**Journal of Research in Medical and Dental Science**
**2020, Volume 8, Issue 5, Page No: 33-36**

Available Online at: www.jrmds.in
eISSN No.2347-2367: pISSN No.2347-2545

# Antimicrobial Assay of Novel Zirconia and Silver Phyto Nanoparticles Biosynthesized using *Ocimum Sanctum* and *Syzygium Aromaticum* Extract-A Preliminary Study

**Sunayna Chowdhary[1*], A. Sumathi Felicita[1], Rajeshkumar Shanmugam[2]**

[1]*Department of Orthodontics and Dentofacial orthopedics, Saveetha Dental College and Hospital, Saveetha University, India*

[2]*Department of Pharmacology, Saveetha Dental College and Hospital, Saveetha University, India*

## ABSTRACT

*Introduction: Dental caries is caused by acidogenic bacteria and pathogens present in oral cavity. In orthodontics, white spot lesions or incipient caries is an iatrogenic side effect that occurs due to the fixed appliances placed in oral cavity, promoting niche for acidogenic bacteria. New vistas of caries management involve introduction of nanotechnology in toothpastes, bonding agents, varnishes, remineralizing agents, mouth rinses etc. Through this preliminary study we demonstrate the antimicrobial activity of novel silver and zirconia nanoparticle synthesized using Ocimum sanctum and Syzygium aromaticum extract against Streptococcus mutans, Lactobacillus and Candida albicans.*

*Materials and Methods: Using extracts of Ocimum sanctum and Syzygium aromaticum, silver and zirconia nanoparticles were synthesized. Formation of Silver and Zirconia nanoparticles was confirmed by surface plasmon spectra using UV- Vis spectrophotometer. The morphology of the crystalline phase of nanoparticles was determined from transmission electron microscopy. The average sizes of the Silver and zirconia nanoparticles was in the range of 20-25nm and 85nm, respectively. The antimicrobial activity was assessed against Streptococcus mutans, Lactobacillus and Candida albicans.*

*Results: Silver and zirconia nanoparticles exhibited good antimicrobial activity against the pathogens. The antimicrobial activity of Silver nanoparticle was more pronounced than zirconia nanoparticle.*

*Conclusion: silver and zirconia nanoparticles synthesized using Ocimum sanctum and Syzygium demonstrated good antimicrobial activity. The plant-based nanoparticle synthesis is a fast, novel and eco-friendly method and the nanoparticles generated by this method shows excellent antimicrobial activity against streptococcus mutans, lactobacillus and candida albicans.*

**Key words:** Nano particles, *Lactobacillus*, *Candida albicans*, Antimicrobial activity, *Streptococcus mutans*

**HOW TO CITE THIS ARTICLE:** Sunayna Chowdhary, A Sumathi Felicita, Rajeshkumar ShanmugamAntimicrobial Assay of Novel Zirconia and Silver Phyto Nanoparticles Biosynthesized using *Ocimum Sanctum* and *Syzygium Aromaticum* Extract-A Preliminary Study, J Res Med Dent Sci, 2020, 8(5): 33-36

**Corresponding author:** Sunayna Chowdhary
**e-mail**✉: dr.sunaynachowdhary@gmail.com
**Received: 01/07/2020**
**Accepted: 10/08/2020**

## INTRODUCTION

Nanotechnology is the newest and one of the most promising areas of research in modern medical science. Nanoparticles exhibit new and improved properties based on size, distribution, and morphology than larger particles of the bulk materials from which the nanoparticles are made [1]. The surface to volume ratio of nanoparticles is inversely proportional to their size. The biological effectiveness of nanoparticles can increase proportionately with an increase in the specific surface area due to the increase in their surface energy and catalytic reactivity [2]. Although there are many routes available for the synthesis of nanoparticles, there is an increasing need to develop high-yield, low cost, non-toxic and environmentally friendly procedures. Therefore, the biological approach for the synthesis of nanoparticles becomes essential [3]. Biological molecules have qualities by which they can undergo highly controlled and hierarchical assembly, which makes them suitable for the development of a reliable and eco-friendly process for metal nanoparticle synthesis [4].

Silver has long been recognized as an effective antimicrobial agent that exhibits low toxicity in humans and has diverse in vitro and in vivo applications [5]. Currently, silver-based topical dressings are widely used to treat infections in open wounds and chronic ulcers. These dressings also protect the host material from oxidation and discoloration [4].

Zirconium oxide nanoparticle is a metal oxide-based nanoparticle. Zirconium oxide nanoparticles ($ZrO_2$) are present in the forms of nanofluids and nanocrystals and nanodots having a white surface area. Zirconium oxide nanoparticles are often doped with yttrium oxide or magnesia. Zirconium is a Block D, Period 5 element and oxygen is a Block P, Period 2 element. Zirconium oxide is also known as, zirconium, zirconia anhydride, zirconia and zircol.

Drugs derived from natural sources, i.e. herbal source, play an important function in the treatment and prevention of disease, which affects human beings. In most of the developing countries like India, Brazil, traditional medicine is believed to be one of the primary healthcare systems. About 60%-65% of new drugs which was developed between years of 1980 and 2002 made use of natural products, i.e. herbal products as one of the raw material and they are very successful even now, specifically in the zones of infectious disease caused by bacteria, fungi, etc. and also effective in treating cancer [6]. Current trends, however, show that the discovery rate of active novel chemical entities is dropping due to adverse effects caused by chemicals [7]. Natural products prepared from plants may give a new source of antimicrobial/anti-fungal agents with successful novel mechanisms of action [8]. Through this study, we aim to synthesize silver and zirconia nanoparticles using Ocimum sanctum and Syzygium aromaticum extract and assess the antimicrobial properties of the particles generated.

## MATERIALS AND METHODS

### Collection of plant materials

The dried leaf of *Ocimum sanctum* and flower of *Syzygium aromaticum* was collected from local market in Chennai, India. These were rinsed with deionized water thrice to remove the fine dust materials and then was air dried to completely remove the moisture.

### Preparation of extracts

The dried leaves and flowers were pulverized well with mortar and pestle to make a powder. Five grams of powder sample was mixed into 100 ml of deionized water and the mixture was boiled for 10 min. After cooling, the extract was filtered with Whatman No. 1 filter paper. The filtrate was stored at 4◦C for further use.

### Synthesis of silver and zirconia nanoparticles

The 100 ml of aqueous filtrate extract of *Ocimum sanctum* and *Syzygium aromaticum* was taken into two 250 ml of Erlenmeyer flask. Then the extract was mixed into Silver nitrate ($AgNO_3$) in one flask and zirconium chloride in another to make the final volume concentration of 1 mM solution each. The reaction mixture was kept into dark room condition until the colour changed. The reaction solution colour changes were observed for the characterization of silver nanoparticles.

### Characterization of nanoparticles

The synthesized silver nanoparticles were characterized by UV – vis spectroscopy periodically for a week to observe rapid reduction silver and zirconia nanoparticles by the action of plant extracts. The biologically reduced brown colour solution mixture was scanned by the spectroscopy instrument operated at a resolution of 1 nm. In this analysis the extract without adding the silver nitrate and zirconium chloride was used as a control. The FTIR is performed to the extract which was exposed before and after addition to the silver nitrate solution. The samples were mixed with KBr to make a pellet and it was placed into the sample holder. The spectrum was recorded at a resolution of 4 $cm^{-1}$. X-ray diffraction pattern was obtained from lyophilized silver nanoparticles by powder diffraction method, where it gives grain size and shape of the particles by h, k, and l index value. The morphological analysis of the particle was done with transmission electron microscopy (TEM). The drop of aqueous silver and zirconia nanoparticle sample was loaded on carbon-coated copper grid and it could dry for an hour. The TEM micrograph images were recorded on JEOL instrument 1200 EX instrument on carbon coated copper grids with an accelerating voltage of 80 kV. The clear microscopic views were observed and documented in different range of magnification.

### Screening of antibacterial activity against micro-organisms

The antibacterial activity was assayed by the Agar well diffusion method. All the glassware, media, reagents were sterilised prior to the procedure in an autoclave at 121◦C for 20 minutes. The synthesized silver and zirconia nanoparticles antibacterial activity was observed against several microorganisms such as *Streptococcus mutans*, *Lactobacillus acidophilus* and *Candida albicans*. The bacterial suspension was swabbed on the Muller Hinton Agar (MHA) plates using sterile cotton swab. Well of 6 mm diameter was made on the MHA using gel puncture. Using micropipette different concentrations like 50 µl, 100 µl and 150 µl of silver and zirconia nanoparticles solution was poured, with *Ocimum sanctum* and *Syzygium aromaticum* extract used as a control. The plates were incubated at 35◦C for 24 h. After the incubation period, the zone of inhibition was observed and tabulated.

## RESULTS

The anti-microbial activity was measured as diameter of zone of inhibition (ZOI) around the well with the nanoparticles against the test strains. The ZOI was expressed in millimeters (mm) for the test pathogens. The various concentration of nanoparticle solution used was 50 µl, 100 µl and 150 µl. Table 1 shows antimicrobial activity of both the nanoparticles against streptococcus mutans. Table 2 shows the antimicrobial activity against *Lactobacillus* and Table 3 shows activity against *Candida albicans*. Table 1 demonstrates that silver nanoparticle has greater zone of inhibition with increasing concentration as compared to zirconia against *streptococcus mutans*. Table 2 shows the zone of inhibition is more for silver nanoparticles and it increases with concentration when tested against lactobacillus species. Table 3 shows that the zone of inhibition increased only for 150 µl concentration for silver nanoparticle. Zirconia nanoparticle shows less zone of inhibition compared to silver for all three pathogens. Zone of inhibition was clearer in the culture plates for silver than in culture plates with Zirconia for all the 3 microorganisms.

**Table 1: Zone of Inhibition for *Streptococcus mutans*.**

| Concentration µl | Silver-ZOI mm | Zirconia- ZOI mm |
|---|---|---|
| 50 | 11.22 ± 0.4 | 8. 2 ± 0.02 |
| 100 | 15.13 ± 0.46 | 8.09 ± 0.3 |
| 150 | 20.33 ± 0.63 | 8.9 ± 0.9 |
| Control | 10 ± 0.42 | 10 ± 0.42 |

**Table 2: Zone of Inhibition for *Lactobacillus acidophilus*.**

| Concentration µl | Silver- ZOI mm | Zirconia- ZOI mm |
|---|---|---|
| 50 | 12 ± 0.27 | 7.33 ± 0.32 |
| 100 | 14.33 ± 0.42 | 8.7 ± 0.32 |
| 150 | 17.66 ± 0.95 | 8.6 ± 0.35 |
| Control | 13 ± 0.67 | 13 ± 0.67 |

**Table 3: Zone of Inhibition for *Candida albicans*.**

| Concentration µl | Silver-ZOI mm | Zirconia-ZOI mm |
|---|---|---|
| 50 | 12.69 ± 0.77 | 8.66 ± 0.27 |
| 100 | 12.8 ± 0.8 | 8.37 ± 0.42 |
| 150 | 15.56 ± 0.67 | 8.66 ± 0.78 |
| Control | 11 ± 0.35 | 11 ± 0.35 |

## DISCUSSION

The addition of *Ocimum sanctum* and *Syzygium aromaticum* extract to silver nitrate solution resulted in colour change of the solution from transparent to dark yellow due to the production of silver nanoparticles. These colour changes arise because of the excitation of surface plasmon vibrations with the silver nanoparticles [9-11]. Antimicrobial assay of biosynthesized silver nanoparticles against *S. mutans, L. acidophilus* and *candida albicans* microorganisms at different concentrations showed that they revealed a strong dose-dependent antimicrobial activity against all the test microorganisms. It was seen that, as the concentration of biosynthesized nanoparticles were increased, microbial growth decreases in these cases. Biosynthesized silver nanoparticles were observed to exhibit more antimicrobial activity on *S mutans*. The results indicated that silver nanoparticles synthesized from *Ocimum sanctum* and *Syzygium aromaticum* have stronger activity than standard antibiotic ciprofloxacin. Antimicrobial property of silver nanoparticles synthesized by chemical method has also been studied by Huang et al. [12]. They reported that silver nanoparticles in size range 10–25 nm are effective antimicrobial agents. Moreover, Shrivastava et al. studied the interaction stage between Ag nanoparticles and bacteria (*E. coli*) [13]. They found that at initial stage of the interaction Ag nanoparticles adhere to bacterial cell wall subsequently penetrate the bacteria and kill bacterial cell by destroying cell membrane.

The Zirconia nanoparticle however showed a lesser antimicrobial action. This is contradictory to experiments by Gowri et al, where the zirconia nanoparticles synthesized using Nyctanthes showed excellent antimicrobial activity against S. aureus and *E. coli* [14].

## CONCLUSION

*Ocimum sanctum* and *Syzygium aromaticum* can be used as reducing agents for the green synthesis of Silver and zirconia nanoparticles. The Zone of inhibition from the microorganism culture studies indicate that the silver nanoparticles hence generated has good antimicrobial properties. In comparison with Zirconia nanoparticles, silver showed much better results. Limitation of this study is that triplicate testing was not done.

This preliminary antimicrobial analysis helps to demonstrate that biosynthesized silver nanoparticle can be further studied upon for its antimicrobial effects on various different microorganisms and can be used as an antimicrobial ingredient in oral rinses and pastes in future.

**REFERENCES**

1. Mubarak Ali D, Thajuddin N, Jeganathan K, et al. Plant extract mediated synthesis of silver and gold nanoparticles and its antibacterial activity against clinically isolated pathogens. Colloids Surf B Biointerfaces 2011; 85:360-365.
2. Ahmad N, Sharma S, Alam KMD, et al. Rapid synthesis of silver nanoparticles using dried medicinal plant of basil. Colloids Surf B Biointerfaces 2010; 81:81-86.
3. Klaus T, Joerger R, Olsson E, et al. Silver-based crystalline nanoparticles, microbially fabricated. Proc Natl Acad Sci USA 1999; 96:13611-13614.
4. Sastry M, Ahmad A, Khan MI, et al. Biosynthesis of metal nanoparticles using fungi and actinomycete. Biosynthesis of metal nanoparticles using fungi and actinomycete. Curr Sci 2003; 85:162-170.
5. Ahmad A, Mukherjee P, Senapati S, et al. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. Colloids Surf B Biointerfaces 2003; 28:313-318.
6. Mubarak Ali D, Sasikala M, Gunasekaran M, et al. Biosynthesis and characterization of silver nanoparticles using marine *cyanobacterium, oscillatoria willei* NTDM01 Digest J Nanomater Biostruct 2011; 6:385-390.
7. Vigneshwaran N, Nachane RP, Balasubramanya RH, et al. A novel one-pot 'green' synthesis of stable silver nanoparticles using soluble starch. Carbohydr Res 2006; 341:2012-2018.
8. Prathna TC, Chandrasekaran N, Raichur AM, et al. Biomimetic synthesis of silver nanoparticles by Citrus limon (lemon) aqueous extract and theoretical prediction of particle size. Colloids Surf B Biointerfaces 2011; 82:152-159.
9. Mulvaney P. Surface plasmon spectroscopy of nanosized metal particles. Langmuir 1996; 12:788–800.
10. Eftink MR, Ghiron CA. Fluorescence quenching studies with proteins. Analytical Biochem 1981; 114:199-227.
11. Fayaz AM, Balaji K, Girilal M, et al. Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: A study against gram-positive and gram-negative bacteria. Nanomed Nanotechnol Biology Med 2010; 6:103-109.
12. Huang NM, Lim HN, Radiman S, et al. Sucrose ester micellar-mediated synthesis of Ag nanoparticles and the antibacterial properties. Colloids Surfaces A: Physicochemical Engineering Aspects 2010; 353:69-76.
13. Shrivastava S, Bera T, Roy A, et al. Characterization of enhanced antibacterial effects of novel silver nanoparticles. Nanotechnol 2007; 18:225103
14. Gowri S, Gandhi RR, Senthil S, et al. Effect of calcination temperature on nyctanthes plant mediated zirconia nanoparticles, optical and antibacterial activity for optimized zirconia. J Bionanosci 2015; 9:181-189.