albicans were acquired. The pathogenic cultures were subcultured and maintained. In the antibacterial assays, ZnNPs (50 and 100 μg/mL) were poured into the wells of Mueller–Hinton agar (MHA) plates, respectively, after which they were incubated for 24 h at 37 °C and 25 °C, respectively. Chloramphenicol was used as a positive control. The growth inhibition zones were measured by the zone inhibition scale (Hi- Media, India).

## Statistical analysis

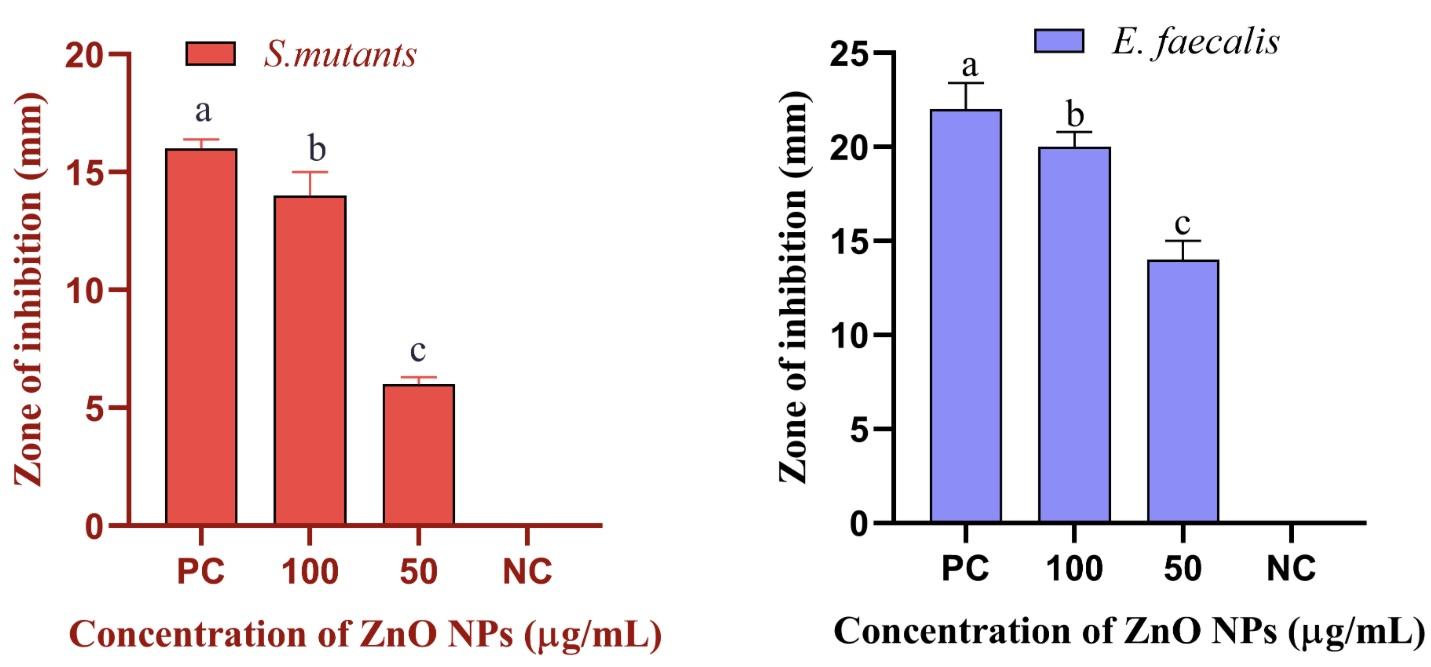
The data were subjected to statistical analysis by means of two-way analysis of variance (ANOVA) along with Tukey’s multiple HSD test. Significance values were maintained at p<0.05.

# Result



**Fig 1:-**Antibacterial activity of ZnO NPs against S. mutans and E. faecalis

The zone of inhibition of Rosa damascena petal extract synthesized ZnO nanoparticles against *S. mutans* and *E. faecalis* carried out by well diffusion method shows an increased rate of inhibition with an increased rate of extract concentration from 50 to 100µg/ml.



**Fig 2:**Zone of inhibition of ZnO NPs against S. mutans and E. faecalis

It was observed that ZnO NPs synthesis from Rosa Damascena petal extract showed promising antibacterial activity.

# Discussion

Several phytoconstituents were identified in the Rosa damascena plant based on their ability to bioaccumulate metal ions and act as bio reductants and stabilisers [(Hamdy et al., 2023; Sreevarun et al., 2023)](https://paperpile.com/c/3YEuvW/CHlu+GOfP). The reduction of Zn nitrate into ZnO NPs, through colour change, may be attributed to excitation of surface plasmon vibrations which results in Surface Plasmon Resonance(Rafi et al., 2024).The Rosa damascena petals extract (RD-ZnO NPs) was applied in order to generate zinc oxide nanoparticles using the phyto nanotechnology approach [(Awwad, 2013; Solanki et al., 2023)](https://paperpile.com/c/3YEuvW/oQTx+qFPM). The mechanochemical process is a cheap and simple method of obtaining nanoparticles on a large scale. It involves high-energy dry milling, which initiates a reaction through ball–powder impacts in a ball mill, at low temperature [(Chokkattu et al., 2023; PradeepKumar et al., 2016)](https://paperpile.com/c/3YEuvW/Q9XA+yKPS). Zinc oxide has also been precipitated from aqueous solutions of zinc chloride and zinc acetate (Tuluwengjiang et al., 2024). Controlled parameters in this process included the concentration of the reagents, the rate of addition of substrates, and the reaction temperature [(Sharma, 2011)](https://paperpile.com/c/3YEuvW/h9FA). Zinc oxide was produced with a monomodal particle size distribution and high surface area [(Muthuswamy Pandian et al., 2022; Neelakantan et al., 2016)](https://paperpile.com/c/3YEuvW/F3To+oyEx).A study conducted by Mohadesse in the poultry industry, showed dried rose dreg (by-product) decreased the occurrence of pathogens without any effect on broiler performance and feed conversion ratio [(Neelakantan, Cheng, et al., 2015)](https://paperpile.com/c/3YEuvW/bHR5). The analgesic, anti-inflammatory effects of R. damascena ethanol, chloroform extracts have been shown in animal models in a study performed by Athal.[(Shoaei et al., 2022)](https://paperpile.com/c/3YEuvW/qceJ) Indeed, the component(s) that have analgesic effects in ethanol extract were not found in rose essential oil [(Oopath et al., 2023)](https://paperpile.com/c/3YEuvW/FgUN).The biofilm matrix and its associated structure can limit the diffusion and permeability of antimicrobial agents toward the core of biofilms, allowing the embedded bacteria to survive and develop resistance mechanisms against such agents [(Govindaraju et al., 2017)](https://paperpile.com/c/3YEuvW/slH4). In this study, the antibacterial effect of EO against the planktonic and biofilm growth of S. mutans and E. faecalis was prominent, indicating its potential use against thick and mature dental biofilms [(Anti-Inflammatory Potential of a Mouthwash Formulated Using Clove and Ginger Mediated by Zinc Oxide Nanoparticles: An In Vitro Study, n.d.; Shoaei et al., 2022)](https://paperpile.com/c/3YEuvW/qceJ+2wwA).Previous studies suggested that ZnO NPs exhibit a characteristic broad absorption peak between 330–460 nm peaking at 374 nm without any other peaks confirms the synthesis of pure ZnO.NPs with the aid of active biomolecules in the plant extract in reduction and stabilisation of synthesised nanoparticles.[(Laghari et al., 2023; Neelakantan, Sharma, et al., 2015)](https://paperpile.com/c/3YEuvW/zScv+YgNX) This absorption peak could be attributed to the intrinsic band-gap absorption of ZnO due to the electron transitions from the valence band to the conduction band (O2p→Zn3d) as explained by Zak et al.[(Abdallah et al., 2023)](https://paperpile.com/c/3YEuvW/tlBb).

# Conclusion

Petal extract of *Rosa damascena* was used for the green synthesis of ZnO nanoparticles. Their phytochemical screening revealed increased active constituents with a high antibacterial content that have the ability to chelate metal ions and aid in the bactericidal effects on S. mutants and E. faecalis.

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