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ORIGINAL ARTICLE

Study of Anti-Bacterial Activity of Silver Nano Particles from Insulin Plant *Costus igneus* Against Oral Bacteria in Human's

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ABSTRACT
Insulin plant, Costus igneus commonly known as Fiery costus, Step ladder or Spiral flag or Insulin plant is native to South and Central America. It is an herbal cure for diabetes and hence commonly called as insulin plant. In today's world, nanotechnology research has emerged as a solution to the challenges we face now and in the future. The biosynthesis of nanoparticles using plants exemplifies green synthesis, which offers advantages over chemical and physical methods as it is more cost-effective, environmentally friendly, and can be easily scaled up for large-scale production. Different bacteria and plants have been utilized to successfully carry out the green synthesis of silver nanoparticles. Consequently, the aim of this study is to focus on the green synthesis of silver nanoparticles using the insulin plant, Costus igneus N. E. Br. and their applications against oral bacteria. Methanol was used to investigate phytochemicals present in plant with the help of Soxhlet. Silver nanoparticles were produced by using plant extract and silver nitrate. Synthesis of silver nanoparticles was confirmed with various characterization techniques like UV-Vis Spectroscopy, Scanning Electron Microscopy, Transmission electron Microscopy, X-ray diffraction Crystallography. This nanoparticle then used against five different oral bacteria like E.coli, Streptococci, Lactobacilli, Staphylococci and Corynebacteria. This antibacterial activity of silver nanoparticles was done by using Disc diffusion method at 37°c. It is found that this plant derived silver nanoparticles have great potential against oral bacteria which is shown in results. 100 mg/ml concentration of nanoparticles was found most effective against bacteria as compared to other concentrations. From the study it is concluded that, the plant Costus igneus has great potential of secondary metabolites in them. By using these secondary metabolites, silver nanoparticles were produced successfully. The synthesized nanoparticle has antibacterial activity in them.
Keywords: *Costus igneus, Phytochemical Screening, Insulin plant, silver nanoparticles, Anti-Bacterial Activity, UV-Vis Spectroscopy, Scanning Electron Microscopy, Transmission electron Microscopy, X-ray diffraction Crystallography.*

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INTRODUCTION

The medicinal plant which includes various types of plants used in practice of the medicinal and therapeutic use. These plants are also used as food, flavonoid, medicine, perfumes, aesthetic values and spiritual activities in India. These plants have been used from pre historical period as a medicine. It has been used in Unani, Indian Ayurveda, European and Mediterranean culture since long over 4000 years as medicine. The various cultures such as Rome, Egypt, Iran, Africa, and America used plants in their healing rituals also developed traditional medicinal systems such as Unani, Ayurveda and Chinese Medicine with respect to the therapeutic use systemically. [1] The plant which was used for study, was *Costus igneus* which belongs to the family Costaceae. The Costaceae was first raised to the rank of family by Nakai on the basis of spirally arranged leaves and rhizomes being free from aromatic essential oils. [2] Before the elevation to family status, Engler and Prantlin 1886 recognized Costoideal as a subfamily under Zingiberaceae. Several anatomical and morphological features support this isolated position including well developed aerial shoot with distinct, rigid, and commonly branched stems. [3] The leaves are inserted in a low spiral with divergences. The family Costaceae consists of 4 genera and approximately 200 species. The genus Costus is the largest in the family with about 150 species that are mainly tropical in distribution. [4] Human beings facing dental problems like Periodontal diseases and dental caries.

Problems like Periodontal diseases and dental caries are because of plaque producing bacteria and fungi, in the oral cavity. Dental treatments are not affordable especially in developing countries. [5]

MATERIAL AND METHODS

Collection of plant materials: The fresh and healthy leaves, roots and stem of the *Costus igneus* were collected from the Botanical Garden and was identified with the help of Department of Botany, PVP College, Pravaranagar. Then all parts of plant were washed thoroughly with running tap water followed by rinsing with distilled water and then the different parts were separated and cut into small pieces. Then all parts were shade dried at room temperature then pulverized into powder. Powdered samples were stored in an air tight container till further use.

Preparation of plant extract: The powder of leaves was extracted by using various solvents like Methanol, Ethanol, Distill Water, Acetone, chloroform and Petroleum Ether. About 5gm of each sample was extracted with 200 ml solvent by using Soxhlet apparatus. This extract was used for the phytochemical investigation by qualitative methods.

Qualitative Phytochemical Screening: The qualitative analysis of plant samples from various extracts was done by using various chemicals. Total 16 different types of qualitative phytochemical test were carried out. In that Carbohydrate Test, Tannins test, Saponin Test, Alkaloids Test, Flavonoids Test, Phenols Test etc. were included. [6]

Biosynthesis of Silver Nanoparticles: Synthesis of silver nano particles was done by using 25 ml of methanolic extract of leaves powder and 225 ml of 100 mM silver nitrate solution and incubates at 72 hours at room temperature. Characterization of synthesized nanoparticles was done using Ultraviolet-Visible Spectroscopy (UV-Vis Spectroscopy), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), X-ray diffraction Spectroscopy (XRD), and Fourier-Transform Infrared Spectroscopy (FTIR) analysis. [7]

Characterization of Silver Nanoparticles

1. Ultraviolet-Visible Spectroscopy (UV-Vis Spectroscopy): The UV spectrums will show highest peak in the 415 nm region that will be confirmed the presence of silver nanoparticles. [8]
2. Scanning Electron Microscopy (SEM): SEM allow surface and morphological characterization at both nanometer and micrometer scale. [9]
3. Fourier-Transform Infrared Spectroscopy (FTIR): FTIR is a technique used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. An FTIR spectrometer collects high-spectral-resolution data over a wide spectral range. [10]
4. X-ray diffraction Spectroscopy (XRD): The XRD will indicate the nanoparticles' structure and topography. The AgNP's formed by the reduction of Ag ions using the *Costus igneus* leaves solvent extract. [8]
5. Transmission Electron Microscopy (TEM): TEM is more accurate than SEM and is used to determine the exact size and shape of the synthesized AgNP's. [8]

Antibacterial Activity: Antibacterial activity test was carried out against five different species of oral bacteria like *E.coli* (MCC 2412), *Streptococcus salivarius* (MCC 4643), *Lactobacillus fermentum* (MCC 4458), *Staphylococcus aureus* (MCC 2408) and *Corynebacterium diptheria* (MCC 2058). These all bacteria are present in oral cavity of human being. Disc Diffusion Method was use for study of antibacterial activity of silver nanoparticles against these five species of bacteria. These bacterial cultures were taken from NCCS-NCMR, Pune. Theses bacterial cultures were inoculated over nutrient agar plates. These plates were autoclaved properly before inoculation. Sterile discs were prepared by using Whatman no. 1 filter paper and then autoclaved. Various concentrations of silver nanoparticles like 25,50,75 and 100 microgram per milliliter were prepared. Distilled water was used as negative control and Penicillin was used as standard drug and silver nitrate solution was used to check antimicrobial activity of silver nitrate. After that sterile filter discs were soaked in the test samples and then inoculated over nutrient agar plate. After incubation of 24 hours at 37^{o}C, the diameter of inhibition zones was calculated in millimeter. [11]

RESULTS AND DISCUSSION

Results for qualitative analysis: Mostly plant extracts of polar solvents like Methanol, Ethanol and DW shows maximum presence of secondary metabolites. Carbohydrates, Tannins, Saponins, Alkaloids, Flavonoids, Steroids, Quinones, Phenols were likely to be present mostly in Methanol, Ethanol and Distilled Water. Acetone extract was with carbohydrates, Tannins, Alkaloids and Flavonoids Chloroform and Pet Ether extracts were not having suitable number of secondary metabolites.

B. Biosynthesis of silver nanoparticles from plant extract: Methanolic-Plant leaves Extract was prepared by using Soxhlet. After that 10 mM silver nitrate Solution was prepared. Then 25 ml (1 mg/ml)

plant-solvent extract and 225 ml silver nitrate (100mM) were added to each other and kept for incubation at 37 ^{0}c over magnetic stirrer at 100 RPM in dark condition. After 72 hours the solution was turn into brown color. Again this solution was kept in hot air over for drying the solution. Fine silver nano particles were collected after complete evaporation of solution. Synthesized silver nanoparticles were stored in dark condition for further analysis [Fig 1].

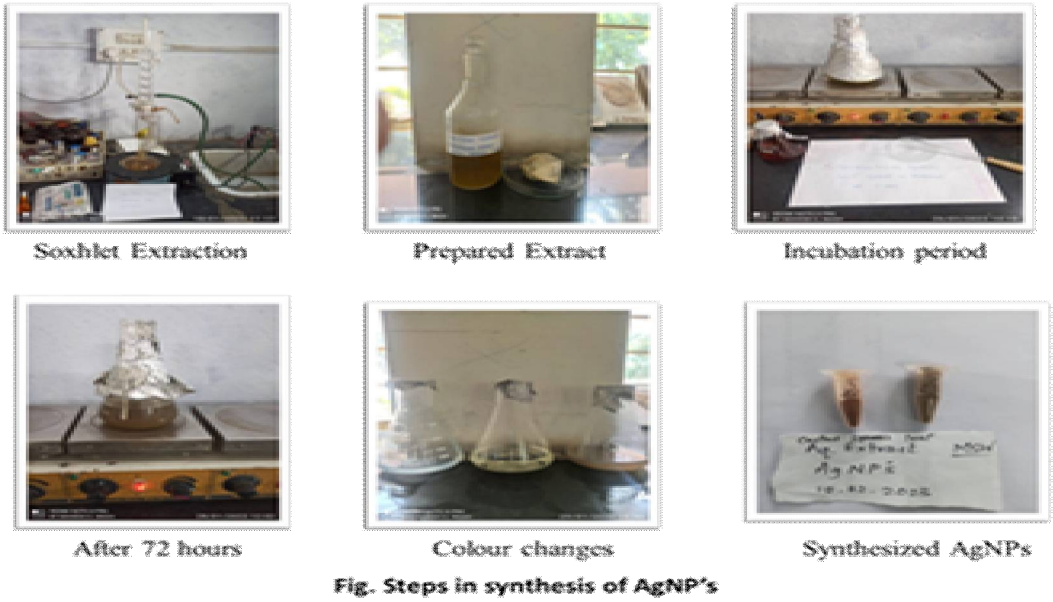

Fig1. Various steps in Silver Nanoparticles synthesis

Characterization of Silver Nano Particles

UV Vis Spectroscopy: UV Vis spectroscopy has great application in optical properties determination of solution. Basically, it works with the principle of Lamberts – Beers Law. A light is passed through the solution, and absorbance can be relate to concentration of solution. Peak around 400 nm range of UV light confirms the presence of silver nanoparticles. Data shows that maximum absorbance is at around 410 nm which ultimately indicates that the silver nanoparticles were successfully synthesized [Fig 2].

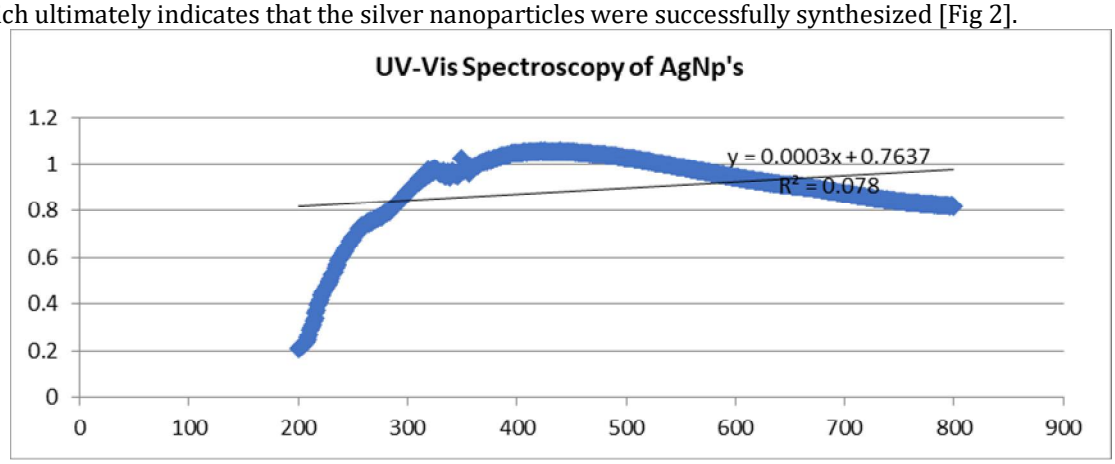

Fig 2. UV-Vis Spectrometer Graph of synthesized silver nanoparticles.

Scanning electron microscopy (SEM): Scanning electron microscopy analysis is used to ascertain the morphologies, sizes, and shapes of generated nanoparticles. When high-resolution images of a sample's surface are required, SEM provides them. The scanning electron microscope operates on the same principles as an optical microscope, but instead of measuring photons, it measures electrons that are scattered from the sample. An electric potential can accelerate electrons, allowing for a shorter wavelength than that of photons. As a result, the SEM can magnify images up to 200,000 times. By using ImageJ software, the average size of nanoparticles was found to be around 50 nm. The SEM-EDS file shows presence of silver material in the particles so it can be concluded that silver nanoparticles were successfully synthesized [Fig 3].

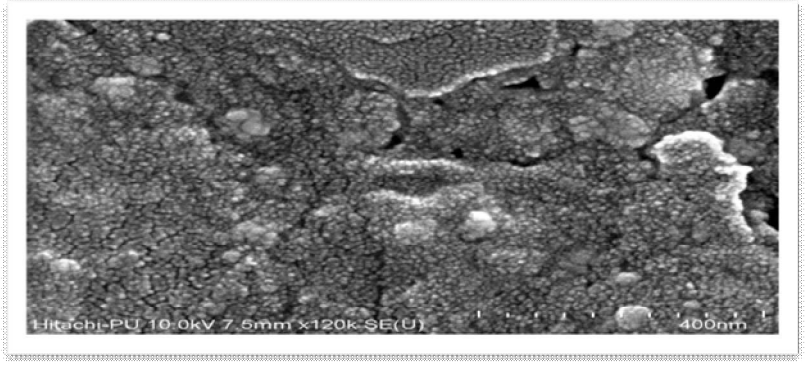

Fig3. Scanning Electron Microscopic Image of silver nanoparticles

Fourier-Transform Infrared Spectroscopy (FTIR): It assesses the relationship between infrared intensity and light wavelength in order to identify related functional categories and structural characteristics of nanoparticle-containing by the biological extracts. The computed spectra distinctly show the well-known dependency of the optical characteristics of nanoparticles. The results revealed unique peaks. The results of FTIR analysis of this study show different stretches of bonds shown at different peaks; 3154.10(–C≡C–H: C-H stretch with alkynes at terminal), 2917.68 (C–H stretch with alkanes), 2844.11(C–H stretch with alkanes), 1649.61(–C=C– stretch with alkanes), 1384.05(C–H rock with alkanes), 1020.10(C–N stretch with aliphatic amines), 824.90(C–Cl stretch with alkyl halides), 717.38(C–Cl stretch with alkyl halides), 458.94 cm^{-1}(C–Cl stretch with alkyl halides). The below figure is showing FTIR analysis of silver nanoparticles [Fig 4].

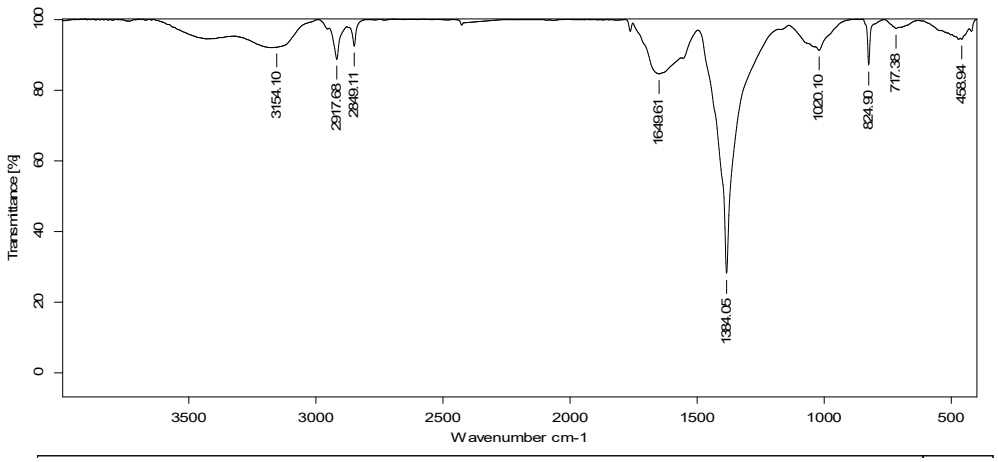

Fig 4. FTIR analysis of Silver Nanoparticles

X-ray diffraction Spectroscopy (XRD): One common method for determining the morphology and structure of crystals is X-ray diffraction. Depending on the number of constituents, the intensity increases or decreases. This technique, which determines the metallic nature of particles, provides information on the size, shape, and translational symmetry of the unit cell from peak positions. It also provides information on the electron density inside the unit cell, or the location of the atoms, from peak intensities. The XRD analysis of synthesized nano particles had 6 different peaks at 27.16, 32.32, 38.21, 44.38, 46.32 and 64.54 angles and the highest peak was found at 38.21. The crystallite size of Nano particles was calculated by Scherrer equation which is, $d=K\lambda/\beta\cos\theta$ Where k = 0.9 is the shape factor, λ is the X-ray wavelength of Ag Kα radiation (1.54 Å), θ is the Bragg diffraction angle, and β is the full width at half maximum (FWHM) of the respective diffraction peak [11]. After using this equation, average crystallite size was found to be 48.08 nm [Fig 5].

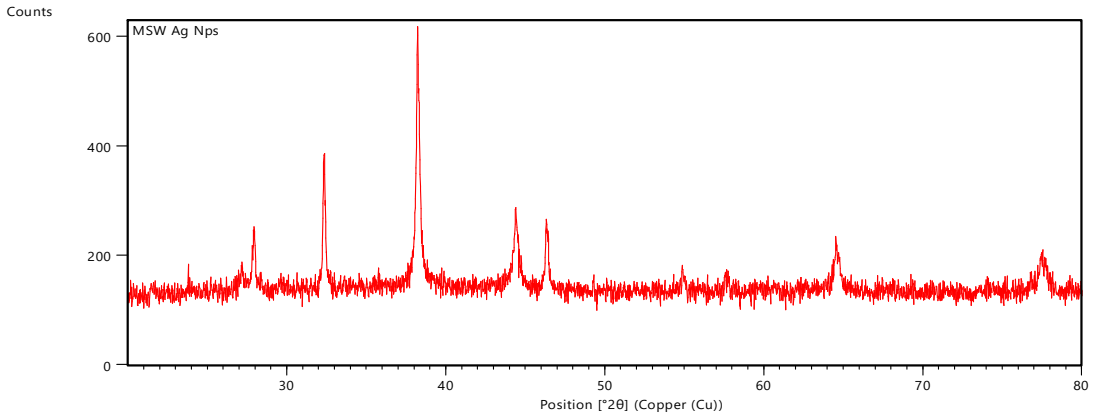

Fig 5. XRD analysis of Silver Nano particles

Transmission Electron Microscopy (TEM): Transmission electron microscopy is a type of microscopy in which an ultra-thin specimen is passed through and interacts with the electron beam as it does so. The interaction of the electrons that are sent through the specimen creates an image, which is then focused and enlarged to be seen on a fluorescent screen, a layer of photographic film, or by a sensor like a CCD camera. The average size of nanoparticles was found to be around 50 nm.

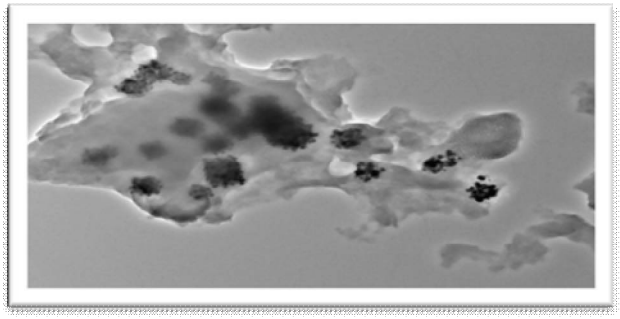

Fig 6. TEM Image of silver nanoparticles.

Results for Antibacterial activity:

Antibacterial activity test was carried out against five different species of oral bacteria like *E.coli*, Streptococci, Lactobacilli, Staphylococci and Corynebacteria according to S.C. Sati *et.al,* [11]. Penicillin was used as standard drug. It is observed that AgNP's showing great results against oral bacteria. For all of the bacteria, 100 microgram/ml concentration show maximum inhibition and 25 microgram/ml concentration show minimum inhibition. In the case of *E. coli* it shows 08±0.57 mm of zone of inhibition at 25 µg/ml concentration and 10±1.20 mm, 12±0 mm and 15±1.20 mm of zone of inhibition for the concentration of 50, 75 and 100 µg/ml respectively. For Streptococcus salivarius, zone of inhibition was 05±1.15 mm, 06±0.57 mm, 07±0.57mm, 09±0.33 mm for various concentrations shown in table. For Lactobacillus fermentum, it was 05±0.57mm, 06±0.33mm, 08±0.33mm, 10±1.15mm of zone of inhibition observed for concentration of AgNP's 25,50,75,100 µg/ml respectively. In the case of Staphylococcus aureus and *Corynebacterium diptheria*, zone of inhibition was found to be 07±1.15 mm, 08±1.52 mm, 10±0.57 mm, 12±1.20mm and 06±0 mm, 08±0.57 mm, 08±1.20 mm, 13±1.52 mm for concentration of AgNP's 25,50,75,100 µg/ml respectively. No zone of inhibition for Distilled water and zone of inhibition for Penicillin was 18±1.15 mm, 13±0.57mm, 20±1.15mm, 14±0.33 mm, and 24±0.33 mm against each different species of bacteria [Table. 1].

Table 1: Zone of Inhibition shown by various bacteria against silver nanoparticles

		Concentrations in microgram/ml						
Sr.No	**Species**	**25**	**50**	**75**	**100**	**N.C.**	**P.C.**	**$AgNo_3$**
1	***E.coli***	08±0.57	10±1.20	12±0	15±1.20	00	18±1.15	07±0
2	***Streptococcus salivarius***	05±1.15	06±0.57	07±0.57	09±0.33	00	13±0.57	05±1.52
3	***Lactobacillus fermentum***	05±0.57	06±0.33	08±0.33	10±1.15	00	20±1.15	05±0.57
4	***Staphyloccus aureus***	07±1.15	08±1.52	10±0.57	12±1.20	00	14±0.33	5±0.33
5	***Corynebacterium diptheria***	06±0	08±0.57	08±1.20	13±1.52	00	24±0.33	03±1.15

(Zone of inhibition in millimeter)

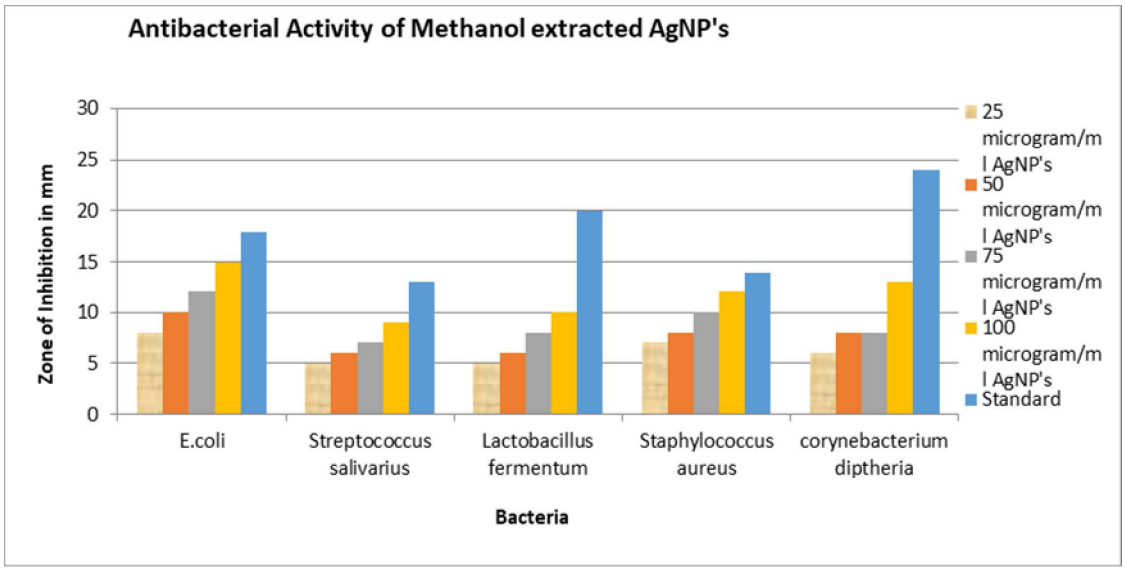

Fig. Antibacterial Activity of Methanol extracted AgNP's against oral bacteria

CONCLUSION

The phytochemical screening showed that phytochemicals such as alkaloids, carbohydrates, saponins, phenols, Quinones, flavonoids, phenols, annins , Cardiac Glycosides, Ninhydrin, Coumarins, Anthraquiones, steroids, Phlobatanins, Anthracyanine terpenoids and glycosides are present in the plant *Costus igneus*. From various characterization techniques it was found that Silver Nanoparticles were synthesized successfully. These nanoparticles are with great potential against various barcteria especially those were found in oral cavity of human being. So, in future these silver nanoparticles will play important role in human's health.

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