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Synthesis of Triphala Incorporated Zinc Oxide Nanoparticles and Assessment of its Antimicrobial Activity Against Oral Pathogens : An In-Vitro Study

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ABSTRACT

Triphala, a traditional Ayurveda herbal formulation consisting of dried fruits of three medicinal plants, is known to have effective antimicrobial properties. Inorganic elements like zinc oxide have also been known to have antimicrobial properties against strains of gram positive and gram negative bacteria, both. Green synthesis of nanoparticles is being encouraged, because of its positive effects on the environment and its cost-effectiveness. Hence, the study was conducted to synthesize Triphala augmented Zinc oxide nanoparticles and assess its antimicrobial activity against oral pathogens. Aqueous extract of Triphala was formulated and incorporated to zinc oxide solution to formulate the nanoparticle. Agar well diffusion method was used to assess the antimicrobial activity against S. Mutans, C. albicans, S. aureus, E.faecalis. Triphala incorporated zinc oxide nanoparticles produced a zone of inhibition more than that of ampicillin/cycloheximide against S. aureus and at 50 µl was almost as effective as antibiotic against the gram positive bacteria. Triphala incorporated ZnONPs as a cost effective, eco-friendly yet efficient method and they show great potential as oral antimicrobial agents.

KEY WORDS: GREEN SYNTHESIS NANOPARTICLES, HERBAL DENTISTRY, NANO-SCIENCE, TRIPHALA, STREPTOCOCCUS MUTANS.

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INTRODUCTION

Dental caries can be defined as a multi-factorial microbial disease characterized by de-mineralization of the inorganic and destruction of the organic substance of the tooth. Dental caries start with bacterial adherence to the tooth structures gradually leading to formation of plaque followed by de-mineralization of enamel due to fermentation of dietary carbohydrates (Mittal et al., 2011; Snehal and Srinivasan, 2015) . The initial caries lesion manifest as white spot lesion and it occurs mainly due to major mineral loss at the subsurface area beneath a relatively intact area(Welbury, Duggal and Hosey, 2018), caused by microorganisms like Streptococcus mutans, Lactobacillus acidophilus which encourage the accumulation and adherence of plaque biofilm by metabolizing sucrose into sticky glycan (Loesche, 2007). Studies have shown that dental caries is not a continuous process, but rather a cyclic phase of demineralization and remineralization (Carounanidy and Sathyanarayanan, 2009). Since 1980, fluorides have been known to control carious lesion facilitating the re-mineralization process in enamel, but high concentration of fluoride in dentifrices and systemic fluorides led to fluoride toxicity, which in turn gave rise to the non-fluoride based re-mineralizing agents like CPP-ACP, Bioactive glass, CPP, Xylitol, nanoparticles (Arifa, Ephraim and Rajamani, 2019).

Among these, nanoparticles have gained popularity in the field of scientific research, owing to their size (1-100 nm), it has shown tremendous potential even as an antibacterial agent because of their enhanced physicochemical properties, large surface area to mass and increased chemical reactivity (Saafan et al., 2018). Among the nanoparticles, metal oxide such as Zinc Oxide (ZnO) has gathered attention from researchers, as it is found to be stable under varying environments, also it has shown antimicrobial activity against both gram positive and gram negative microorganisms and antibacterial activity against spores. Also, they are non toxic, biocompatible in nature which makes it a desirable choice of material (Tiwari et al., 2018). Synthesis of ZnO nanoparticles can be achieved through physical processes, chemical processes like sol-gel, pyrolysis, electro-deposition, which are useful for mass synthesis but at the same time prove to be detrimental to the environment (Su and Chang, 2018).

That's when green synthesis of nanoparticles were introduced where secondary metabolites of plant products were used as reducing agents for synthesizing and stabilizing the nanoparticles. Triphala is a botanical preparation consisting of equal parts of three herbal fruits, known to have excellent antibacterial, antimicrobial, antiviral properties, also exhibits potent antioxidant properties due to its essential phytochemicals (Jagadish, Anand Kumar and Kaviyarasan, 2009). We have successfully completed numerous epidemiological studies for the betterment of our community (Prabakar, et al., 2018a, 2018b; Prabakar et al., 2018; Vishnu Prasad et al., 2018; Khatri et al., 2019; Manchery et al., 2019; Shenoy, Salam and Varghese, 2019). In this research we are studying/analyzing the Therefore, this study was conducted to synthesize Triphala incorporated zinc oxide nanoparticles and assess its antimicrobial activity against oral pathogens.

MATERIAL AND METHODS

Study design: In-vitro

Ethical approval: Prior to the start of the study, ethical approval was obtained from Scientific Review Board, Saveetha Dental College, SIMATS.

Study method

1. Synthesis of Triphala incorporated zinc nanoparticles: Tripahla was obtained from a dedicated ayurvedic pharmacy and inspected for purity and phytochemical composition prior to use. Aqueous extract of Triphala was prepared by boiling 10gm of Triphala powder in 100 ml of double distilled water in a water broth at 70 0C for ten minutes to obtain 1% of the Triphala extract and it was filter paper was used to filter the solution and the obtained filtrate was used for nanoparticle synthesis. 1 ml of the filtrate was mixed with the solution zinc oxide acid solution and ascorbic acid. The solution was then placed in an incubator at 250 rpm until there was evidence of colour change suggestive of nanoparticle synthesis, following which the solution was centrifuged at 10000 rpm for 30 minutes. The pellet obtained was washed with double distilled water, followed by absolute ethanol and dried in a hot air oven for 2 hours and stored in an air-tight container. Confirmation of the ZnONPs was performed using UV-Visible spectrophotometer at 1, 12, 18, 24, 48 and 72 hours.

2. Antimicrobial activity of Triphala incorporated zinc nanoparticles: Agar well diffusion method was used to determine the antibacterial activity of different concentrations of ZnONPs against oral pathogens such as *S. mutans, E. faecalis, C. albicans* and *S. aureus.* Secondary cultures of microbial suspension were dispersed evenly on the surface of Muller Hinton agar and rose Bengal agar plates using a sterile spreader. Different concentrations of nanoparticles (25, 50 & 100 µl) were incorporated through a sterile micropipette into the wells created on the agar plate using sterile cork borer. The plates were then incubated at 37°C for 24 h to 48 h. Commercial antibiotic ampicillin (50mg/ml) was used as positive control for *S. mutans, E. faecalis, S. aureus* but for *C. albicans* cycloheximide was used and the zone of inhibition (mm) was recorded for each plate and compared with control. All the tests were replicated in triplicate for analysis.

RESULTS AND DISCUSSION

Zinc oxide nanoparticles have proved to be one of the most important metal oxide nanoparticles, owing to their peculiar physical and chemical properties, (Smijs and Pavel, 2011). From being used in rubber industries (Kołodziejczak-Radzimska and Jesionowski, 2014),

skin products (Newman, Stotland and Ellis, 2009), food additives (Rasmussen et al., 2010), it has received much recognition in the field of biomedical research. In comparison to other metal oxide nanoparticles, zinc oxide NPs have relatively less toxicity and excellent biomedical applications, like drug delivery, antibacterial, anticancer, anti-inflammation (Mishra et al., 2017). Of many methods of preparation, green synthesis of nanoparticles by biological systems (Fakhari, Jamzad and Kabiri Fard, 2019) by plant extracts has become an emerging field in nanotechnology. In this study, Triphala, an ancient herb with equiproportional mixture of *Terminalia chebula*, *Terminalia belerica*, and *Emblica officinalis*, was incorporated into Zinc nanoparticles and its antimicrobial activity was assessed against oral pathogens. Previous research also shows efficacy of Triphala against potent microorganisms (Chainani et al., 2015), (Prabhakar et al., 2014) (Srinagesh, Krishnappa and Somanna, 2012). Similarly, studies involving the antimicrobial efficacy of zinc oxide nanoparticles have also been conducted (Siddiqi et al., 2018) (Souza et al., 2019).

The green synthesis of the zinc oxide nanoparticles, was confirmed after visual observation, where after the addition of the zinc oxide solution with Triphala plant extract, the colour changed to brown with slight viscosity in nature, indicating the formation of zinc oxide nanoparticles, similar to other studies (Rajeshkumar et al., 2018). In the current study, the mean zone of inhibition (ZOI) was found to increase as the concentration of NPs increased. Maximum ZOI for St.mutans was observed at 100µl concentration, 25mm, which was even more than that of the commercial antibiotic used (Figure I). Against St. Aureus, Triphala incorporated ZnONP showed a zone of inhibition maximum of 30 mm with the commercial antibiotic, however at 100µl and 25µl concentrations also it showed good potential with 26 mm and 24 mm of ZOI (Figure II). Similarly, for E.faecalis maximum ZOI was observed with the commercial antibiotic at 27 mm, followed by 24 mm at 100µl concentration and 20 mm for 50µl concentration (Figure III).

However, in the current study Triphala incorporated ZnONPs were not as potent as the commercial antibiotic against C.albicans, as the zone of inhibition was highest at 30 mm for AB, and 20 mm at 100µl concentration (Figure IV). Various other studies have reported efficacy of Triphala against gram positive bacteria, gram negative bacteria and yeast as well (Bajaj and Tandon, 2011) (Azizi-Lalabadi et al., 2019). Zinc oxide nanoparticles have also proven to be effective against a variety of gram positive and gram negative bacteria in other studies (Santhoshkumar, Venkat Kumar and Rajeshkumar, 2017) (Happy Agarwal et al., 2018). However, the current study is the first of its kind where antimicrobial efficacy of Triphala incorporated ZnONP is tested against oral pathogens, hence evidence is less. Therefore, further animal studies/in-vivo research should be conducted to validate the above findings.

Figure 1: Zone of inhibition at different concentrations against *St. mutans*

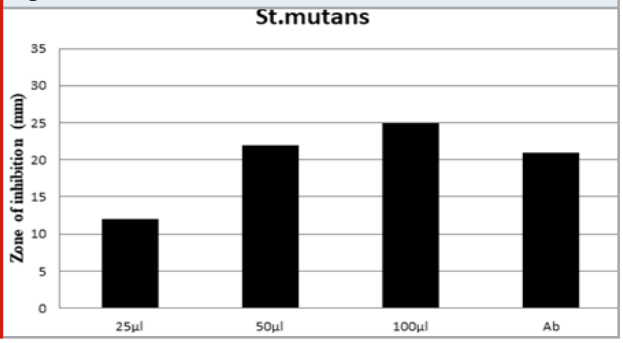


Figure 2: Zone of inhibition at different concentrations against St. aureus

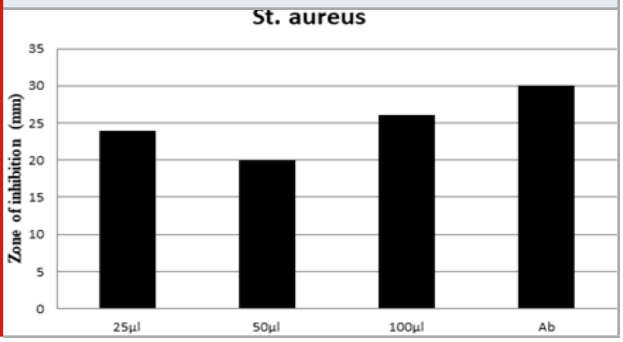


Figure 3: Zone of inhibition at different concentrations against E. faecalis

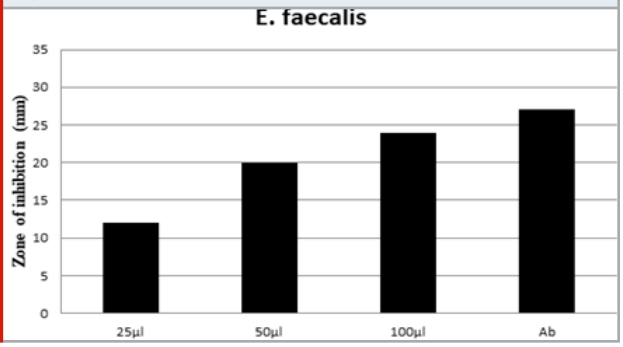
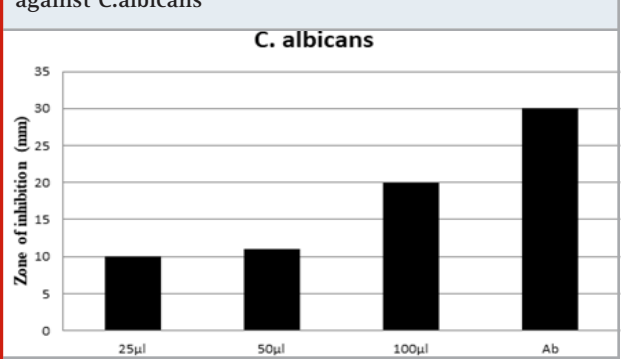


Figure 4: Zone of inhibition at different concentrations against C.albicans



CONCLUSION

The synthesized Triphala incorporated Zinc Oxide nanoparticles show excellent potential as an oral antimicrobial agent against strains of gram positive bacteria and are also bio-friendly and inexpensive in nature. However, animal studies and in-vivo research has to be conducted to establish the above findings.

Conflict of interest: Nil.

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