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Antibacterial and antioxidant effect of synthesized silver nanoparticles from aqueous leaf extract of *Sechium edule*

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Abstract
Objectives: The primary focus of this study is to present antibacterial and anti oxidant efficacy of Silver nanoparticles (AgNPs) synthesized from the aqueous leaf extract of *Sechium edule* (*S. edule*) plant by ultrasonication assisted method.
Methods: Silver nanoparticles were synthesized by ultrasonic assistance using aqueous leaf extract of *Sechium edule* (*S. edule*) as a reducing agent. Characterization of AgNPs was done by UV-Visible spectroscopy, FTIR, SEM and TEM. Antibacterial activity was analyzed by using disc diffusion method and 2, 2- Diphenyl 1- picryl hydrazyl (DPPH) scavenging assay for antioxidant evaluation.
Results and Discussion: Visual observation of brown color indicated the formation of AgNPs. Further confirmation was done by using UV-Visible, FTIR spectroscopy. Morphological analysis by SEM and TEM technique indicated the triangular pyramidal shape with 10 nm in size.
Conclusion: Results obtained suggest that AgNPs from aqueous extract of *Sechium edule* leaf extract show enhanced antibacterial and antioxidant efficacy.

Keywords: *Sechium edule*, silver nanoparticles, antibacterial activity, DPPH

1. Introduction
Nano science has blossomed due to its infinite applications which are growing very rapidly. It has found its way in the medicine we take, food we eat, chemicals we use and much more. In the creation of a nanostructure, nanoparticles are the most essential component and are very ordinary in the nature. Safety use of Nanoparticles has lead to the development of many new facets of research. Synthesis and fabrication of nanomaterials form an important part of nanotechnology. Hence synthesis of nanoparticles with unique and specific properties has attracted a great deal of attention from research groups [1]. Diverse sizes and shapes of nanoparticles depend on the synthesis procedure adopted. The use of the silver has been rediscovered and is coming back into mainstream medicine as an anti-microbial and anti-infective agent[2]. Silver in metallic state is readily oxidized by dissolved oxygen traces in the solution. Thus, the use of protecting or capping agents in the preparation of AgNPs is critical. The role of such protecting agents, however, goes beyond merely preventing surface oxidation. Therefore plant extract which act as both reducing and capping agent play a vital role in the synthesis of stable nanoparticles. This charge controls for the formation of complexes with biomolecules and eventually cell-nanoparticles interaction [3, 4].
Silver nanoparticles are the most effective among all because of its good antimicrobial efficacy against bacteria, viruses and other Eukaryotic micro organisms. They are most widely used as antimicrobial agent in textile industries for water treatment [5]. There is a possibility that AgNPs with nanosizes can freely permeate inside the cell membrane. Baker and colleagues also showed that AgNPs antimicrobial properties were directly related to the total surface area of the nanoparticles [6]. AgNPs within the size range of 10–100 nm have strong bactericidal potential against both Gram-positive and Gram-negative bacteria [7]. Silver nanoparticles of different shapes and sizes show unique interactions with bacteria and viruses [8]. AgNPs exhibit a multilevel antibacterial effect on cells and this taken together with the low rates of acquired resistance emergence in many bacterial species, AgNPs are particularly promising as antimicrobials. Silver readily binds to the bacterial cell wall and cell membrane and evidently inhibits the respiration chain [9].
Application of ultrasonication method helps in dispersing of materials in liquids in order to break particles agglomerates. This leads to smaller particles, increased size uniformity and

improves the material transfer at particle surface.
Sechium edule (*S. edule*) commonly called chayote is mainly used for human consumption. It has various medicinal uses. Both leaves and fruits have diuretic, cardiovascular and anti inflammatory properties. Infusions of leaves are used to dissolve kidney stone and in the treatment of arteriosclerosis and hypertension, fruits are used to alleviate urine retention [10]. The leaves contain the highest amount of flavonoids [11]. So the present study has focused on aqueous leaf extract of *Sechium edule* to synthesize AgNPs to evaluate its antibacterial and antioxidant efficacy by ultrasonication assisted method.

2. Materials and Methods
Silver nitrate (AgNO3) was purchased from Fischer; deionized water was used throughout the experiments.

2.1 preparation of aqueous leaf extract of *Sechium edule* plant
The fine powder of *S. edule* was obtained from dried leaves by using kitchen blender. 5 gram of powder was taken into a 250-ml conical flask with 100 ml of sterile distilled water and boiled for I hour at 80o C. The leaf extract was filtered through whattman filter paper (no.41) and was used as reducing as well as capping agent.

2.2. Phytosynthesis of silver nanoparticles
The plant extract-mediated bioreduction involves mixing the 10 ml aqueous leaf extract with 90 ml of 2mM AgNO3 in 100 ml Erlenmeyer flask. To study the optimum factors for the synthesis of silver nanoparticles, the experiments were carried out at different conditions of varying temperature, pH and Silver ion concentrations. The effect of these parameters on the synthesis of silver nanoparticles was monitored by UV-Visible spectrophotometer.

2.3 Characterization
2.3.1 UV-visible spectroscopy
Formation of AgNPs was confirmed by UV-Visible spectral analysis. The absorbance spectra were recorded using Ultra violet – Visible spectroscopy (specrtoscan UV-2600) at a wavelength of 300- 700 nm.

2.3.2 Fourier Transform infrared spectroscopy
Fourier Transform infrared spectroscopy (FT-IR) was performed on Bruker FTIR spectrophotometer in the range of 500-4000 cm-1 to detect the possible functional groups responsible for bioreduction and stabilization of silver nanoparticles.

2.3.3. Scanning Electron Microscopy (SEM)
Morphology of synthesized AgNPs were examined by Quanta 200 FEG to identify the shape of AgNPs

3.3.4 Transmission electron microscopy (TEM)
Particle size (TEM) and crystalline nature of nanoparticles (SAED) were analyzed using Transmission electron microscopy (TEM –JEO 2100).

2.4 Antibacterial Activity of AgNPs
Gram negative Escherichia coli (MTCC 443) and Gram positive *Staphylococcus aureus* (MTCC 96) bacterial pathogens were used for antimicrobial activity. The antibacterial activity was determined by well diffusion methods [12]. The test samples were dissolved in DMSO and loaded in to wells with various concentrations such as 25 µg/well, 50 µg/well, 75 µg/well and 100 µg/well. The streptomycin added well served as positive control. The solvent alone served as negative control. The antibacterial activity was determined by measuring the diameter of the zone of inhibition around the well using antibiotic zone scale (Himedia, Mumbai, India).

2.5. Antioxidant Activity: The ability of the samples to annihilate the DPPH radical (1,1-diphenyl-2-picrylhydrazyl) was investigated by the method described by (Blois 1958) [13]. Stock solution of compound was prepared to the concentration of 10 mg/ml. Different concentration of the extract (200, 400, 600, 800, 1000 µg) of sample were added, at an equal volume to methanolic solution of DPPH (0.1mM). The reaction mixture is incubated for 30min at room temperature; the absorbance was recorded at 517 nm. Ascorbic acid was used as standard control. The annihilation activity of free radicals was calculated in percentage inhibition according to the following formula

% of Inhibition = (Absorbance of control – Absorbance of Test)/Absorbance of control * 100

3. Results and Discussion
Visual formation of silver nanoparticles are well known by changing of color from yellow to brown and finally to colloidal brown indicating the formation of silver nanoparticles while adding leaf extract with silver ion solution due to the excitation of free electron in the nanoparticles [14].

3.1 Characterizations
3.1.1 UV-visible absorption study
The reduction of silver ion (Ag+) to metallic silver nanoparticles (Ag) was spectrometrically identified by double beam UV-visible spectrophotometer at different wavelength 300 – 700 nm. Strong Surface Plasmon Resonance (SPR) peak was observed at 414.5 nm (Fig.1).

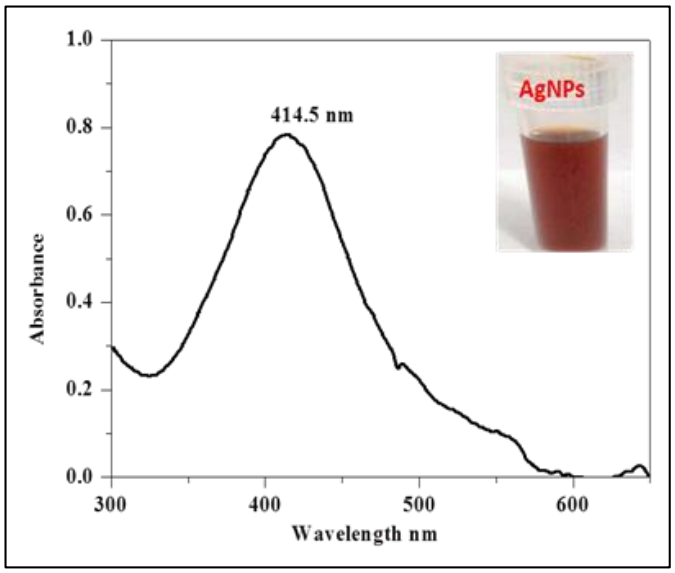


Fig 1: UV-Visible spectra of synthesized silver nanoparticles from *S. edule* leaf extract. Inset; photo of synthesized AgNPs

3.1.2 Fourier transforms infrared spectral analysis
FTIR measurements were carried out to identify the presence

of various functional groups responsible for bioreduction and stabilization of AgNPs. FTIR spectrum shows absorption band at 2922.18 cm^{-1} indicate the presence of capping agent responsible for the formation of AgNPs (Fig 2). Bands at 1737.23cm^{-1} was assigned for C-C stretching. The band at 1689.90 cm -1 corresponds to C-N and C-C stretching indicating the presence of proteins [15].

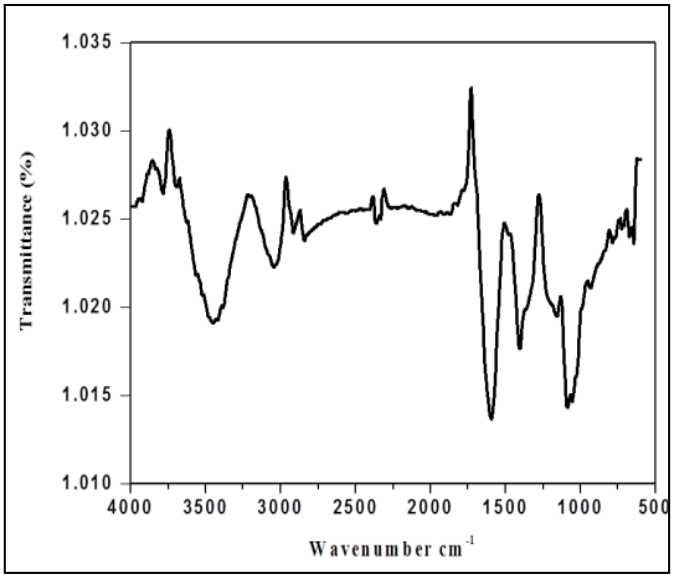


Fig 2: FTIR spectrum of synthesized silver nanoparticles

3.1.3 Scanning electron microscopy

SEM studies revealed triangular pyramidal shape of nanoparticles synthesized from *S. edule* aqueous leaf extract (Fig 3).

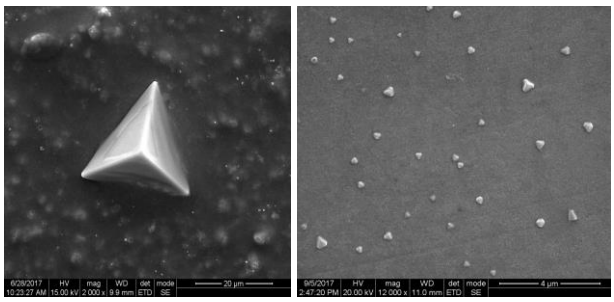


Fig 3: SEM images of synthesized silver nanoparticles from *S. edule* extract

3.1.4 Transmissions electron microscopy

TEM images at different magnifications provided the insight into the size of AgNPs (Fig.4). The size of AgNPS shown by TEM images exhibits the average diameter ranging from 10 nm to 17 nm.

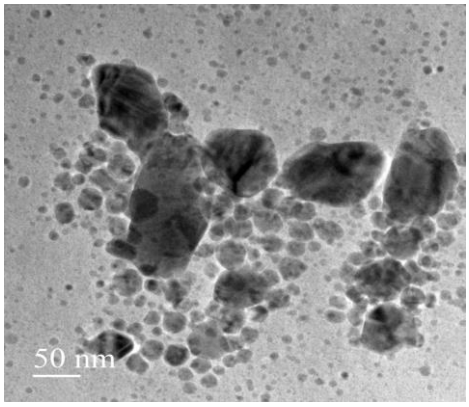
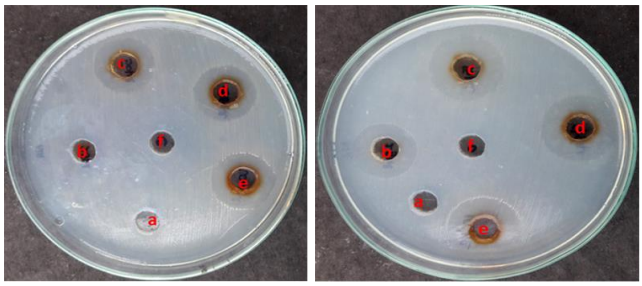


Fig 4: TEM images Of AgNPs from *Sechium edule*

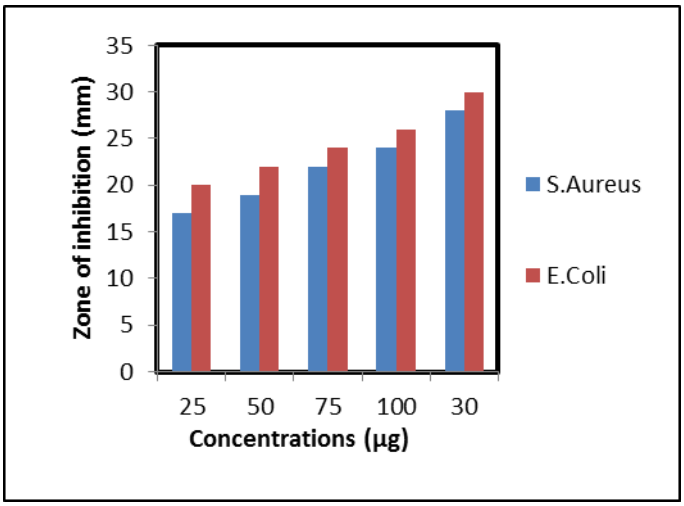
3.2. Antibacterial activity of AgNPs

The antibacterial activities of photosynthesized AgNPs were analyzed against gram positive *Staphylococcus aureus* and gram negative Escherichia coli (fig.6).The triangular pyramidal shape of AgNPs exhibited potential antibacterial activity. In the present study, zone of inhibition was found to be highest 26mm against *E.Coli* and lowest 17 mm against S.Aureus (table 1). Antibacterial activities were found to be increasing with the increase in concentration of AgNPs against control inhibition value of 30 mm Azithromycin (Graph 1). These findings are in agreement with earlier studies examined for antibacterial efficacy by Ghosh *et al* [16]. The antimicrobial efficacy of silver nanoparticles depends on the size of nanoparticles [17, 18]. The possibility could be that AgNPs with nanosize can freely permeate inside the cell membrane. Baker and colleagues also showed that antimicrobial properties of AgNPs were directly related to the total surface area of the nanoparticles [19]. AgNPs binds on the bacterial cell wall weakening it and eventually leading to its rupture. It promotes cell disruption via hydroxyl radicals and other reactive oxygen species by poisoning respiratory enzymes and components of the microbial electron transport system.



Staphylococcus aureus *Escherichia coli*

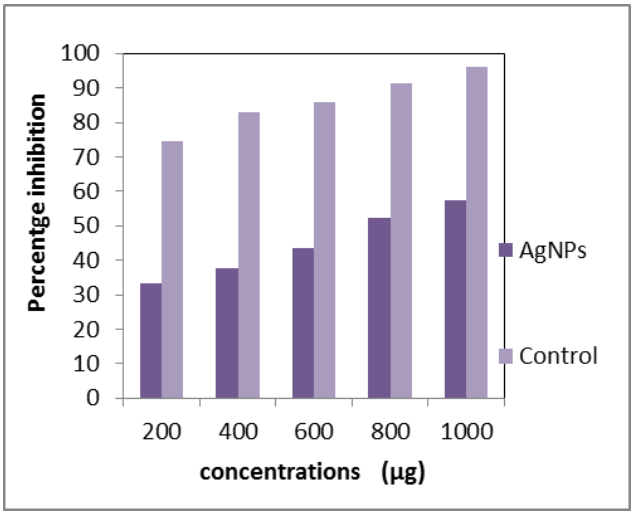
Fig 5: The antibacterial activity determination by well diffusion methods. (a: 0µl/well; b: 25µl/well; c: 50µl/well; d: 75µl/well; e: 100µl/well; f: 30µg/well (Azithromycin).



Graph 1: Bar diagram showing the zone of inhibition.

3.3 Antioxidant Assay

The antioxidant activity of synthesized silver nanoparticles was estimated by comparing the percentage inhibition of DPPH radicals with that of ascorbic acid. Silver nanoparticles showed potential antioxidant activity compared to ascorbic acid (Table 2). Calculated IC_{50} value for the synthesized AgNPS from *S. edule* was found to be 764.508µg/ml. Result obtained showed good antioxidant activity as compared to ascorbic acid as standard antioxidant. Flavonoids present in plant extract are the medium for excellent antioxidant capacity. Therefore it could be concluded that AgNPs from *S. edule* extract can be used efficiently in the production of potential antioxidant for commercial application.



Graph 2: Graphical representation of DPPH activity of synthesized silver nanoparticles

4. Conclusions

Silver nanoparticles synthesized from the aqueous leaf extract of *Sechium edule* was found to exhibit potential antibacterial and antioxidant property. Synthesized silver nanoparticles with triangular pyramidal shape were capable of penetrating into cell membrane making potential impact in different biological media. Based on the result obtained biosynthesized AgNPs from *Sechium edule* could be of immense use in the medical field for their efficient antibacterial and antioxidant function

Conflict of Interests: The author declares that there is no conflict of interests regarding the publication of this paper.

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Table 1: Antibacterial activity of synthesized AgNPs from aqueous extract of P. edulis

Concentrations (µg/well)	Zone of inhibition (mm) *Staphylococcus aureus*	Zone of inhibition (mm) Escherichia. Coli
25	17	20
50	19	22
75	22	24
100	24	26

Table 2: Antioxidant activity of synthesized AgNPs from aqueous extract of *P. edulis*

Concentrations (µg)	Percentage inhibition sample	Control
200	33.30	74.58
400	37.65	82.98
600	43.45	85.80
800	52.18	91.49
1000	57.56	96.14

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