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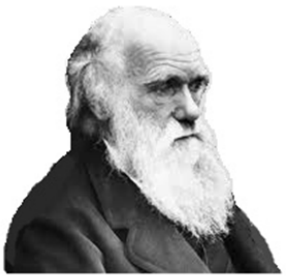

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Investigation on the Role of Nanoparticle on Microbial Nano Interface to Understand Pathogenecity

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ABSTRACT

Nano-biotechnology represents the intersection of nanotechnology and biotechnology which is an emerging field in creation, productivity and utility of nanoscale structures for advanced biotechnology. Plant and plant extract are considered as green and effective paths in the synthesis of gold and silver nanoparticles. The aim of the present study is to analyze the interaction of silver nanoparticles synthesized from herbal source (*Cinnamon zeylanicum*) with pathogenic bacteria *Salmonella typhi* (*S. typhi*) in order to know the microbe pathogenicity. The micro-nano interface disturbed the growth of microbes. Under the influence of different concentration of nanoparticles (50 μL to 1,000 μL), the microbes exhibited slow growth as compared to control. The viable cell count also decreased as the nanoparticles concentration increased. This shows major difference in the bacterial culture of with and without nano particle. Under normal culture, without nanoparticles intervention, the microbes show lag phase at 0-4 h, the log phase begins and its exponential growth occurs between 4-8 h and after 12 h, stationary phase was attained. There was a slow growth observed when the culture was exposed to silver nanoparticles. This clearly shows the antimicrobial effect of nanoparticles. In this study we used green synthesized nanoparticles, which have shown a good antibacterial efficacy against the pathogenic microorganism *Salmonella typhi.*

***Keywords*:** *Salmonella typhi*, Silver nanoparticles, Antimicrobial

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1. INTRODUCTION

Nano-biotechnology represents the intersection of nanotechnology and biotechnology which is an emerging field in creation, productivity and utility of nano-scale structures for advanced biotechnology [1]. An important area of research in this field is the synthesis of nanoparticles with different chemical compositions, sizes and shapes. During the last two decades, the biosynthesis of metal nanoparticle (silver, gold, platinum and palladium) has received considerable attention due to the growing need to develop environmentally sociable technologies in material synthesis [2]. The nanoparticles are of great interest due to their extremely small size, a large surface-to-volume ratio and exhibit utterly novel characteristics as compared to the large particles of bulk material [3]. Nanoparticles of metals, viz. gold, silver, platinum and palladium are widely used for production of commercially available products such as shampoos, soaps, detergents, shoes, and toothpaste besides their applications in medical and pharmaceutical products [4]. Silver nanoparticles have been employed in sensor technology, biological levelling and many other biomedical applications [5, 6].

Synthesis of nanoparticles can be performed using a number of routinely used chemical and physical methods. However, these methods are energy and capital intensive; employ toxic chemicals and non-polar solvents in the synthesis procedure and later on synthetic additives or capping agents, thus precluding their applications in clinical and biomedical fields. Therefore, the need for the development of clean, reliable, bio-compatible, benign and eco-friendly process to synthesize nanoparticle leads to turning researchers towards 'green' chemistry and bioprocesses. In recent years, biological synthesis of nanoparticles has been emerged as a promising field of research in nano- biotechnology. Microorganisms such as bacteria, fungi, actinomycetes, yeasts and viruses are reported to have the innate potential to produce metal nanoparticles either intra- or extra-cellular and considered as potential bio-factories for nanoparticle synthesis. A great deal of effort has been devoted towards the biosynthesis of metal nanoparticles, using bacteria, [6] fungi [7], actinomycetes [8], yeast [9] and viruses [10], in addition to the above mentioned synthesis methods.

Phytosynthesis which utilizes parts of whole plants as biological factories to synthesize the metallic nanoparticles is under exploitation and must be an advantageous and profitable approach [11]. In comparison to microorganisms, phytosynthesis method is devoid of complex and multistep processes like microbial isolation, culturing, maintenance, etc., and is also very rapid and cost effective that can be easily scaled up for bulk production of nanoparticles [12]. In addition, phytosynthesis is truly a 'green' synthesis route in comparison to other known methods of nanoparticle synthesis. Plants are known to harbour's a broad range of metabolites. However, their potential is yet to be fully utilized in full throttle for synthesizing metallic nanoparticles [13]. By using plant tissue culture techniques and optimizing the downstream processing, it is possible to synthesize metal nanoparticles at industrial scale. Earlier reports cite the use of microorganism such as algae [14], bacteria [15], yeast [16] and fungi [17] for the biosynthesis of NPs.

Recently, several plant extracts [18], diatoms [19] and human cells [20] have been emerged as novel resources for their ability to produce safe and non-toxic nanoparticles such as iron, cobalt, gold, silver, platinum, iron oxide, alloys, sulphides, quantum dots, etc. Products from nature or those derived from natural products have been used as reducing and capping agents during synthesis. The techniques involved are usually simple, environmentally friendly and naturally cordial one pot processes.

It has been verified by various studies that the reductive capabilities of the proteins and metabolites present in these biological systems can transform inorganic metal ions into metal NPs [21-23].

In this study, we've reported the biosynthesized AgNPs via reduction of silver ions using Cinnamon Bark extract (*Cinnamomum zeylanicum* Blue), and studied the antibacterial activity of these biosynthesized AgNPs against pathogenic bacteria *Salmonella typhi* in order to understand its pathogenicity.

2. MATERIALS AND METHODS:

2. 1. Growth of *Salmonella typhi*

The bacterial culture was grown in Nutrient Broth (pH 7.3 ±0.2) [10 g peptone 10 g beef extract, 5 g sodium chloride/L] under aerobic cultivation at 37 °C for overnight. Then 10 mL of overnight cultured broth (10% v/v) was taken and centrifuged at 10,000 rpm .The pellet was inoculated to 100 mL nutrient broth incubated in a shaker at 150 rpm, 37 °C. Then the culture was stored for further experimental use.

2. 2. Preparation of AgNPs from Cinnamon:

The barks of the *Cinnamomum zeylanicum* Blume were taken and shadow dried, then it was pulverized into fine coarse powder using blender. To a 100 mL of Distilled water 2.5 gram *Cinnamomum zeylanicum* Blume bark powder was added and boiled for 5 minutes. The extract was filtered and further used for reaction. To synthesis metal nanoparticles (Ag), 1 mL of plant extract and 50 mL of 1 mM $AgNO_3$ solution was added and kept under stirring for 8 h, simultaneously the solution is exposed to microwave irradiation for different time intervals such as 10 s, 20 s, 40 s and 60 s. The phytoconstituents present in the bark are strongly acted as reducing agents to form a spherical shaped silver nanoparticle that was confirmed through characterization study. The SEM and DLS studies revealed that the average particle size is 10 to 50 nm. The synthesized AgNPs was used for antibacterial activity.

2. 3. Growth of *Salmonella typhi* with Nanoparticles

Single colony of *S. typhi* was inoculated into 20 mL of nutrient broth under aseptic condition. Broth was incubated at room temperature for 12 h, then the overnight culture was used as an inoculum. The known concentration of silver nano particle was added to a culture and incubated in a shaker at 150 rpm in 37 °C. Growth of *S. typhi* was observed under the influence of nanoparticles by observing optical density at 600 nm using UV-Vis Spectrophotometer (Elico), simultaneously viability of cell count observed through spread plate technique.

2. 4. Cell Viability under different concentrations of Nanoparticles

The silver nanoparticles of different concentration (50 µL to 1,000 µL) was introduced to the culture of *S. typhi* and kept for incubation at 150 rpm at room temperature in shaker for 12 h and the growth was observed in terms of optical density. All the samples which contain nanoparticles of (50-1,000 µL) were subjected to cell viability test. Serial dilution was carried out to know the colony forming unit of *S. typhi*.

3. RESULTS AND DISCUSSION

When *S. typhi* was grown under the influence of nanoparticles, this shows major difference from in the culture of with and without nano particle. The culture without AgNPs, attained lag phase at 0-4 h then the log phase begins and its exponential growth occurs between 4-8 h, then stationary phase was attained.

There was slow growth observed when the culture exposed to silver nanoparticles. This clearly shows the antimicrobial effect of AgNP. **Figure 1 s**hows the growth of *S. typhi* with and without the influence of nanoparticle. Normally cells are active during mid log phase. For better understanding the pathogenicity effect under the influence of AgNPs, next we introduced nanoparticles during early log phase. **Figure 2** shows the growth of *S. typhi* after the introduction of particles during log phase. Under the influence of nanoparticles the sluggish growth of bacteria shows that AgNPs inhibit the bacteria by breaking the cell wall.

Figure 3 shows the growth of culture under the influence of different concentration of nanoparticle (50 µL to 1,000 µL) and the decreased optical density exhibited the particles role in the growth of *S. typhi*.

The viability cell count method is mainly used to check the viability, the decreased growth rate in the culture with silver nanoparticle has shown that the nanoparticles suppressed the growth of cells. The colony forming unit per mL was decreased in the culture having nanoparticle exposure. Here the nanoparticles were added in various concentration from 50 to 1,000 µL, the initially the growth is high because of less concentration of nanoparticles and its growth decreased as the concentration of nanoparticle increased. The viability cell count method is mainly used to check the viable rate of the culture, the decreased growth rate in the culture has shown that the nanoparticles are suppressed the growth of cells. The colonies (cfu/mL) was high which is not having the influence of nano particle.

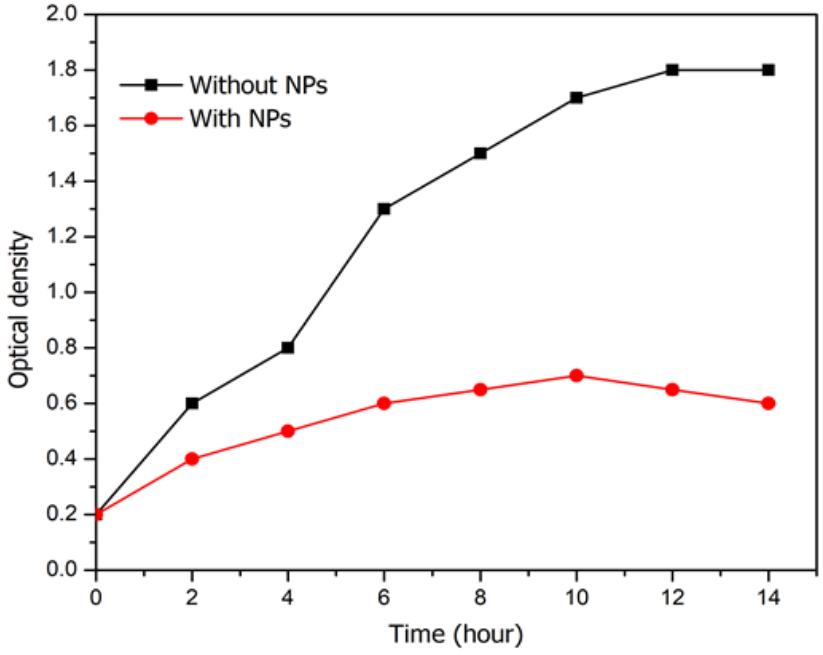

Figure 1. Growth of *S.typhi* with and without the presence of AgNPs synthesized

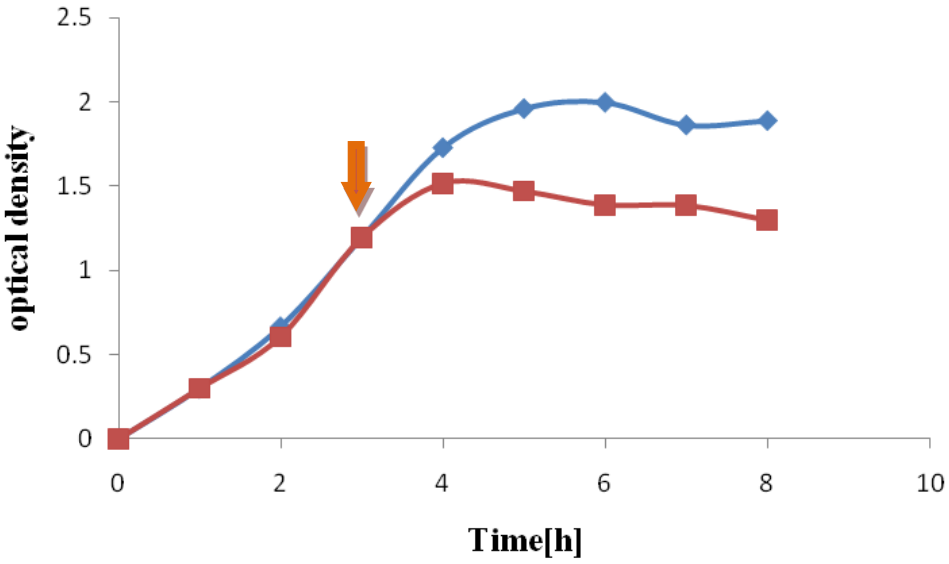

Figure 2. Growth of *S. typhi* under the influence of AgNPs during Log Phase

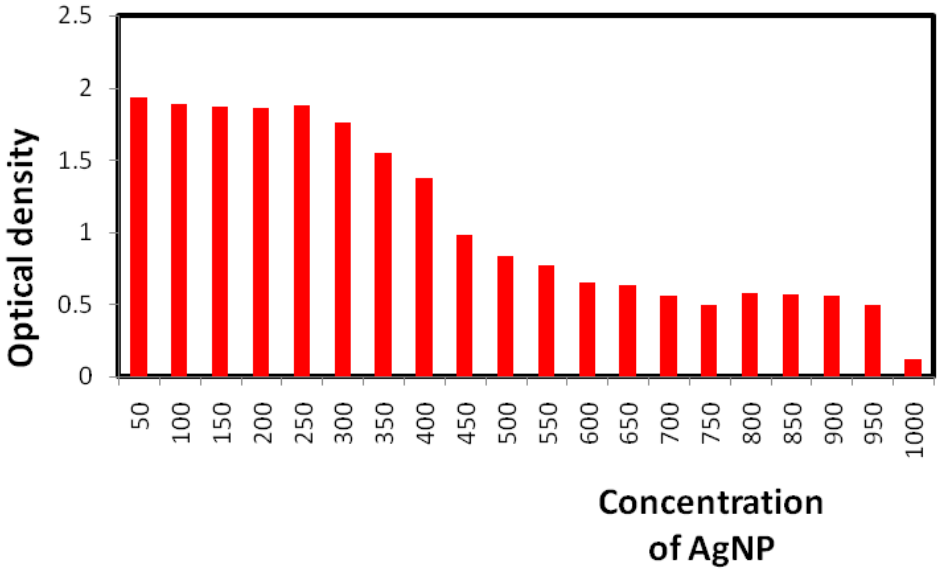

Figure 3. Growth of *S. typhi* under different concentration of AgNPs (µl) synthesized from *Cinnamomum zeylanicum*

4. CONCLUSION

Antimicrobial and antibiotic resistances are an increasingly serious threat to human health. It is necessary to overcome it with the help of nature. Therefore, there is an increase in the investigation of nanoparticles role against human infectious diseases management. The current study about the AgNPs application as antimicrobail agent is an alternative solution from

the therapeutic medications. The biosynthesized AgNPs have shown good antibacterial efficacy and hence it has a potential to be used as antibacterial agent against *Salmonella typhi.*

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