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In-vitro Antidiabetic and Anti-Microbial activity of Silver nanoparticles synthesized using Medicinal plant - *Aegle marmelos*

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Abstract

The advance and very applicable technology is nanotechnology and it was derived from the term of nano it is the billionth of meter or 10^{-9} m. It played an important role in many of the recent trends related to human life improvement. There were many fields which interact with the nanotechnology and resulted to the good need for the human beings. In this study, we report the synthesis of silver nanoparticles by using reduction of silver nitrate. The biosynthesized nanoparticles were characterized by using UV - visible spectrophotometer, the surface plasmon resonance observed at 420 nm to 450 nm due to the presence of silver nanoparticles. The spherical shaped and 50–100 nm sized nanoparticles were viewed by Scanning electron microscope, Atomic force microscopic image of silver nanoparticles were analysed and to investigate the in-vitro antidiabetic potentials and anti-microbial activity of the silver nanoparticles synthesized from *Aegle marmelos* leaf extract. In-vitro antidiabetic activity was analyzed through Alpha-amylase inhibitory test, the silver nanoparticle exhibited 100µg/mL maximum inhibitory effect on the enzyme in a dose-dependent manner. The anti-microbial activity of the silver nanoparticles was investigated the *Klebsiella planticola* having a maximum zone of inhibition at 50 µL of AgNPs solution when compared to another organism. It is one of the simple and ecofriendly method, it has cost effective and there is no side effect.

Keywords: Silver nanoparticles; *Aegle marmelos;* In-vitro antidiabetic activity; Anti-microbial activity.

INTRODUCTION

Diabetes remains to be one of the most prevalent chronic disorders in both developing and developed countries [1]. Type 1 diabetes is characterized by impaired insulin secretion of the pancreatic β cells, whereas type 2 diabetes, which accounts for more than 90% of diabetic cases, is characterized by insulin resistance and progressive β-cell dysfunction. Although many hypoglycemic agents have been developed into the market, their various side effects greatly limit their wide application in the clinic [2]. Thus, there is still a need for more effective and safe oral antidiabetic agents. Botanicals are a valuable source of therapeutics for metabolic disease including diabetes. Because various kinds of chemical constituents present in botanicals act on a variety of targets by different modes and mechanisms, they can exert distinctively therapeutic effects in diabetes and/or its complications. Due to relatively easy accessibility and availability of dietary botanicals compared to prescription pharmaceuticals, scientific research supporting the efficacy and safety of botanical therapies is of paramount priority [3].

The advance and very applicable technology is nanotechnology and it was derived from the term of nano, it is the billionth of a meter or 10^{-9} m. The Nano comes ultimately from the Greek word for dwarf and is also related to the Spanish word Nino [4]. It played an important role in many of the recent trends related to human life improvement. There is a many of the fields were interact with the nanotechnology and resulted to the good need for the human beings. The medical applications such as treatment and disease diagnosis are coming under the nanomedical technology [5]. The present study, we investigated the antidiabetic potentials and anti-microbial activity of the silver nanoparticles synthesized from *Aegle marmelos* leaf extract.

MATERIALS AND METHODS

Chemicals

Nutrient broth, Nutrient agar and silver nitrate were purchased from Hi-Media, Mumbai. Glucose test kits were purchased from Beijing BHKT clinical reagent Co., Ltd Beijing, China). All the experiments were performed by using double distilled water.

Plant material

The leaf of *Aegle marmelos* was collected in August 2015, Adhiparasakthi college Agricultural college medicinal garden, kalavai, and identified by professor Jayaraman, Plant Anatomy Research Center, Chennai, Tamilnadu (Voucher specimen no. PARC-2017/2443) has been deposited in the herbarium of the center. The experiment was conducted using the stored plant materials.

Preparation of *Aegle marmelos* leaf extract

5 g of fresh leaves were surface sterilized using Tween 20 and double distilled water. Then the leaves were cut into fine pieces and dispersed in 100 ml distilled water and boiled for 10 min at 70°C. Thereafter aqueous extract of *Aegle marmelos* was filtered and concentrated under

reduced pressure and finally vacuum dried at temperature 30°C and the pressure 760 torr to 1 bar. The yield of the aqueous extract was 12.5% w/w [6].

Synthesis of Silver nanoparticles

For silver nanoparticles synthesis, 1 mM silver nitrate solution was prepared in 90 ml of distilled water and the solution was taken in 250 ml Erlenmeyer flask. About 10 ml of plant extract added to silver nitrate solution and kept the flask at room temperature. A control was also maintained without the addition of leaf extract. The colour changes observed visually and the synthesis of silver nanoparticles at different time intervals were monitored by UV-vis spectrophotometer of the solution [7].

Characterization of silver nanoparticles

About 10 ml of leaf extract was added to 90 ml of silver nitrate solution. The colour changes measured by UV –vis spectrophotometer at specific time intervals of 30 min, 1 h, 2 h, 4 h, 6 h, and 12 h.

Characterization studies

UV–vis spectrophotometer

The reduction of silver ions was monitored by using double beam UV-vis spectrophotometer (Perkin Elmer, Singapore) of the reaction medium in the wavelength range of 300-700 nm with 1000 mm quartz cell. The resolution of the UV-vis spectrophotometer was 1 nm. The UV-vis spectra of the resulting solution were recorded. The graph of wavelength on X-axis and absorbance on Y-axis was plotted.

Scanning Electron Microscope

The morphology and size of the silver nanoparticles were found by Scanning Electron Microscope (Philip model CM 200). The thin films of the sample were prepared on a carbon coated copper grids by just dropping a very small amount of the sample, the extra solution was removed by using blotting paper and the grids were allowed to dry by putting it under the mercury lamp for 5 min and the images were recorded.

Energy Dispersive X-ray analysis

Elemental analysis of silver was carried out by EDAX (Philips XL-30). The aqueous suspension of silver nanoparticles sample was prepared for the analysis of EDX by the drop-coating method. EDX analysis was carried out in the spot profile mode with a beam diameter of 1 µm at several places on the sample.

Transmission Electron Microscope and SAED Pattern

The morphology of the nanoparticles was analyzed using the high-resolution images obtained with a JEOL3010 transmission electron microscope (TEM) operated at an accelerating voltage of 300kV. Prior to analysis, AgNPs were sonicated for 5 min and a drop of appropriately

diluted sample was placed onto carbon coated copper grid. The extra solution was removed by using blotting paper. After that, the liquid fraction was allowed to dry at room temperature.

In vitro - Antidiabetic assay (α-Amylase inhibition assay)

Alpha-amylase inhibition was determined by quantifying the amount of maltose liberated during the experiment. Different concentration of nanoparticles (25, 50, 75, 100, 125 µL) were pre-incubated with 100 µL of α-amylase solution (1 U/mL) at room temperature for 30 minutes. 100µL of starch solution (1%w/v) was further added to it and the mixture was incubated at room temperature for 10 minutes. 100µL of 96 mM (3, 5- dinitrosalicylic acid solution) DNSA reagent was added to it to stop the reaction and the solution was heated in a water bath for 5 minutes. Control was maintained where the equal quantity of enzyme extract was replaced by sodium phosphate buffer maintained at a pH value of 6.9. Reading was measured at 540 nm. The experiment was performed in triplicate. Acarbose was used as a positive control [8].

% inhibition was calculated using the formulae-

$$\% \text{ inhibition} = \frac{C - T}{C} * 100$$

Where, C= control, T= test sample.

Antibacterial activity of biosynthesized silver nanoparticles

The silver nanoparticles synthesized using *Aegle marmelos* was tested for antimicrobial activity by agar well diffusion method against pathogenic bacteria *Bacillus subtilis, Klebsiella planticola, Pseudomonas sp, Streptococcus sp* and *Staphylococcus aureus.* Luria Bertani Agar medium was used to cultivate bacteria. The fresh overnight culture of each strain was swabbed uniformly onto the individual plates using sterile cotton swabs. 5 wells were made on each Luria Bertani Agar plates. Then the centrifuged silver nanoparticles (10, 20, 30, 40 and 50 µL) were poured into each well on all plates and incubate for 24 hr at 37°C. After incubation, the different levels of zonation formed around the well was measured [9].

RESULTS AND DISCUSSION

Plant-mediated synthesis of silver nanoparticles - Visual observation

Fig. 1b. shows the appearances of yellowish brown colour suggests the formation of silver nanoparticles through the leaf extract of *A. marmelos*. The brown colour formation started in 2 hr and attained dark brown colour after 6 hr of incubation. The appearance of precipitation notices at the reaction vessel after 6 h of incubation indicates that the reaction was completed. Similarly, the colour change from yellow to brown within 6 hr using the leaf extract of *Pungamia pinnata* were also observed

a. Plant Extract **b. Initial** **c. Final**

Figure 1: Visual observation of silver nanoparticles synthesis process

UV-vis spectroscopic analysis

The synthesis process was started at the 2nd hour and the very good peak was observed at the 6th hour. The good peak was found at 420 nm indicates the synthesis of silver nanoparticles at 4th and 6th hour absorbance. After that, the peak was shifted to 440 nm shows changes in the nanoparticles reaction mixture. It may be the agglomeration may lead to the big size of nanoparticles [10].

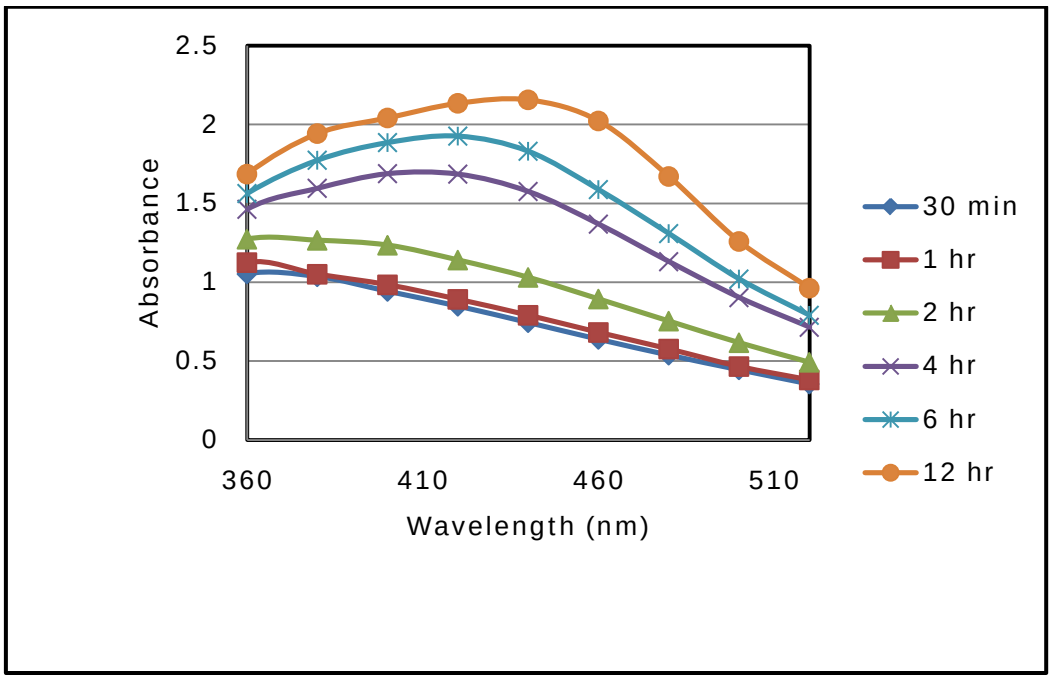

Figure 2: UV-vis spectroscopic analysis of Silver nanoparticles synthesized by *A. marmelos*

Atomic force microscopy

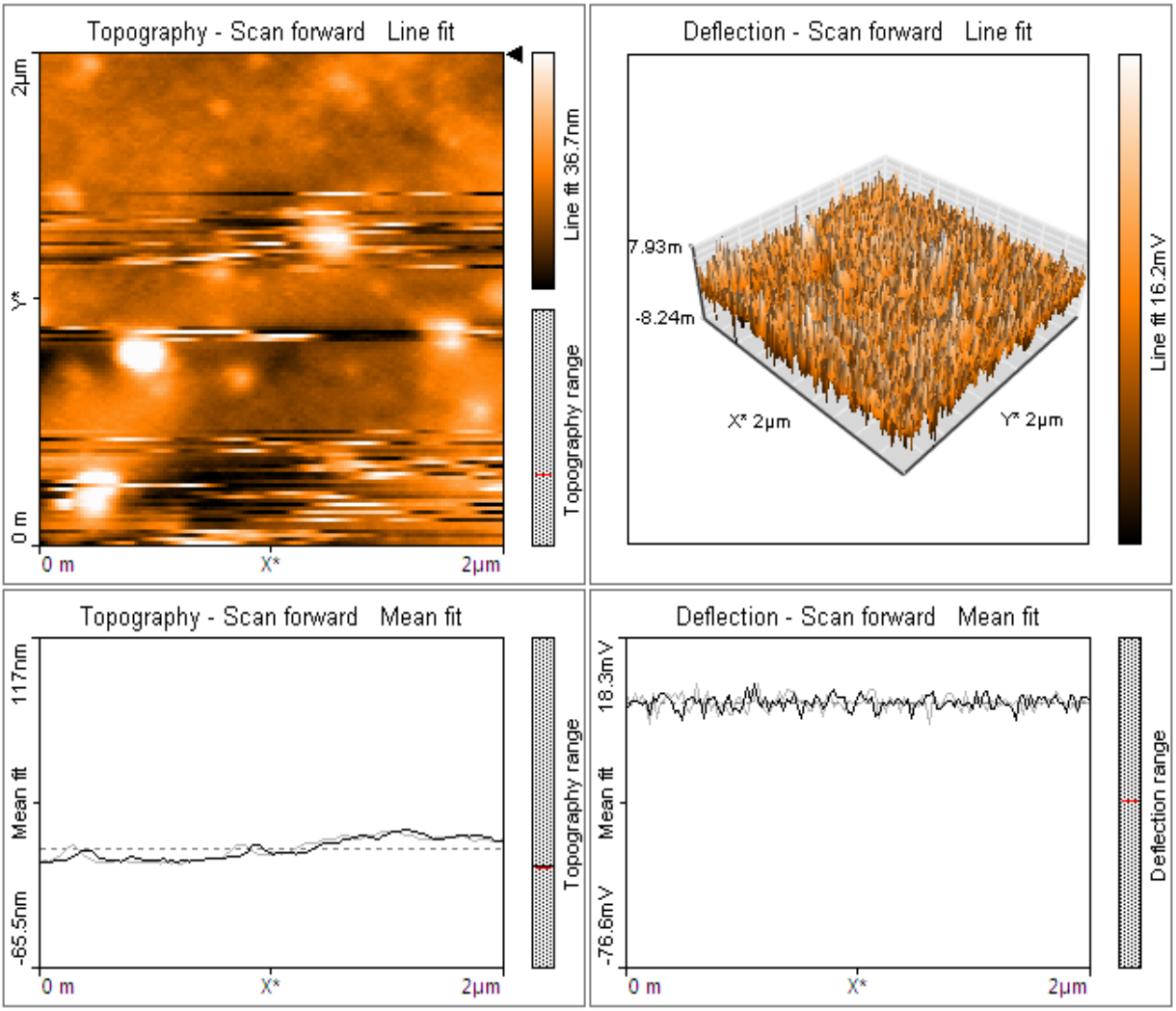

Figure 3: AFM image of Silver nanoparticles synthesized by *A. marmelos*

Figure 3 shows the atomic force microscopic image of silver nanoparticles synthesized using medicinally important plant A. marmelos. The spherical shaped particles were observed in this image and in the background some light colour background also observed may be the phytochemicals of plant extract.

Scanning electron microscope

The SEM image shows the silver nanoparticles synthesized using *A. marmelos* and the size of the particles are 20-60 nm. Most of the silver nanoparticles are spherical shape and many irregular shapes are found. The images are showing so many clumps may be the phytochemicals of plant extract. The plant is having phytochemicals closely binds to the nanoparticles and forms the clumping [11].

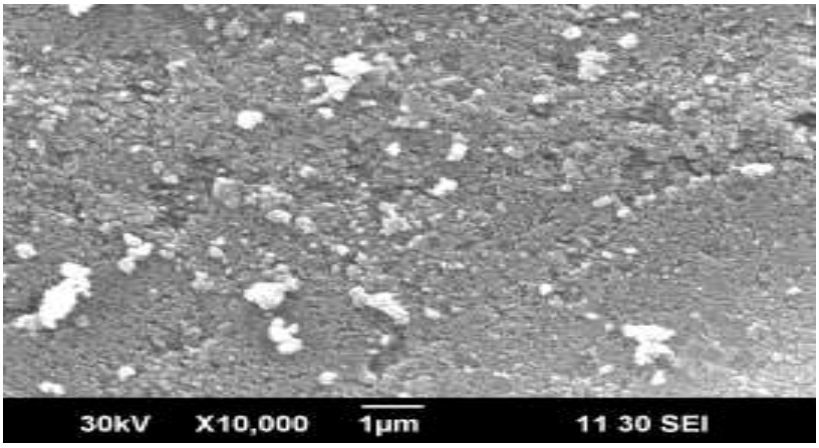

Figure 4: SEM image of Silver nanoparticles synthesized by *A. marmelos*

In vitro antidiabetic activity

Alpha-amylase inhibitory assay

Alpha-amylase is the main enzyme involved in the breakdown of polysaccharides and release of sugar in the main blood stream which further leads to increased blood glucose level and ultimately diabetes. The inhibitory effect of this enzyme can prove to have a potential therapeutic effect on diabetes. Figure 5 represents the % inhibition effect of silver nanoparticle on the enzyme. Silver nanoparticle exhibited an inhibitory effect on the enzyme in a dose-dependent manner and when the dose was increased to 100µg/mL, it resembled the % inhibition exhibited by standard Acarbose. It could be interpreted from the results that silver nanoparticle could be used as an alternative to Acarbose because of the potential inhibitory effect that it has on the enzyme.

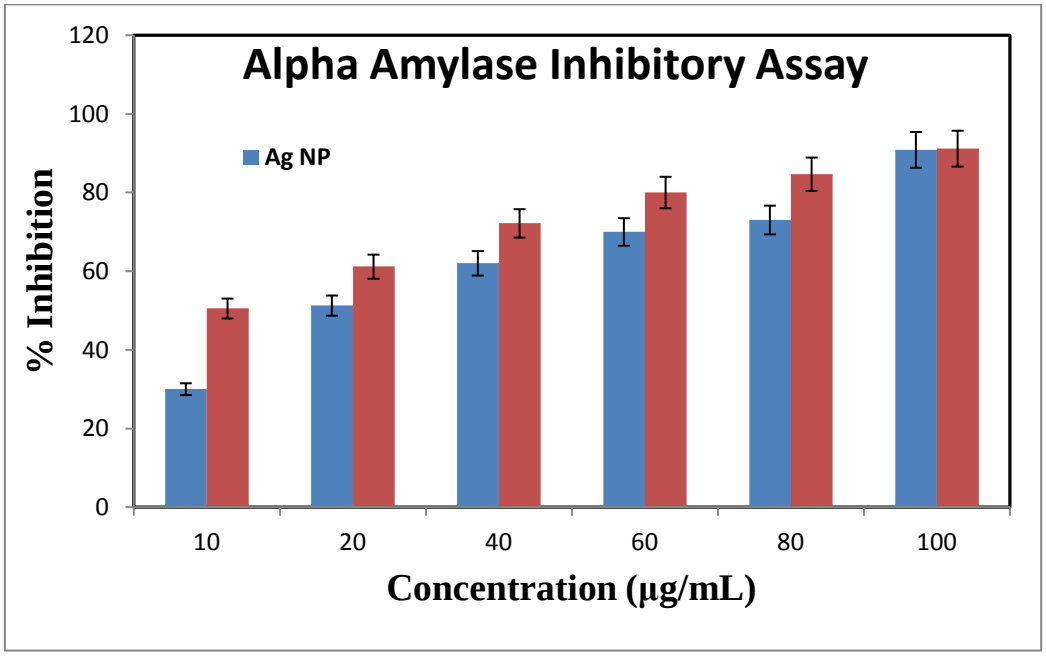

Figure 5: In vitro antidiabetic activity of silver nanoparticles

Antibacterial activity of silver nanoparticles

The antibacterial activity of silver nanoparticles against different bacterial pathogens like *Bacillus subtilis, Klebsiella planticola, Pseudomonas sp, Streptococcus sp.* and *Staphylococcus aureus* were performed. The zone of inhibition against the pathogens is shown in Table 1 and figure 6-10. The silver nanoparticles are having a very good zone of inhibition against most of the pathogens. *Klebsiella planticola* having a maximum zone of inhibition at 50 µL and *Streptococcus sp* has a minimum zone of inhibition in maximum volume of AgNPs solution. Based on this study we will use the silver nanoparticles much biomedical application related to clinical diseases caused by pathogenic microorganisms.

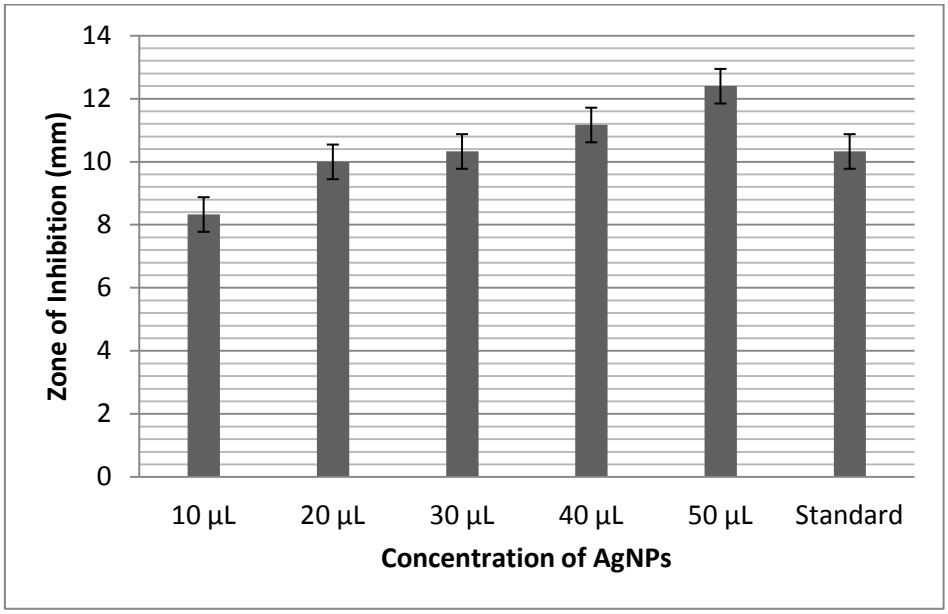

Figure 6: Antibacterial activity of silver nanoparticles synthesized using *A. marmelos* against *Bacillus subtilis*

Table. 1 Antibacterial activity of AgNPs synthesized from *A. marmelos* leaf extract

Conc. of AgNPs	*Bacillus subtilis*	*Klebsiella planticola*	*Pseudomonas sp.*	*Streptococcus sp.*	*Staphylococcus aureus*
10 µL	08.33±0.167	10.40±0.100	10.00±0.000	08.47±0.034	08.50±0.289
20 µL	10.00±0.501	11.97±0.261	10.13±0.467	09.07±0.067	09.17±0.167
30 µL	10.33±0.441	12.77±0.234	10.67±0.334	10.43±0.434	10.67±0.334
40 µL	11.17±0.334	12.83±0.167	12.50±0.289	11.37±0.134	11.83±0.273
50 µL	12.40±0.100	14.17±0.334	13.33±0.167	11.50±0.289	12.07±0.115
Standard	10.33±0.467	11.370.501	12.07±0.167	11.17±0.289	12.77±0.000

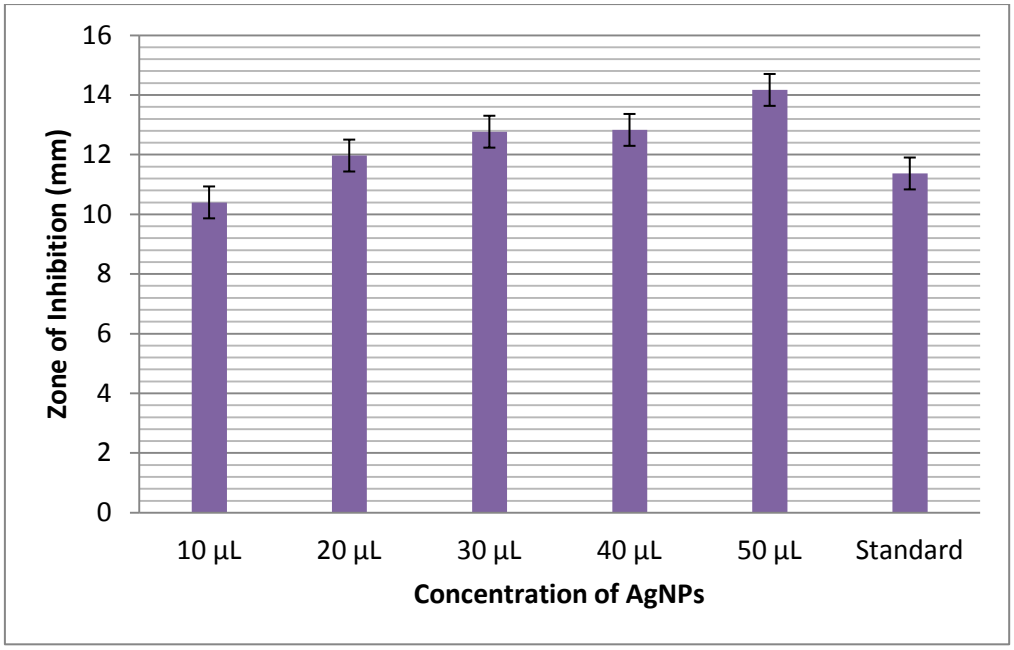

Figure 7: Antibacterial activity of silver nanoparticles synthesized using *A. marmelos* against *Klebsiella planticola*

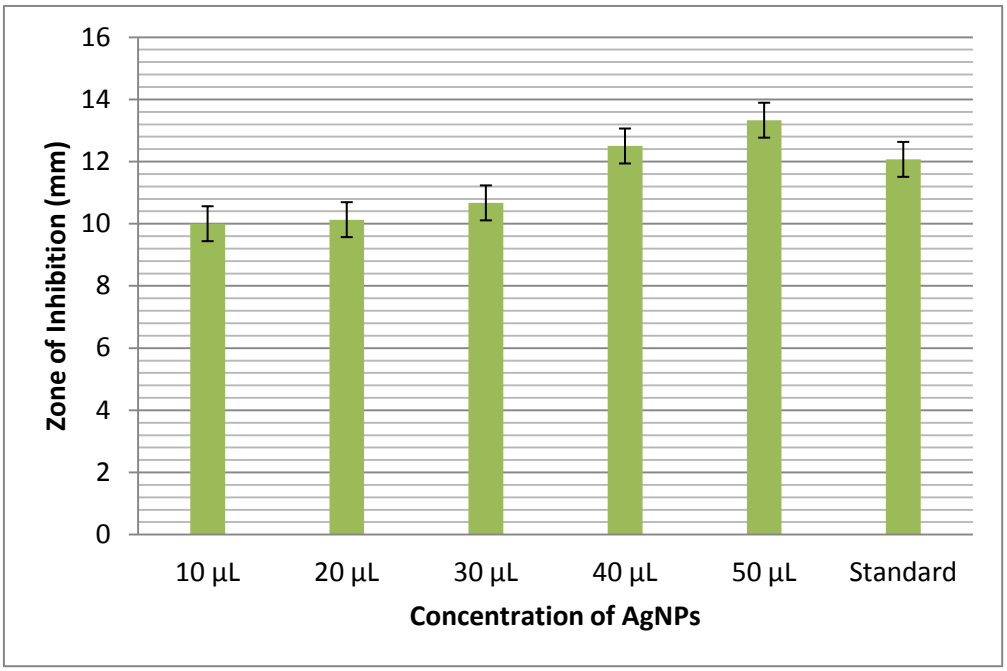

Figure 8: Antibacterial activity of silver nanoparticles synthesized using *A. marmelos* against *Pseudomonas sp*

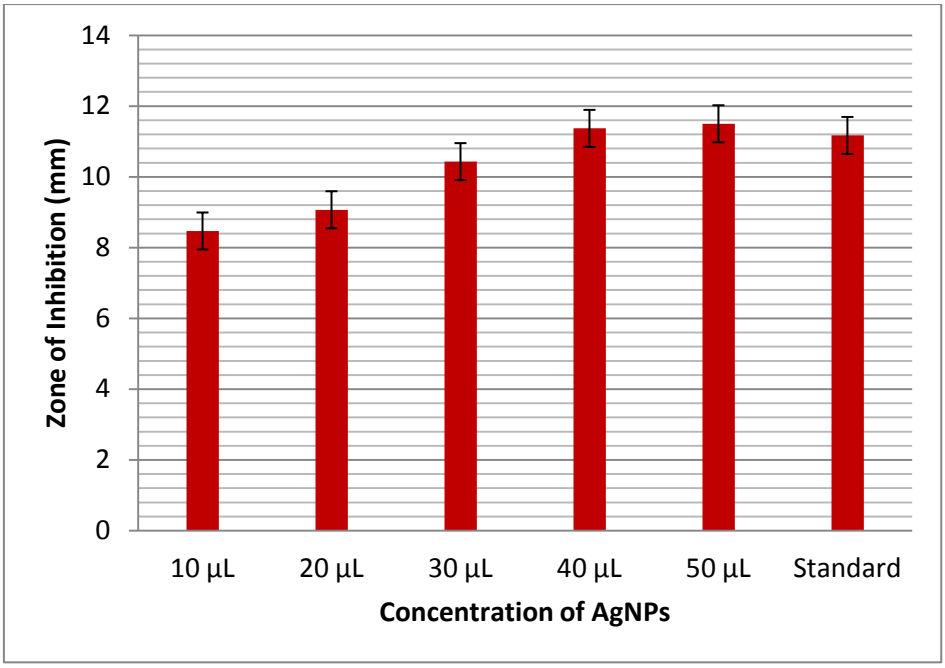

Figure 9: Antibacterial activity of silver nanoparticles synthesized using A. marmelos against Streptococcus sp

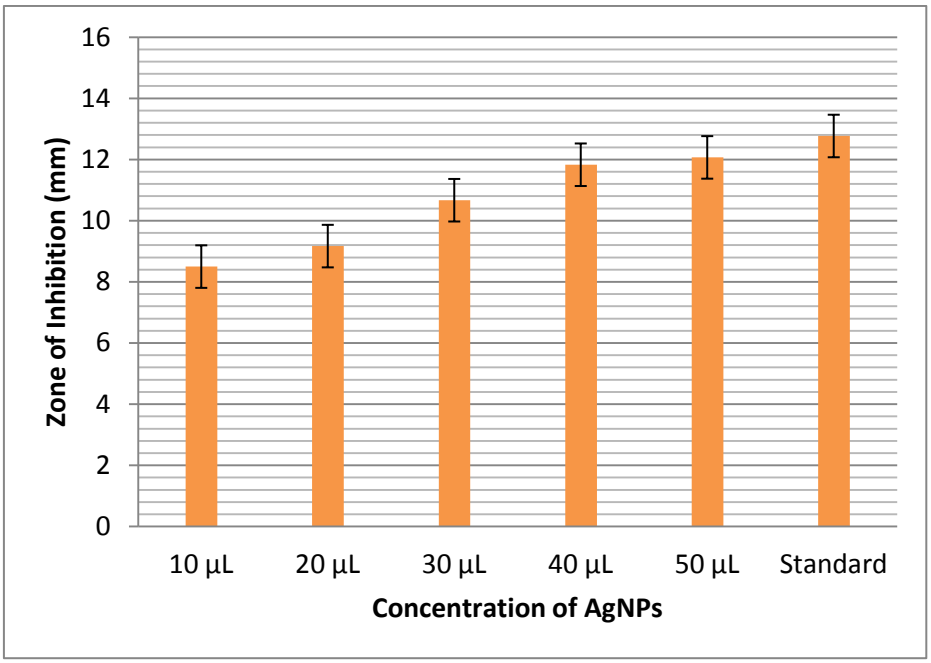

Figure 10: Antibacterial activity of silver nanoparticles synthesized using *A. marmelos* against *Staphylococcus aureus*

Conclusion

In this present investigation, we used the most applicable and ancient traditional and medicinal plant *Aegle marmelos* for the green synthesis of silver nanoparticles. The characterization results are showing the synthesized nanoparticles are very good quality. The

UV-vis spectrophotometer exhibit the peak at 420 nm clearly indicates the silver nanoparticles surface Plasmon resonance. The microscope like atomic force microscope and scanning electron microscope clearly indicate the silver nanoparticles are spherical and many nanoparticles are get agglomerated in nature due to the *Aegle marmelos* plant phyto compounds. Finally, the in-vitro antidiabetic activities of silver nanoparticles are highly important in this study. Based on these results, silver nanoparticles may be used for the anti-diabetic drug in future. The silver nanoparticles are very much used in controlling disease causing and disease spreading microorganisms. The silver nanoparticles synthesized using *Aegle marmelos* showing very good growth inhibition of disease-causing pathogens. It is one of the simple and ecofriendly methods because when compared to the chemical and physical method it has cost effective and there is no side effect.

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