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# GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM *MUNTINGIA CALABURA* FRUIT EXTRACT AND ITS BIOLOGICAL ACTIVITY

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**Keywords:**

*Muntingia calabura*, $AgNO_3$, Scanning Electron Microscopy, Energy Dispersive X-ray spectroscopy, DLS/ ZETA POTENTIAL analysis

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**ABSTRACT:** Synthesis of silver nanoparticles using fruit extract has been gaining considerable attention as an eco-friendly approach in green chemistry. The present work reports the synthesis of *Muntingia calabura* silver nanoparticles (McAgNPs) by the addition of an appropriate amount of *Muntingia calabura* fruit extract to the aqueous $AgNO_3$ solution under constant stirring at room temperature for 24 h. The addition of fruit extract leads to the formation of stable colloidal silver nanoparticles. The peak obtained at 460 nm using UV-VIS spectroscopy confirmed the formation of silver nanoparticles. Scanning electron microscopy analysis showed the spherical and oval morphology of the silver nanoparticles. Energy-dispersive X-ray spectroscopy (EDS) analysis indicated the abundance of silver nanoparticles in chemical composition. DLS/ZETA POTENTIAL analysis was applied in order to measure the particle size distribution and nature of the surface charge of the McAgNPs. Further, the antibacterial activity against the silver nanoparticles was established.

**INTRODUCTION:** Nanotechnology is the most promising leading science in modern key technology development. Green synthesis of nanoparticles aims at minimizing generated waste and implementing sustainable processes. Recently, green processes using fewer toxic substances have been emphasized in the development of nanotechnology for promoting environmental sustainability.

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Nanoparticles are in great interest due to their extremely small size and large surface area, which lead to both chemical and physical differences in their properties such as mechanical properties, biological and catalytic activity, thermal and electrical conductivity, optical absorption and melting point compared to bulk of the same chemical composition [1]. Most of the physical and chemical methods for synthesizing silver nanoparticles (AgNPs) are too expensive and are found to be responsible for various biological risks.

However, the Green synthesis of metal oxide nanoparticles has discovered the new possibilities of nanotechnology [2]. Synthesis of nanoparticles

using plant material extracts is the most preferred method as green and environmentally friendly for the synthesis of nanoparticles. Plants are widely distributed, easily available, much safer to handle and act as sources of several metabolites rich in pharmacological constituents who act as bio-reducing agents in the synthesis of nanoparticles. Plant-mediated synthesis of nanomaterials has been increasingly gaining popularity due to its eco-friendly nature and cost-effectiveness [3].

The plant extract, which acts as reducing and capping agents for nanoparticles synthesis, are more advantageous over other biological processes as they eliminate the elaborated process of culturing and maintaining the cell and can also be scaled up for large-scale nanoparticle synthesis. Moreover, plant-mediated nanoparticles synthesis is preferred because it is cost-effective, environment friendly, a single-step method for biosynthesis process, and safe for human therapeutic use [4]. The present study was conducted using the fruit of *Muntingia calabura*. The common names for the fruit are Calabur tree, Capulin, Jamaica cherry, Panama berry, Strawberry cherry, Pasito (Colombia), Nigua, Datiles, Bois ramier, Gasagase hannina mara, Seresa, Takhop farang. The fruit is native to the neotropics, from Mexico south to Bolivi, the Caribbean, Central America and Argentina. These are commonly found in South India and usually found in tropical lowland areas. It can grow in poor soil and can tolerate acidic, alkaline conditions and also drought, but it shows slow growth in saline conditions. Due to its high medicinal value the fruit was chosen for production of silver nanoparticles and their antimicrobial efficacy against selected pathogens was assessed.

## MATERIALS AND METHODS:

**Materials:** *Muntingia calabura* (Cherry) fruits were collected from different places of chikkamagalure, Karnataka, India. The bacterial strains (*Salmonella typhi, Staphylococcus pneumonia, Pseudomonas aeruginosa, Klebsiella sp.* and *Xanthomonas campestris*) were obtained from the Department of Biotechnology, IDSG Government College, Chikkamagalure, Karnataka, India. For antimicrobial activity and other biological activity, research-grade chemicals were used. Silver nanoparticles were analyzed with UV-Visible spectrophotometer, DLS, EDS and SEM.

**Methods:**

**Preparation of fruit Extract:** The *Muntingia calabura* were washed with distilled water, and the fruit was peeled off. The fruits were dried in a hot air oven at 50 °C for 48 hours. After 48 hours, the fruit was grinded using a pestle and mortar. Ten grams of dried fruit powder was mixed with 100 ml of double-distilled water and boiled for about 20 min on a plate in a stirred condition. The contents were filtered through Whatman no. 1 filter paper and filtrate obtained was stored in the refrigerator for further use.

**Synthesis of Silver Nanoparticles:** 20ml of aqueous fruit extract was added to 20 ml $AgNO_3$ solution and stirred in the rotor. A solution turned into dark brown color after 24 hours, indicating the production of silver nanoparticles. Thus produced AgNPs were centrifuged at 15,000 rpm for 20 min, and the pellet is collected and stored for further characterization studies.

**Characterization of Nanoparticles:** The preliminary detection of *Muntingia calabura* silver nanoparticles was carried out by visual observation of color change in the solution. The sample was subjected to optical measurement using UV-visible spectrophotometer by scanning the spectra in the range of 200-600 nm. Dynamic light scattering was used to determine the size distribution small particles in suspension. Scanning electron microscope (SEM) was used to determine morphology of nanoparticles. The Energy-dispersive X-ray spectroscopy (EDS) analysis was performed to quantify the elemental constituents of the sample.

**Properties of Synthesized Nanoparticles:**

**Phytochemical Analysis:** Chemical tests were carried out using standard procedure to identify phytoconstituents present in fruit extract. Tannins were tested by using ferric chloride by formation of greenish black colour (Braymer's test). Flavonoids were confirmed by the observation of yellow color formed after the addition of dilute ammonia and concentrated sulphuric acid. Libermann- Burchard test was followed for testing the presence of steroids. Salkowki's test-Reddish brown coloration of interface was observed between the fruit extract, chloroform mixture, and concentrated sulphuric acid confirms the presence of Terpenoids [5]. Glycosides were identified by the formation of

interface brown ring. Presence of Saponins were identified by the formation of froth with distilled water then emulsion by addition of olive oil to froth. The presence of Carbohydrates was confirmed by performing Molisch's, Fehling's and Benedict's tests by following standard procedure. Positive results for Fehling's and Benedict's test indicate the presence of reducing sugars [6].

**Antibacterial Activity:** The microorganisms used for antimicrobial studies were obtained from Department of Biotechnology, IDSG Government College, Chikkamagalure, Karnataka, India. The antibacterial activity of AgNPs was evaluated by using the agar well diffusion method against four different bacterial strains, namely *Salmonella typhi, Staphylococcus pneumonia, Pseudomonas aeruginosa, Bacillus subtilis,* and *Xanthomonas campestris*. The bacterium lawn was prepared and were inoculated with respective strains of bacteria. Wells were creating, and freshly prepared AgNPs were loaded into the wells with concentrations of 15µl, 25µl, 50µl, 75µl and incubated at 37 °C for 24 hrs after 24 hrs and zone of inhibition were measured.

**DPPH Free Radical Scavenging Assay:** The DPPH free radical scavenging activity was conducted according to standard protocols [7]. About a 0.2AgNP solution was taken at different concentrations (20-100µl) and was mixed with 0.8 ml of Tris-HCl buffer Then, a 1ml DPPH solution was added to the above each mixture [8]. The mixture was stirred and incubated for 30 min in room temperature. The absorbance of the solution was measured at 517 nm using UV-Visible Spectro-photometer. All these assays were carried out in triplicates with standard synthetic antioxidant BHA (Butylated hydroxyanisole). Blank was prepared without the addition of DPPH, and for control, 0.2ml of methanol without fruit extracts was added. The lower absorbance of the reaction mixture indicated a higher percentage of scavenging activity [9].

Percentage of DPPH radical scavenging determined using the formula:

%inhibition = {(control OD-sample OD) / control OD} × 100

**Reducing Power Assay:** The reducing power assay was conducted based on protocol [7]. About 1ml of each extract solution was mixed with 2.5ml phosphate buffer (pH 6.6) and 2.5ml of (1%) Potassium ferricyanide. This mixture was incubated at 50 °C for 20 min. About 2.5 ml (10%) trichloro-acetic acid was added to the mixture, which was then centrifuged at 3000rpm for 10 min.

Finally, 2.5ml of the supernatant solution was mixed with 2.5ml of distilled water and 0.5ml (0.1%) ferric chloride allowed to stand for 10min. The absorbance was measured at 700nm in UV-Visible Spectrophotometer. Ascorbic acid was used as standard [10].

## RESULTS AND DISCUSSION:

**Synthesis of Silver Nanoparticles:** Aqueous silver ions were reduced to McAgNPs after the addition of *Muntingia calabura* fruit extract. These mixtures were incubated initially, the mixture showed whitish-yellow colour, ultimately after 24h mixture turned into dark brown colour. This colour was due to surface Plasmon resonance of deposited silver nanoparticles. The preparation of fruit extract and synthesis of McAgNPs were shown in **Fig. 1** and **2**.

Mamta Devi *et al*., (2020) synthesized silver nanoparticles and separated them by centrifugation at 15,000 rpm for 20 min [11]. The process was repeated by dispersion of pellets in water to obtain colorless supernatant, and AgNPs formation was confirmed by observing color change from colorless to dark-brownish, similar to the present work. Mandar Medhi *et al.,* (2014) synthesized AgNPs from *Averrhoa carambola* fruit extract and confirmed the formation of AgNps by colour change of filtrate from pale yellow to dark brown after reduction, similar to present work [12].


**FIG. 1: A- *MUNTINGIA CALABURA* TREE, B- RIPED FRUITS**


**FIG. 2: A- PEELED FRUIT, B- SAMPLE PLACED IN HOT AIR OVEN, C- DRIED SAMPLE, D- BOILED SAMPLE, E- CENTRIFUGED SAMPLE, F- PELLETS**


**FIG. 3: A- AQUEOUS FRUIT EXTRACT; B- 20 ml OF $AgNO_3$ + AQUEOUS FRUIT EXTRACT; C- STIRRED SAMPLE B AFTER 24 h SHOWING DARK BROWN INDICATES THE PRODUCTION OF SILVER NANO PARTICLES**

**Characterization of Synthesized Nanoparticles:**

**Visual Observation:** After 24 hrs of incubation, the color changes were observed in fruit extract where *M. calabura* showed dark brown color indicated synthesis of $AgNO_3$ nanoparticles **Fig. 3C**.

**UV- Spectrophotometer Analysis:** In the present study McAgNPs showed UV absorption peak at around 460nm, and the peak was intense, indicating the formation of AgNPs **Fig. 4**. Kaushik Roy *et al.*, (2013) observed the UV-Vis absorption spectrum of silver nanoparticles in the presence of grapefruit extract, solution which showed a peak at 450 nm similar to the present work [13]. Mane Gavade *et al.*, (2015), observed UV absorption spectra exposed from the reaction of reduction of silver ions by carambola fruit extract, which has dispersed nanoparticles with broadening peaks in the absorbance band at the wavelength of 448 nm, which is also similar to the present work [14]. Mamta Devi *et al.*, (2020), observed the reduction of $Ag^+$ ions to $Ag^\circ$ by *A. marmelos* fruit extract, which was monitored using UV–Vis spectrophotometer by recording the absorption spectra at the wavelength of 300–700 nm [15]. UV–Vis spectral analysis showed that the maximum absorption was at 436 nm, which confirmed the formation of McAgNPs in the solution similar to the present work.

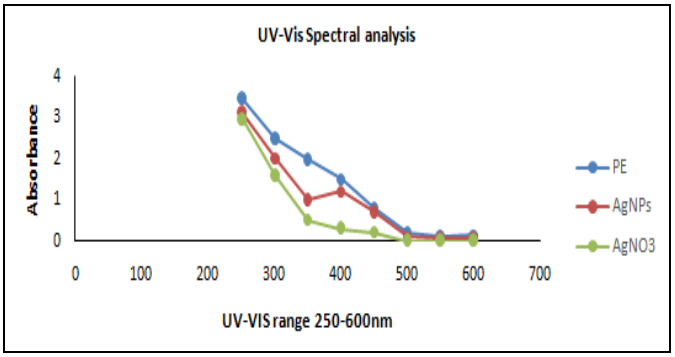


**FIG. 4: UV-VIS ABSORPTION SPECTRUM OF McAgNPs FROM *M. CALABURA* FRUIT**

**SEM Analysis** The AgNPs synthesized from *M. calabura* fruit extract showed oval and spherical when analyzed using SEM **Fig. 5**. It is observed that AgNPs of different shapes were obtained from Cherry fruit extracts which are being used as reducing and capping agents. This may be due to presence of various biomolecules in the fruit extract interacting with silver nanoparticles in different quantities as capping and stabilizing agents. Shani *et al.*, (2018) synthesised silver nanoparticles using *Enicostemma axillare* leaf extract where they found the SEM images similar to the present work and showed spherical nanoparticles [16]. Femina *et al.*, (2019) observed that the clustered AgNPs form well-defined spherical microstructures. The aggregated microspheres are coated by a thin film composed of various phytochemicals contained in the mucilage or gum of aqueous extract of clammy cherry in SEM as similar to present work [17]. Razi *et al.*, (2015) Synthesized silver nanoparticles from the extract of Oak fruit Hull where they obtained spherical nanoparticles, which was confirmed using SEM [18].

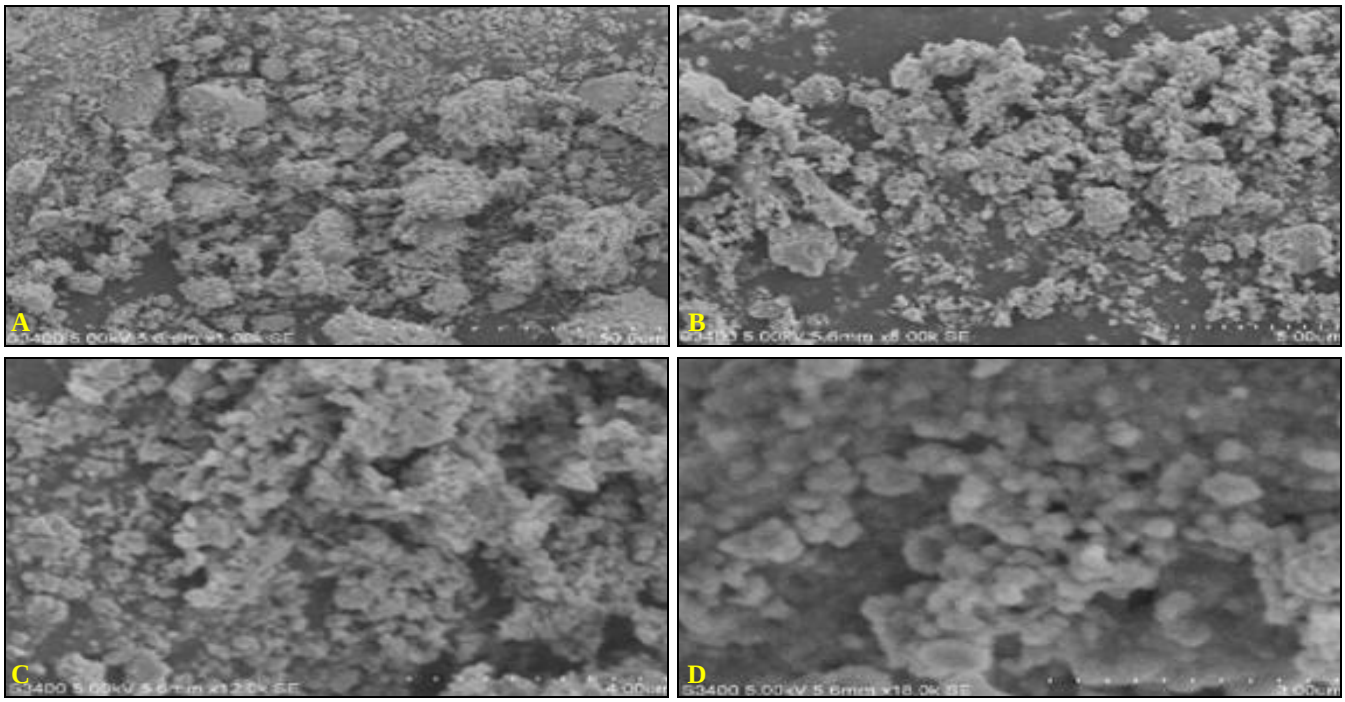


**FIG. 5: SEM IMAGES OF McAgNPs OBTAINED FROM *MUNTINGIA CALABURA* FRUIT EXTRACT**

**EDS Analysis:** The EDS spectra obtained from the McAgNPs powder showed the prominent peaks in EDS indicated the abundance of silver in the composition of metal nanoparticles without any contamination **Fig. 6**. The strong optical absorption peak at 3keV was due to surface Plasmon resonance induced by silver in the nanocrystallite nature. Umoren *et al.*, (2014) synthesised silver nanoparticles using apple (*Malus domestica*) fruit extract where they found strong optical absorption peak at 3keV similar as present work [17]. Fernand *et al.*, (2019) synthesized silver nanoparticles using *Solanum mammosum* fruit extract observed the EDX spectra that shows three characteristic peaks of silver similar to present work under 3keV due to surface plasmon resonance. Silver abundance is about 26.9% normalized mass percent [18]. The other elements are Na (22.7%), Mg (4.6%), S (6.8%), K (36.4%) and Ca (2.5%). Abiola *et al.*, (2019) Synthesized silver nanoparticles using terrestrial fern (*Gleichenia pectinata* wild) and the reduced silver nanoparticles were subjected to EDX analysis with an optical absorption characteristic peak at 3 keV similar to present work [19].

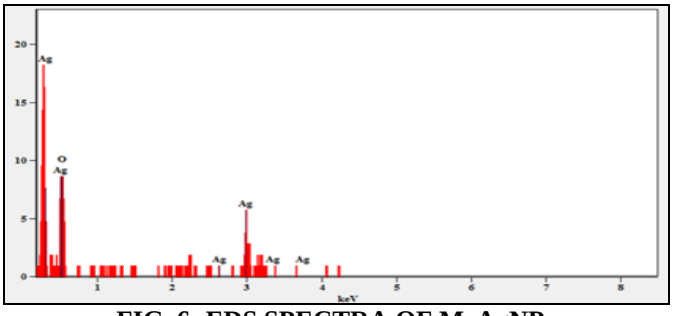


**FIG. 6: EDS SPECTRA OF McAgNPs**

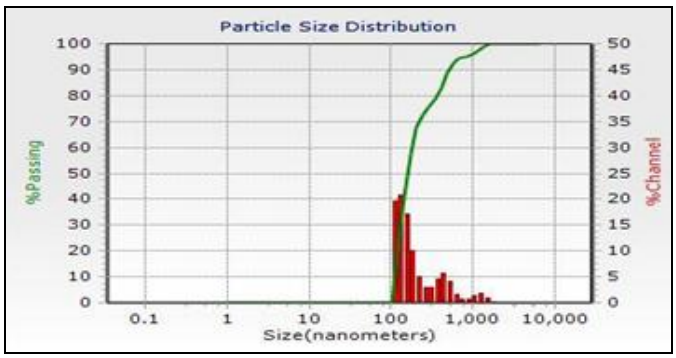


**FIG. 7: PARTICLE SIZE DISTRIBUTION OF McAgNPs OBTAINED FROM DYNAMIC LIGHT SCATTERING ANALYSIS (DLS)**

**DLS Analysis:** Dynamic light scattering analysis (DLS) was used to study the particle size distribution of the McAgNPs. The results indicated that the McAgNPs obtained from *Muntingia calabura* fruit extract was in the range between 96-793 nm **Fig. 7**. The wide range of particle size distribution was due to agglomeration and improper distribution of colloidal McAgNPs in their hydrodynamic state. Zhang *et al.*, (2016) synthesized nanoparticles and characterized the nanoparticles with the particle size range from 2-500 nm [20]. Mamta Devi *et al*., (2020) synthesized silver nanoparticles using methanolic fruit extract of *Aegles marmelos* and obtained DLS graph of methanolic extract of *Aegle marmelos* revealed that the particle size of AgNPs was in the range of 10–200 nm, similar to the present work [10]. Razi *et al.*, (2015) synthesized silver nano-particles using extract of oak fruit hull and they observed the average size of nanoparticles is 40 nm by performing DLS analysis similar to present work [16].

**Applications of Synthesized Nanoparticles:**

**Phytochemical Test:** The synthesized nano-particles were subjected to the phytochemical test, and all the tests showed positive **Table 1** and **Fig. 8A** and **B**. Krishnaveni and Dhanalakshmi (2014) studied phytochemical analysis in *M. calabura* fruit extract. The present work showed a positive result for all the tests as similar for work [21].

**TABLE 1: PHYTOCHEMICAL ANALYSIS**

| S. no. | Experiment | Result |
|---|---|---|
| 1 | Tanins | Positive |
| 2 | Flavonoids | Positive |
| 3 | Steroids | Positive |
| 4 | Terpinoides | Positive |
| 5 | Glycosides | Positive |
| 6 | Saponin | Positive |
| 7 | Alkaloids | Positive |
| 8 | Carbohydrate- | |
| | Molisch's | Positive |
| | Fehling's | Positive |
| | Benedict's | Positive |
| 9 | Proteins | Positive |

**FIG. 8A: TANNIN TEST, FLAVONOID TEST, STEROID TEST, TERPENOID TEST, GLYCOSIDE TEST, SAPONIN TEST**

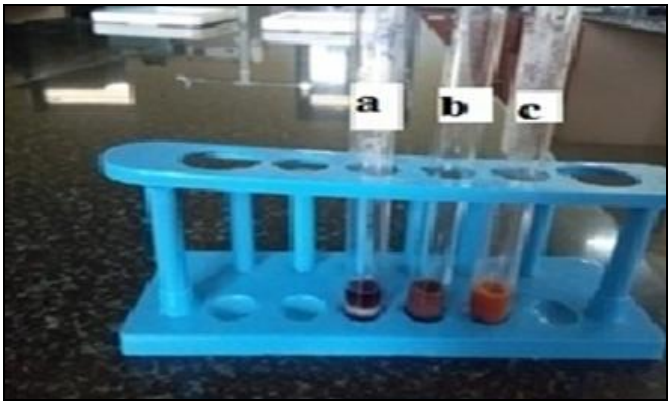


**FIG. 8B: CARBOHYDRATE: A- MOLISCH'S TEST, B- FEHLING'S TEST, C- BENEDICT'S TEST**

**DPPH Radical Scavenging Activity:** Positive DPPH tests demonstrated that McAgNPs are free radical scavengers. The DPPH scavenging assay exhibited effective inhibition activity of McAgNPs **Fig. 9**. The DPPH activity of the nanoparticles was found to increase in a dose-dependent manner. However, the McAgNPs exhibited more inhibition with more than 60% scavenging activity. Reducing powers of the extracts were found to increase with increasing concentrations. The ferric ion reducing activities of McAgNPs didn't differ from DPPH free radical scavenging activities. Interestingly, **Fig. 7** shows the DLS pattern of the biologically synthesized nanoparticles after separating them

from the reaction mixture by ultracentrifugation. The DPPH Radical Scavenging activity clearly shows that the size range of nanoparticles varied within 96-578 nm. The procedure was carried using the protocol of Makari *et al.*, (2008) the result of present work almost similar to the previous work [7].

**TABLE 2: RADICAL SCAVENGING ACTIVITY**

| Concentration (µg/mL) | % Activity |
|---|---|
| 5 | 5 |
| 10 | 7.98 |
| 20 | 13.57 |
| 40 | 16.94 |
| 60 | 23.45 |
| 80 | 28.02 |
| 100 | 36.87 |

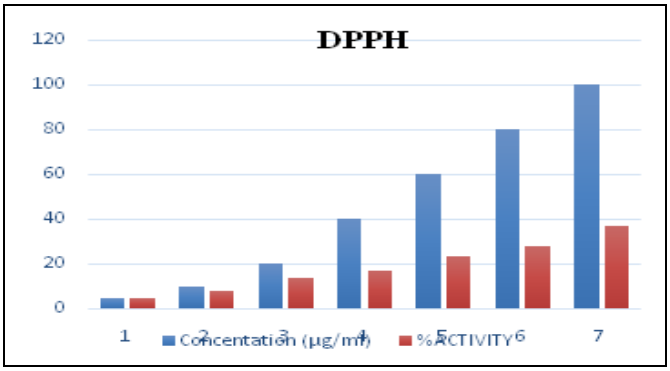


**FIG. 9: DPPH ASSAY OF McAgNPs**

**Reducing Power Assay:** The dose-response for the reducing powers of the McAgNPs **Fig. 10**. Reducing powers of the extracts were found to be rise with an increase in concentrations. The ferric ion reducing activities of the McAgNPs did not differ specifically from their DPPH free radical scavenging activities **Fig. 9**.

The reducing power was consistently higher than those obtained for DPPH scavenging. Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. Compound with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes [22]. So, they can act as primary and secondary antioxidants.

**TABLE 3: REDUCING POWER ASSAY OF McAgNPs**

| Concentration (µg/mL) | % Activity |
|---|---|
| 5 | 1.25 |
| 10 | 3.265 |
| 20 | 9.65 |
| 40 | 13.56 |
| 60 | 18.47 |
| 80 | 26.34 |
| 100 | 36.74 |

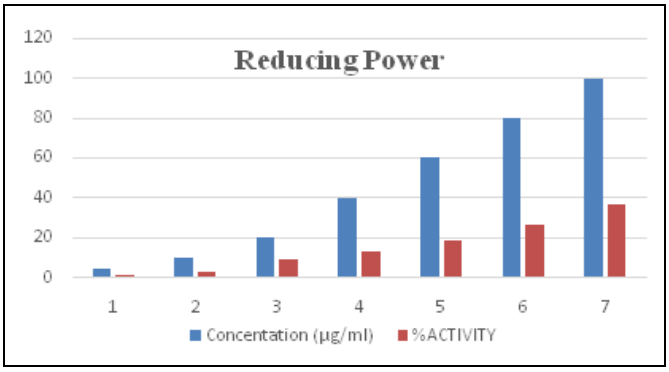


**FIG. 10: REDUCING POWER ASSAY OF McAgNPs**

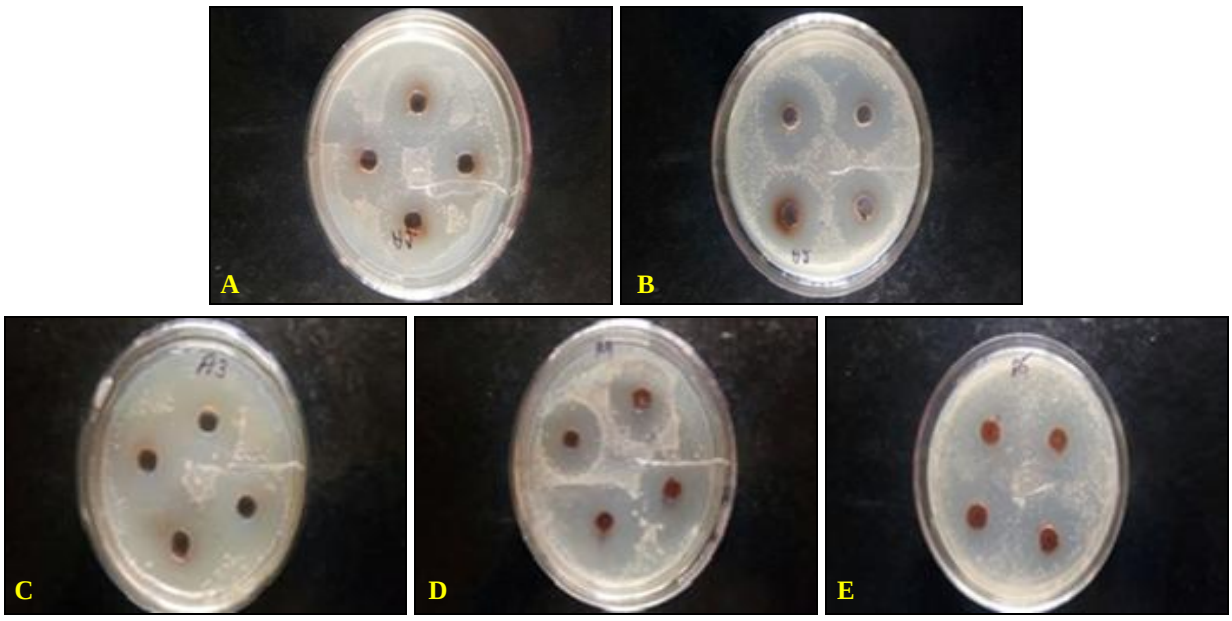


**FIG. 11: ANTIBACTERIAL ACTIVITY OF McAgNPs AGAINST, A- *SALMONELLA TYPHI*, B- *STEPTOCOCCUS PNEUMONIA*, C- *PSEUDOMONAS AERUGINOSA*, D- *BACILLUS SUBTILIS* E- *XANTHOMONAS CAMPESTRIS***

**Antibacterial Assay:** The antibacterial activity of McAgNPs was analyzed against *Salmonella typhi, Steptococcus pneumonia, Pseudomonas aeruginosa, Bacillus subtilis,* and *Xanthomonas campestris* by standard agar well diffusion method. The culture plates treated with McAgNPs exhibited moderate antibacterial activity. The zone of inhibition by McAgNPs was found to be 1.6, 1.5, 1.7, 1.5, 1.5mm, respectively. The zone formation **Fig. 11** indicates the potential antibacterial activity of McAgNPs against the following organisms.

**CONCLUSION:** The present study describes the synthesis, characterization, and antimicrobial activity of silver nanoparticle using *M. calabura*. The used biogenic method here is nontoxic, environmentally friendly, simple, and low cost and has no toxic chemicals. The results confirmed that cherry fruit extract plays an important role in the reduction and stabilization of silver nanoparticle. The brown color appeared after incubation of the mixture for 24 h. UV-Visible spectroscopy showed absorption maxima at 430 nm. The size of the Nanoparticle varied from 96-578 nm. The bio-produced AgNP were characterized using SEM, DLS. In the present study, green synthesized AgNPs showed antibacterial and antioxidant activity; this may be useful in a wide variety of applications in pharmaceutical, biomedical fields, industrial appliances like bandage, food, and water storage. Further research is to be adapted for finding better models in this research.

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**CONFLICTS OF INTEREST:** There is no conflict of interest

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