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CHARACTERIZATION OF ANTIMICROBIAL AND ANTIOXIDANT STUDY OF *AVERRHOA CARAMBOLA* AND SYNTHESIS OF SILVER NANOPARTICLES

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Abstract: **Starfruit botanically known as *Averrhoa carambola* and it belongs to family Oxalidaceae, grows in tropical and subtropical regions throughout the world. The Starfruit can be used raw as vegetable and ripe as fruit. The Starfruit are sweet tasting fruit that possesses high nutritional value. This study was aimed to understand the antimicrobial, antioxidant activities and nutritional content of tropical fruits *Averrhoa carambola* (starfruit). The edible parts of the fruits were analyzed for different phytochemicals and antioxidant activity like phenolics, flavonoids, DPPH, and ascorbic acid were found in starfruits. In the present study, information regarding the extracts of *Averrhoa carambola* were evaluated to investigate antimicrobial activity against Gram positive and Gram negative bacteria by well diffusion method. Phytochemical were present such as saponins, phenols, tannins, steroids, proteins, carbohydrates and flavonoids as well as measured nutrients content like proteins, carbohydrates, vitamins, minerals. In this study well defined silver nanoparticles were synthesized by using carambola fruit extract. The synthesized NPs were analyzed by ultraviolet spectroscopy. The antimicrobial activity of the synthesized AgNPs was obtained against *Escherichia coli* and *Pseudomonas aeruginosa* by agar well diffusion method.**

Keywords: Averrhoa carambola, **Antimicrobial, Antioxidant, Phytochemicals, Nanoparticles.**

1. INTRODUCTION

Averrhoa carambola, commonly known as starfruit bears a grt significance in traditional medicine. Five-lobbed fleshy, yellow-greenish, edible fruits of *Averrhoa carambola* of Oxalidaceae are native of South-East Asia and it cultivated in some parts of India. *Averrhoa carambola* is a small, slow-growing evergreen tree with a short-trunk or a shrub. The compound leaves are soft, medium-green, they are spirally arranged around the branches in an alternate fashion. The fruits are showy with an oblong shape. The fruits have a thin, waxy skin that is orange-yellow colored. A yellow or green tropical fruit with smooth skin and five pointed, curved parts, making a star shape when you cut through it. The juicy fruits are yellow inside when ripe and have a crisp texture and when cut in cross-section are star shaped. Starfruit generally stored at room temperature for maximum of two to five days. Carambola are not too a particular soil, it grows well on sand, heavy clay or limestone and in rich loam.

The fruits are good source of antioxidants and used traditionally in mouth ulcers, toothache, nausea, diarrhea, ascites etc. Pharmacological investigations on *Averrhoa carambola* have demonstrated anti-inflammatory, anti-microbial, anti-fungal, anti-tumor and anti-ulcer activities. In addition, the plant possesses hypocholesterolemic investigations have shown the presence of Phytochemical such as saponins, tannins, alkaloids and flavonoids. Anti-bacterial activities of extracts of different plants against various microorganisms have been reported. Plants have always been a significant source of natural products having therapeutic potential. In India, rich resource of wild or underutilized fruits is available. These underutilized fruits have recently drawn attention of many researchers as a natural source of treatment for curing various

diseases. Some studies on underutilized fruits have claimed them to be better sources of nutrients. Such underutilized tropical fruits provide limitless opportunities for screening of novel drugs. Phenolics possess a wide spectrum of biochemical activities such as antioxidant, anti-mutagenic, anti-carcinogenic. Flavonoids are the largest group of naturally occurring phenolic compounds, which occurs in different plant parts both in free state and as glycosides. The Ascorbic acid levels of the starfruit is believed to be responsible for its sweet or sour taste.

Present study has been aimed to understand the characterization of antimicrobial, nutritional content and antioxidant study of tropical *Averrhoa carambola* (starfruit). we were measured the antimicrobial, antioxidant activity or nutritional content in *Averrhoa carambola* (starfruit) as well as In this study well defined silver nanoparticles were synthesized by using carambola fruit extract. we were reporting the synthesis and characterization of silver nanoparticles by using carambola fruit extract. The synthesized AgNPs were characterized by using characterization techniques and tested against several pathogenic microorganisms.

2. MATERIALS AND METHODS

2.1 Sample collection and preparation

Fruits of *Averrhoa carambola* (starfruits) were collected in September 2017, from markets of Dadar, Mumbai, Maharastra. The fruits were cleaned. The edible portions of the fruits were dried at 50 ºC for 2-3 days, then separately grind into fine powder using a mechanical grinder. The powder was kept in dark coloured glass bottles and subsequently used.

2.2 Preparation of starfruit extract:

5 g of dry powder was mixed in 50 ml sterile methanol (80%). Keep 2 hours at room temperature using orbital shaker at 150 Rpm. Then extract was filtered through Whatman No.1 filter paper. After that extract was evaporated using vacuum rotary evaporator for purpose of dryness and stored in glass vials. These crude solvent extract were diluted with 10% dimethyl sulphoxide (DMSO) which are to be used as negative control.

2.3 Antimicrobial activity:

2.3.1 Test Microorganisms

Bacterial strains were selected for the antimicrobial activities of these fruits study. The strains were used in *Salmonella typhimurium*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus cereus*, All bacterial cultures were maintained on tryptic soy agar (HiMedia) and subcultured regularly. The fungal strain *candida albicans* was grown on Sabouraud dextrose agar (HiMedia). This culture was incubated at 35-37 ºC for 24 hours.

2.3.2 Well diffusion bioassay

Standardized inoculum suspension (0.1ml) of each bacterial strain was spread on Muller Hinton Agar plates with a sterile bent glass rod spreader. Then punch agar plate with a sterile cork borer of 4 mm size and then pour 100 µL of each sample with micropipette in the bore. Allow plates to stand for 30 min. Then incubate the plates at 37°C for 24 h. After incubation measured the zone of inhibition in (mm).

2.4 Phytochemical analysis:

2.4.1) Test for carbohydrates (Fehlings test): 1 ml of Fehling's A and 1ml of Fehling's B solution was added in 0.5 mg of extract and boil it in a water bath. The formation of yellow or red precipitate indicates the presence of reducing sugar.

2.4.2) Test for Saponins (Foam test): Diluted the methanolic extract with distilled water and shake well in a graduated cylinder for 15 min. The persistent foam to a length of 1cm indicates the presence of Saponins.

2.4.3) For steroids and sterols (Salkowski's test): Dissolved 2 ml of methanolic extract in 2 ml of chloroform and 2ml of concentrated sulphuric acid along the sides of the test tube. The upper layer turns red and lower layer turns yellow with fluorescence, indicates the presence of the steroids and sterols compound in the extract.

2.4.4) Test for tannins (lead acetate test): To 2-3 ml of extract, was added in 0.5 ml of 1% lead acetate and the formation of white precipitate indicates the presence of tannins and phenolic compounds.

2.4.5) Test for amino acid (Ninhydrin Test): To a small amount of extract add a few drops of 5% Ninhydrin solution. Then heat the solution in a water bath for 10 mins. Yellow color appeared if amino acids are present.

2.4.6) Test for Terpenoids: Add 4 mg of extract treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then add concentrated solution of sulphuric acid slowly and red violet color will observe for terpenoid.

2.4.7) Test for flavonoid: Crude extract mix with 2ml of 2% solution of NaOH. An intense yellow color formed which turn colorless on addition of few drops of diluted acid which indicated the presence of flavonoids.

2.4.8) Test for phenol: Crude extract was mixed with 2ml of 2% solution of $FeCl_3$.A green or black coloration indicates the presence of phenols.

2.5 Nutrient content:

2.5.1 Ascorbic acid content

In this assay, the yellow colour of test solution changes to various shades of green and blue, exhibit a broad light absorption at 700nm. Calibration curve was prepared by adding 0,1,2, 5, 8, and 10ml of ascorbic acid stock solution into 100ml volumetric flask and dilute to volume with water. Solution concentrations were 0,100, 200, 500, 800, and 1000ug/L ascorbic acid, the effective range of the assay. From sample pipetteing the separate tubes and to it 2.5ml phosphate buffer, and then add 2.5 ml of potassium ferrocyanide solution solution keep at 50^oC for 20mins, then 2.5 ml of trichloroacetic acid add and centrifuge at 10 min., for 3000 rpm, 1.25 ml supernatant was collected and then added 1.25 ml distilled water and 0.25ml of ferric chloride. Measure absobance at 700 nm against blank and plot the graph.

2.5.2 Phosphorous content

Soluble phosphorous is converting to heterocomplex in the presence of molybdate ion, which reduce to in presence on stanous chloride to give a blue colour, it was measured At 660 nm. concentration range between 200 ug/ml, stock used as phosphate solution. In 1ml of sample was added 4ml of distilled water then add 10ml of ammonium molybdate and 0.125 stannous chloride and check absorbance at 660nm. Plot a graph of optical density vs concentration of phosphorous.

2.5.3 Carbohydrate content

Estimation of carbohydrate was used as standard glucose solution range between 100 to 1000 ug/ml. Distilled water used as a diluent, take a 1ml sample in tube add 1ml diluent, then added 1 ml DNSA keep the tube in boiling water bath for 10 min., after boiling 10 ml of diluent was added. Then measured the absorbance at 530 nm. Plotting the graph optical density vs concentration of glucose.

2.5.4 Protein content

Bovine serum albumin standard range between 40 to 200 ug/ml. Diluent used as a distilled water. 1 ml of sample added 5 ml Alkaline $CuSO_4$ then incubate at room tempreture for 10 min. After incubation add 0.5 ml of folin Ciocalteau reagent (1:2 diluted) and mixed at once. Then incubate at room temperature for 30 min., and measured absorbance at 660 nm. Plot the graph optical density vs concentration and calculate the protein content.

2.5.5 Iron:

Prepared the iron stock, 2.41g of ferric ammonium sulphate powder added in 25 ml conc.H_2SO_4. powder soaked at overnight. After overnight soaking to forming acid slurry. This slurry was put in 250ml volumetric flask and adjust volume with the help of distilled water. This solution fully dissolved in several days. Stock range between 1 to 10 ug/ml. sample added in 10ml of ammonium thiocyanate and measured the absorbance at 470nm. Plot the graph and calculate the iron content.

2.6 Antioxidant assay:

2.6.1 Measurement of Total Phenolic Content Using Folin-Ciocalteu Assay

In this method, 1 ml of sample was mixed with 9 ml of distilled water to 1ml of FC reagent was added. The mixture incubated for 5 minute at room temperature. After incubation added 10 ml of 7% Na_2CO_3 , mixed and allow to stand for 90 minutes at room temperature. The absorbance measure at 760 nm. Gallic acid (50-500 mg/L) was used as standard and the TPC express in terms of milligram gallic acid equivalent per 100 gram dry matter (mg GAE/100 g dm).

2.6.2 Measurement of Total Flavonoids

The total flavanoid content of starfruit extract was estimated based on this method sample 1ml was mixed with 4ml of distilled water and subsequently with 0.3ml NaNO2 solution (10%) after 5minute, added 0.3ml $AlCl_3$ solution(10%) and

2ml NaOH solution (1%). Immediately, The absorbance of the solution measure at 400 nm wavelength in a spectrophotometer. Gallic acid is a ubiquitous flavonoid, present in many plant extracts, will be used as a standard to quantify the total flavonoid content. Standard curve was prepared (0-12 ug/ml) and results express in microgram gallic acid equivalents (mg GAE/g)

2.6.3 Ascorbic acid content

In this assay, the yellow colour of test solution changes to various shades of green and blue, exibite a broad light absorbtion at 700nm. Calibration curve will prepare by adding 0,1,2, 5, 8, and 10ml of ascorbic acid stock solution into 100ml volumetric flask and dilute to volume with water. A solution concentration was 0, 100, 200, 500, 800, and 1000ug/L ascorbic acid, the effective range of the assay. From sample was pipetteing the separate tubes and to it 2.5ml phosphate buffer, and then added to 2.5 ml of potassium ferrocyanide solution solution kept at 50^{o}C for 20mins, then 2.5 ml of trichloroacetic acid add and centrifuge at 10 min., for 3000rpm, and 1.25ml supernatant was collected then added 1.25 ml distilled water and 0.25mL of ferric chloride. Measure the absobance at 700 nm against blank and plot the graph.

2.6.4 DPPH free radical scavenging activity

The antioxidant or free radical scavenging activity of the extracts was measured on the basis of decrease in the absorbance of methanol solution of stable DPPH. DPPH means 1,1-diphenyl-2-picrylhydrazyl free radical. DPPH is one of the few stable and commercially available organic nitrogen radicals exhibiting a dark purple color at absorbance 530 nm. When free radicals are scavenged, DPPH will be reduced, producing a light pink coloration reducing the absorbance. 5 ml of DPPH (25 mg /L) solution was added to 1 ml of sample solution. Mixture was shaken vigorously and kept at room temperature for 30 min in dark. Then the absorbance was measured at 530 nm. Scavenging activity was calculated as the percentage inhibition (1%) using the following formula:

$$\% \text{ DPPH Anti-radical Activity} = \frac{(\text{Control Absorbance} - \text{Sample Absorbance}) \times 100}{\text{Control Absorbance}}$$

2.7 Synthesis of silver nanoparticle and its characterization:

Starfruit was washed with distilled water and cut into small pieces. The aqueous fruit extract was prepared 5 g of fruit pieces with 100 ml distilled water boiled for 15 min. The extract was cooled at room temperature and filtered through whatman filter paper No.1. The filtrate was collected and used for further experiment of synthesis of AgNPs. A stock solution of 4 Mmol L^{-1} $AgNO_3$ will be prepared. 10 ml of fruit extract taken and 25 ml $AgNO_3$ stock solution and constant stirring at 40°C. The colourless fruit extract solution changes to reddish brown slowly indicating the formation of AgNPs. And to check the bactericidal activity against *Escherichia coli*, *Pseudomonas aeroginosa* by using agar well diffusion method. The synthesized AgNPs were characterized by using various analytical techniques. The reduction of the pure Ag+ ions was monitored by measuring the absorbance of the reaction medium with UV spectrophotometer.

3. RESULTS

3.1 Antimicrobial Activity:

The well diffusion assay showed that the fruit extracts have different degrees of bacterial and fungal growth inhibition, depending on the strains **(Table No. 1)**. fruits of *Averrhoa carambola* showed better antimicrobial activities. Starfruit extract was not very effective against fungus *Candida albicans* and bacteria like *Salmonella typhimurium* But starfruit showed stronger antimicrobial activities bacteria like *Bacillus cereus. Averrhoa carambola* found to have potential antibacterial and antifungal activity against four medically important bacterial and fungal strain. fruit extract of *A. carambola* exhibited a maximum inhibition zone of 20 mm and 17mm against *Bacillus cereus and klebseilla pneumoniae*. *Averrhoa carambola* found to have potential antibacterial and antifungal activity against four medically important bacterial and fungal strain.

Table No. 1: Antibacterial activities, indicated by diameter of inhibition zone of selected samples against the micro-organisms.

Fruit	*Bacillus cereus*	*klebseilla pneumoniae*	*Escherichia coli*	*Staphylococcus aureus*	*Salmonella typhimurium*	*Candida albicans*
Averrhoa carambola	20mm	17mm	12mm	14mm	10mm	09mm

3.2 Phytochemicals

The edible parts of the fruits were analyzed for different phytochemicals **(Table 2)**. The phenolics, flavonoids, tannin, steroids, carbohydrates, and proteins were present in *Averrhoa carambola* as well as terpenoids were absent in *Averrhoa carambola* as shown in **Fig.No.1.**

Table 2: Phytochemical qualitative evaluation of *Averrhoa carambola*

Phytochemicals	***Averrhoa carambola***
Carbohydrates	Present
Saponins	Present
Steroids	Present
Tannins	Present
Amino acids	Present
Terpenoids	Absent
Flavanoids	Present
Phenols	Present

Figure No.1 Qualitative tests of *A. carambola*

3.4 Nutrient content:

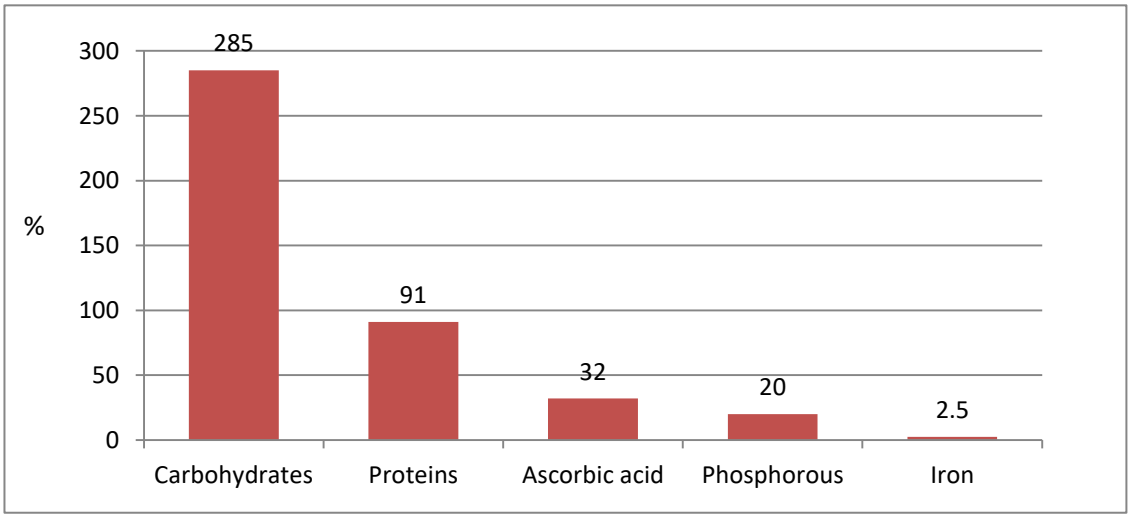

Figure 2: Nutritional content of *Averrhoa carambola*

In this study the nutritional content of the starfruit was measured. In 100g of starfruit, 91mg/ml of protein, 285mg/ml of carbohydrates, minerals like 20 mg/ml of phosphorous, 2.5 mg/ml and 32mg/ml Ascorbic acid or vitamin C was obtained. This result highlighted in **fig.2.**

3.4 Antioxidant Activity:

In the present study methanol were used for extraction of antioxidant compounds. The results obtained in the present study are highlighted in **Table 3**. Methanol extracts can be attributed to the differences in the polarity and viscosity. These features makes it possible for the solvent like methanol to easily diffuse into the pores of the fruit materials and leach-out the bioactive compounds (like that of antioxidants).The high contents of phenolic compounds indicated that these compounds contribute to the antioxidant activity.

Table 3: Antioxidant activities of fruit extracts

Phytochemicals	***Averrhoa carambola***
Phenol (mg/ml)	95
Ascorbic acid (mg/ml)	67
Flavonoids(mg/ml)	2.85
DPPH (mg/ml)	7.5

Extracts of fruits contain higher amounts of phenol and ascorbate. DPPH scavenging assay is applied extensively for the determination of free radical scavenging or antioxidant activity of compound. In DPPH assay measures the capability of the extract to donate hydrogen to the radical. DPPH assay the lower the IC50 the better it is able to scavenge the radical. Starfruit extract showed better antioxidant activity.

3.5 Synthesis of Nanoparticle And its characterization

Synthesis of AgNPs as shown in **figure 3 (A)**. AgNPs are extensively used in the pharmaceutical industries and have inhibitory activities on various microorganisms. Biosynthesized AgNPs were analyzed for their antibacterial activity against organism *Escherichia coli* and *Pseudomonas aeruginosa* by agar well diffusion method. The antibacterial activity was assayed by measuring the diameter of zone of inhibition around the well. The images for antibacterial acivity of biosynthesized AgNP's against *Escherichia coli* and *Pseudomonas aeruginosa* were shown in **figures 3 (b) and (c)**. The **Table 4** shows summarized results of antibacterial activity.

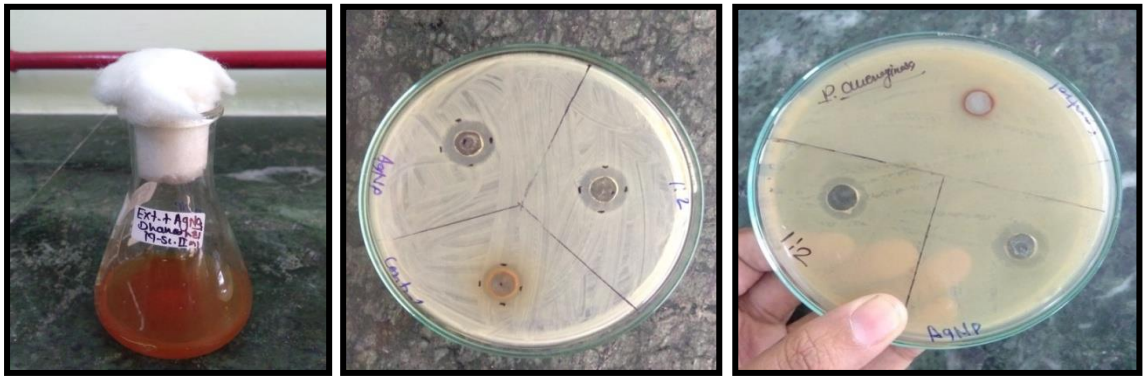

Figure 3: A) Silver Nanoparticles, B) Antibacterial acivity of biosynthesized against (b) *Escherichia coli* And (c) *Pseudomonas aeruginosa.*

Table 4. Antibacterial activity of AgNPs.

Test microorganism	**Carambola Fruit Extract**	**AgNPs (Undiluted)**	**AgNPs (Diluted)**
Escherichia coli	11 mm	14 mm	12 mm
Pseudomonas aeruginosa	0 mm	13 mm	11 mm

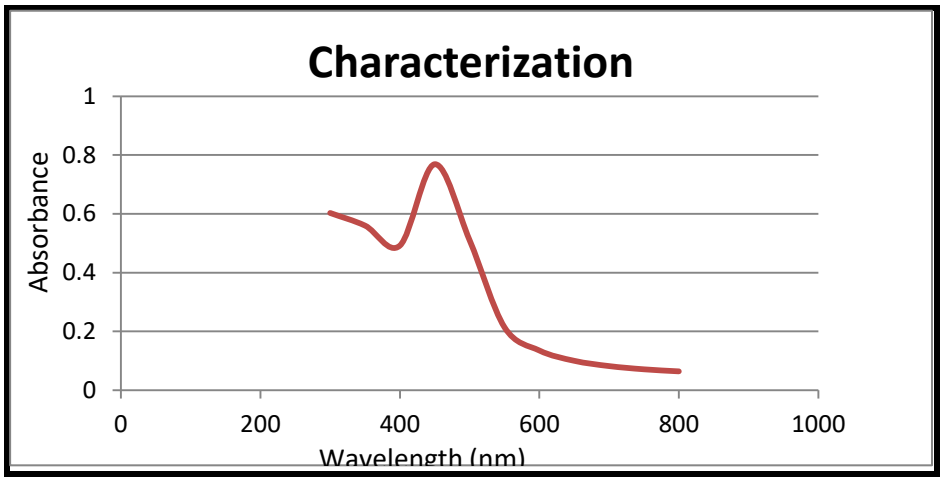

Figure 4. UV-absorption spectra of AgNPs

UV– spectral analysis of AgNPs the formation of AgNPs in an aqueous colloidal solution was investigated by using UV–spectrophotometer analysis as shown in **Fig.4**. AgNPs turned yellowish brown in the aqueous solution, which has been reported and check the absorbance with the help of UV spectrophotometer. It has been noticed that the absorption peak width gradually became narrower with time, and this suggests the narrow size distribution of newly formed AgNPs. Characterization of silver nanoparticles is done by UV-Spectrophotometer. The maximum absorption of silver nanoparticles was found in 450nm.

4. CONCLUSION

It could be concluded that *Averrhoa carambola* is an excellent plant due to its multifaceted medicinal properties like, antimicrobial, antioxidant activity and nutritional content. The tested extract of fruits was shown antimicrobial efficacy against most of microbes examined. Agar well diffusion assay was showed the fruit extracts have different degrees of bacterial and fungal growth inhibition depending on the strain. The edible parts of the fruits were analyzed for different phytochemicals like phenolics, flavonoids, alkaloids, Tannins, steroids, present in starfruits. They were showed strongest antioxidant activity. Starfruit present nutrients content like proteins, carbohydrates, vitamins, minerals; this content are useful to increase our energy. Synthesis of AgNPs with the help of starfruit is the industrial application. Synthesize AgNPs was showed the better bactericidal activity and characterization of silver nanoparticles is done by using UV Spectrophotometer.

5. DISCUSSION

In this study discussing and determining the antimicrobial activity using different strains as well as study the synthesis and characterization of silver nanoparticles. Characterization of silver nanoparticles with the help of UV spectrophotometrically as well as antioxidant activity and nutritional content by calorimetrically.

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