

Antimicrobial activity of silymarin mediated zinc oxide and hydroxy apatite nanoparticles against oral pathogens

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Abstract:

The nanoparticles such as hydroxyapatite, zinc oxide, titanium dioxide and zirconium nanoparticles have application in dentistry. Therefore, it is of interest to document the antimicrobial activity of silymarin mediated zinc oxide and hydroxy apatite nanoparticles against oral pathogens. Hence, we synthesized hydroxyapatite and zinc oxide nanoparticles with silymarin and characterized by UV-visible spectrophotometer. Data shows that silymarin mediated HAP and ZnO nanoparticles have antimicrobial activity against oral pathogens such as *Pseudomonas sp*, *Staphylococcus aureus*, *Streptococcus mutans*, *Enterococcus faecalis* and *Candida albicans*.

Keywords: Hydroxyapatite nanoparticles, green synthesis, silymarin, zinc oxide nanoparticles, oral pathogens.

Background:

Nanotechnology is employed to treat microbial infections [1]. Nanoparticles are incorporated in antibacterial coatings for implants in biomaterials to prevent bacterial infection and promote wound healing. It is also used in microbial diagnosis [2,3]. Nano materials due to its particle size, larger surface area, increased chemical reactivity, proven to be broad-spectrum antimicrobial agent for treating dental infections caused by bacterial accumulation in the oral cavity [4]. Nanoparticles are used in various fields of dentistry including endodontics, prosthodontics, periodontics and orthodontics. Dental plaque/biofilm accumulation is commonly seen in orthodontic appliances/treatments. This will enhance the chance of enamel demineralization, which is commonly called as white spot lesions caused due to organic acid produced by the microbial biofilms [5-6]. This can be managed by providing oral hygiene education. Moreover, mechanical therapy can prevent and remove the plaque biofilm. However, more effective methods are employed to treat the plaque formation. Natural products/herbal based compounds are proven to be effective treatment due to their antimicrobial, antioxidant and anti-inflammatory effects. Nonetheless, the exact mechanism of action is still unknown. Addition of antimicrobial nanoparticles to orthodontic adhesives and resin modified glass ionomeric cements prevents the invading microbes in the oral activity [7]. It is known that hydroxyapatite nano fillers had good antimicrobial property and shear bond strength [8]. Zinc oxide nanoparticles used in the field of biomedicine for their physicochemical properties is popular due to its UV absorption properties [9-10]. It shows significant antimicrobial, antioxidant and wound healing efficacy. It is cost effective with low toxicity metal oxide [11]. The antibacterial and anti cancer effects are exhibited by activation of apoptotic signalling pathway, destroying the membrane integrity and Intracellular through reactive oxygen species [12-13]. Silymarin derived from milk thistle *Silybum marianum* is a known hepato protective agent [14]. It has been shown to possess various pharmacological activities like hepato protective, antioxidant, anti inflammatory, anticancer, and cardio protective effects [15]. Silymarin is primarily used in management of periodontal infections and oral cancer. It is effective antimicrobial drug to treat dental caries dental plaque. It also inhibits the growth of oral bacteria either alone or in combination with other antibiotics [16, 17, 18]. Therefore, it is of interest to document the antimicrobial activity of silymarin mediated zinc oxide and hydroxy apatite nanoparticles against oral pathogens such as *Streptococcus mutans*, *Lactobacillus acidophilus*, *E. faecalis*.

Materials and Methods:

Preparation of silymarin extract:

0.5g of silymarin powder was measured and dissolved in 100mL distilled water and boiled for 10minutes at 60-80°C using a heating mantle. The boiled mixture was filtered using Whatmann No.1 filter paper. The filtered extract was stored in refrigerator.

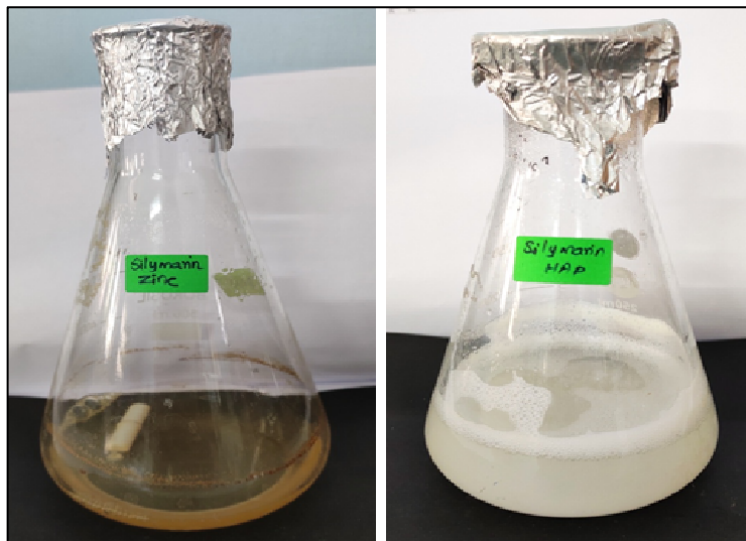


Figure 1: Silymarin mediated zinc oxide and HAP nanoparticles

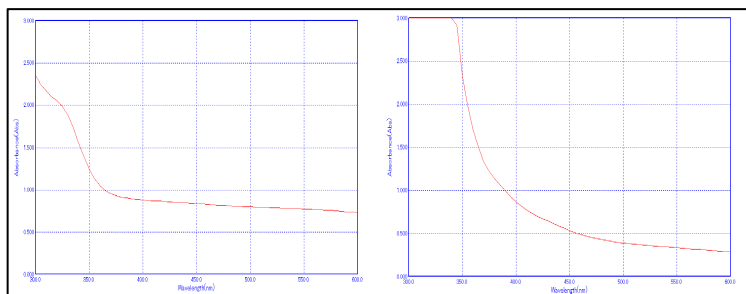


Figure 2: UV-visible spectroscopic analysis of silymarin mediated HAP and ZnO nanoparticles

Preparation of zinc oxide nanoparticles (silymarin)

20 millimolar Zinc acetate was measured (0.876g) and dissolved in 60 ml of distilled water. To that 40ml of filtered silymarin extract was added. To obtain uniform dispersion the prepared solution was maintained in an orbital shaker at 120rpm for 3-4 days. To confirm the synthesis of zinc nanoparticles UV Visible spectrophotometry was taken at regular specific intervals in the wavelength range of 250-650nm.

Preparation of hydroxy apatite nanoparticles (silymarin)

10 millimolar Hydroxy apatite (0.502g) was measured and dissolved in 60ml of distilled water. To that 40ml of filtered silymarin extract was added. To obtain uniform dispersion the prepared solution was maintained in an orbital shaker at 120 rpm for 3-4 days. To confirm the synthesis of HAP nanoparticles UV Visible spectrophotometry was taken at regular specific intervals in the wavelength range of 250-650 nm.

with silymarin zinc acetate pellet solution in the concentration range of 25 μ L, 50 μ L, 100 μ L and the fourth well loaded with a standard antibiotic (amoxycillin). Then the plates were incubated at 37°C for 24 hours. After incubation, the plates were observed and measured for Zone of inhibition around the nanoparticle loaded wells. Rose Bengal Agar was prepared as the medium for *C. albicans* and the inoculated plates were incubated at 37°C for 48 hours.

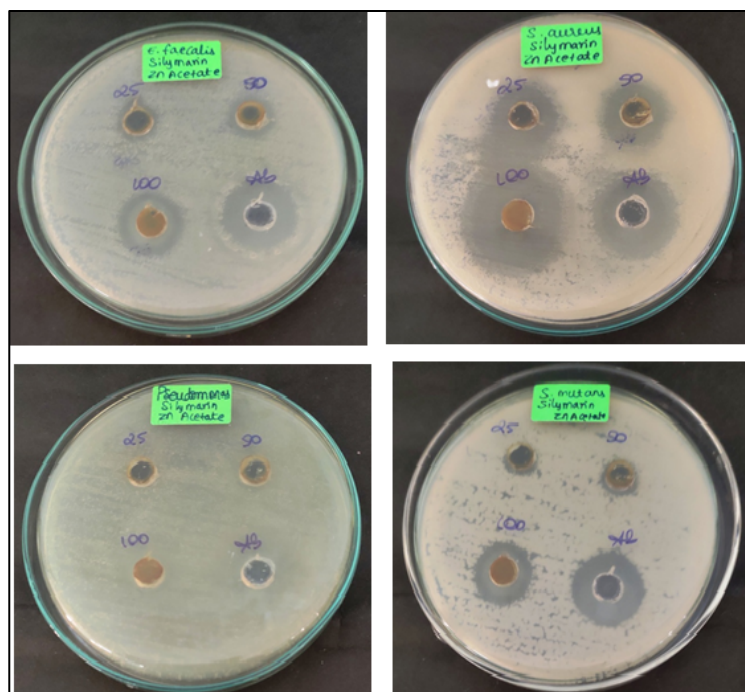


Figure 3: Antibacterial activity of HAP nanoparticles

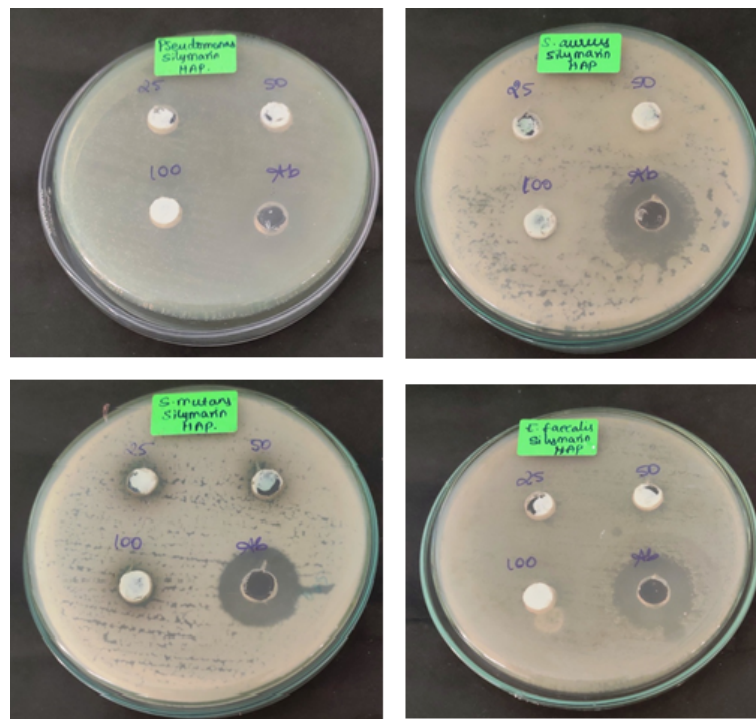


Figure 4: Antibacterial activity of zinc oxide nanoparticles

Centrifugation:

The synthesized nanoparticle solution was centrifuged at 8000rpm for 10 mins. After centrifugation, the supernatant was discarded and the pellet was stored.

Antimicrobial activity (agar well diffusion technique):

Mueller Hinton Agar was prepared, and sterilized using autoclave at 121°C for 15-20 minutes. The sterile MHA media was poured on the surface of the sterile Petri plates and allowed for solidification. After solidification, the organisms such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus mutans*, *Enterococcus faecalis* were swabbed using sterile cotton buds. The wells were made using a T-shaped well cutter. Among four wells per plate 3 wells were loaded

Results and Discussion:

Visual observation:

Figure 1 shows the green synthesis of hydroxyapatite and zinc oxide nanoparticles. The silymarin reacts with the hydroxyapatite and zinc acetate and forming the nanoparticles. When compared with initial addition in the 24 h the formation cloudiness and slight modifications in the solution indicates the nanoparticles formation [19, 20].

UV-visible spectroscopy:

The broad peak at 540 nm confirms the synthesis of hydroxyapatite nanoparticles and peak at 340 nm confirms the zinc oxide nanoparticles using silymarin. Figure 2 clearly shows the surface

plasmon resonance of nano Hap and ZnO nanoparticles. The zinc oxide nanoparticles synthesized using different plant extracts also confirms the nanoparticles synthesis [21, 22].

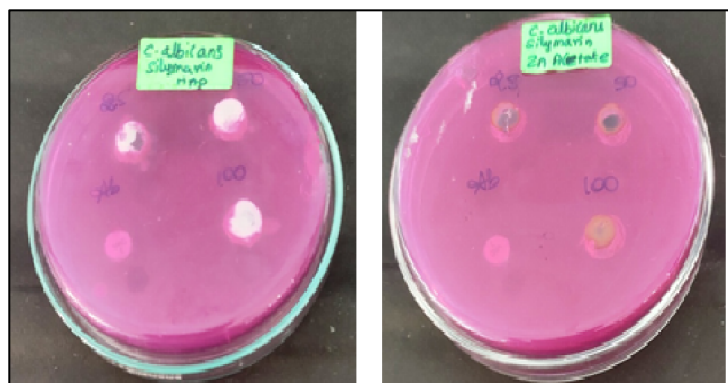


Figure 5: Antifungal activity of HAP and zinc oxide nanoparticles

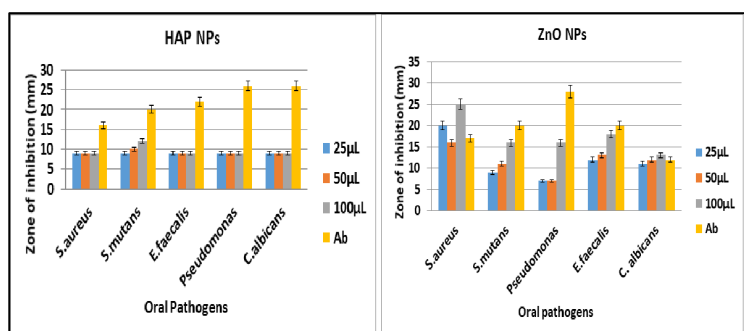


Figure 6: Antimicrobial activity of HAP and zinc oxide nanoparticles

Antimicrobial activity

The figure 3 and 6 shows the antibacterial activity of HAP nanoparticles against *pseudomonas sp*, *S. aureus*, *S. mutans* and *E. faecalis* in that the HAP nanoparticles shows zone of inhibition against *S. mutans*.

The zinc oxide nanoparticles shows very good antibacterial and antifungal activity against all oral pathogens such as *pseudomonas sp*, *S. aureus*, *S. mutans*, *C. albicans* and *E. faecalis*. In that the zinc oxide nanoparticles shows more activity when compared with standard drug. Figures 4, 5, and 6 clearly shows the antimicrobial activity of silymarin mediated zinc oxide nanoparticles. The zinc oxide nanoparticles synthesized using

different plant extract shows good antibacterial, antifungal and anticancer activity [23,24,25,26].

Conclusion:

The application of nanoparticles in dentistry is gaining momentum. Results show that silymarin mediated HAP and ZnO nanoparticles have antimicrobial activity against oral pathogens such as *Pseudomonas sp*, *Staphylococcus aureus*, *Streptococcus mutans*, *Enterococcus faecalis* and *Candida albicans*.

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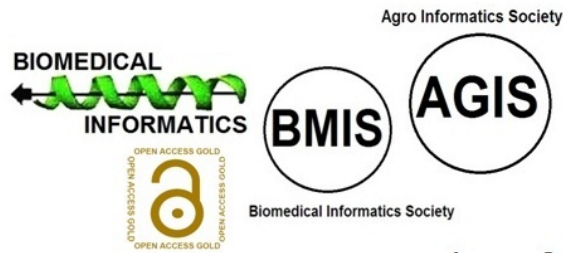
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