



## Eco-friendly Synthesis of Silver Nanoparticles using *Capparis decidua* (Forsk.) Edgew Stem Extract: An Investigation for Potential Antimicrobial Agent

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### ABSTRACT

Nanotechnology refers to the modification and synthesis of extremely small nanosized particles or devices and depend on the characteristics and interaction of atoms. These devices help in diagnosing and treating various disease as it is cheap, portable, safe and easy to access. The most commonly synthesized nanoparticle, that is, silver nanoparticle has various important beneficial properties due to their peculiar biocidal activity. *Capparis decidua* is one of the traditional remedies used plants for various medicinal treatments. Biological synthesis of metal NPs, a long established method that utilize plant extracts which has the ability for the control of disease, besides being safe and no phytotoxic effects. The method of green synthesizing silver nanoparticles using medicinal plants were found to be highly toxic against different pathogenic microorganisms. The synthesis involves reacting the silver metal stock solution with the stem extract of different stoichiometric ratio resulting in unique shapes and sizes of NPs. Yet another noteworthy feature of the present work is that the synthesized nanoparticles has significant antioxidant potential and antimicrobial efficacy. This enables the synthesis of silver nanoparticles by facile, non-hazardous, energy-frugal, and inexpensive methods; while gainfully utilizing the arid plant *Capparis decidua* (Forsk.).

**Keywords:** *Capparis decidua*, silver nanoparticles, antioxidant, antibacterial agent, antifungal agent

### INTRODUCTION

The field of Nanotechnology is an emerging area of modern research that involves with the design, synthesis and utilization of nanoparticle with potential in the field of medical and biotechnology [Abbasi *et al.*, 2009; Dubchak *et al.*, 2010]. The high availability, functional property, compatibility and ability to target drugs make metallic nanoparticles widely used in the field of today's industry. Bio-nanotechnology hire biological concepts and physical, chemical strategies to supply nanosized particles that have very precise features. That is due to relatively larger extent, accelerated stability and reactivity for reaction and stepped forward mechanical strength [Abbasi *et al.*, 2016; Ealias and Saravanakumar, 2017; Younas *et al.*, 2023].

Nanoparticle synthesis in the field of biotechnology is getting immense awareness in view of the various application like drug transport, biomedicine, most cancers remedy, molecular based totally analysis, bio-imaging and many others. Biological process for the nanoparticle synthesis must be value effective, smooth, non-hazardous, non-lethal, and eco-friendly [Awwad *et al.*, 2013]. Nanoparticle are of immense interest these days because to its varying properties like size, distribution, morphology from which one can easily study the biomedical outcomes like catalytic activity, optical properties, antibacterial, and electrical and magnetic properties in other fields [Abbasi *et al.*, 2012; Banerjee *et al.*, 2014].

Nanotechnology is the creation and utilization of particle having nanoscale size and has the potential in the field of medical and biotechnology. Metallic NPs have an extensive application in the field of industry due to high availability, compatible, functional and capacity to target drugs [Navaladian *et al.*, 2007 and Rajasekharreddy *et al.*, 2010; Anuradha *et al.*, 2011].

Bio-nanotechnology employ biological principles and physical, chemical methods to produce nanosized particles which have specific functions. For the production of stable novel nanosized particles which uses the green biosynthesis method researchers mainly focuses on the plant-based extracts having medicinal or therapeutic properties [Khan *et al.*, 2018]. There applications are notable appropriate for biological molecules, cause of their variant properties. The technique of biological molecules to synthesis metal nanoparticles are especially controlled which was found to be reliable and ecofriendly [Bar *et al.*, 2009].

The easiest and biogenic efficient method for synthesis of nanoparticle is by using plant as a source of medium. The plant extract contains natural substances like phenol, flavanoids, alkaloids that have a huge reducing tendency and promotes the nanomaterial synthesis. Recently biosynthesis of silver nanoparticle are done using variety of plants that has possess medicinal traits like *Dracaena Cochinchinensis*, *Eucommia ulmoides*, *Gloriosa superba*, *Ocimum sanctum*, *Euphorbia nivulia*, *Eclipta prostrata*, *Fagonia Cretica*, *Oryza sativa*.

Plant mediated synthesis of nanoparticle is of highly growing importance in today's world due to its simplicity, easy synthesis process with diversifying nature of particles. Involvement of protein factors with redox potential act as reducing agents in metal reduction. Plant has a wide network of metabolites and enzymes that act together to prevent damages to cellular components [Malik *et al.*, 2014]. The nanoarticle size, its growth can also be controlled by altering the medium conditions like pH, reductant concentration, temperature, mixing ratio of the reactants etc [Ahlawat *et al.*, 2015].

The *C. Decidua* plant has found huge groups of medicinal applications in conjunction with its dietary property. *C. decidua* own numerous pharmacological attributes such as antidiabetic, anthelmintic, antibacterial, antifungal, analgesic, anti-nociceptive, antirheumatic, hypolipidemic, antiatherosclerotic, anti-tumor, anti-giardial, antioxidant, anti-inflammatory, hepatoprotective, and antiepilepticactivities [Nazar *et al.*, 2010].

The biogenic synthesis procedure is unique perspective where the reaction go down which can be oxidation or reduction reaction whereas the chemical procedure leads to toxicity due to the existence of chemicals that are absorbed (Parasharu, 2009). Over the years, the silver nanoparticles are gaining tremendous attention as it is been proved effective in antimicrobial efficacy, diagnosis and treatment of various diseases [Khan *et al.*, 2014]. In medical and industrial processes, metallic silver particles inhibit the microorganism and majorly used as skin ointment, prevent skin burn, medical devices [Jiang *et al.*, 2004]. Silver nanoparticles these days have huge applications due to its unique properties which include antimicrobial applications, biosensor, cosmetic products, electronic components and many more [Mody *et al.*, 2010]. Silver nanoparticles has broad range antimicrobial activity that show cell toxicity releasing ROS and inhibit the microbial pathogens that penetrates the bacterial cells resulting in cell membrane damage. (Nel *et al.*, 2009).

The study on green synthesis of silver nanoparticle show immense popularity and its wide beneficial nature in modern science field [Song and Kim, 2009]. Chaudhary *et al.* reported that Ayurvedic bhasmas are in nanometer dimensions and are considered as nanomedicine which are free of toxicity used for therapeutic purpose. Bhasmas are produced by biological methods which are used as medicines in the ayurvedic field and is one of the ancient applications of nanotechnology [Prasanta *et.al* 2009]. The biologically active phytochemical molecules present in the plant extracts promote the synthesis and preparation of the different nanoparticles by acting as reducing agents [Shaik *et al.*, 2018]. Chandran and his team has used Aloe vera plant extract for synthesizing AgNPs showed reduction of silver ion and led to the formation of spherical shaped NPs [Chandran *et al.*, 2006]. Similarly, another study was carried out by Krishnaraj and team using leaf extracts of *Acalypha indica*, where they synthesized AgNPs rapidly within 30 min of incubation period showed brown color changes, with the addition of leaf extract. Amount of brown color increased in direct proportion to the incubation period [Krishnaraj *et al.*, 2010].

Ruparelia and their colleagues worked on antimicrobial activity of copper and silver nanoparticles against different bacterial strains of *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* and that showed better antimicrobial efficacy against *Escherichia coli* and *Staphylococcus aureus*. Based on the notable antimicrobial characteristic, colloidal silver is commercially available to treat infection and wounds. Medical equipment's such as surgical instrument, catheters and needles are also covered with silver nanoparticles for surgical procedures to avoid any infection during handling [Ruparelia *et al.*, 2008]. A component named Phyllanthin from *Phyllanthus amarus* was reported as capping agents in the synthesis of silver and gold nanoparticles [Kasthuri *et al.*, 2009].

Another work on the antibacterial efficacy was carried by Shivaji and his team by developing stable AgNPs in dark environment using cell-free culture supernatants of psychrophilic bacteria *Pseudomonas antarctica*, *Pseudomonas proteolytica*, *Pseudomonas meridiana*, *Arthrobacter kerguelensis*, *Arthrobacter gangotriensis*, *Bacillus indicus* and *Bacillus cecembensis* [Shivaji *et al.*, 2011]

In this regards, there are not more detailed study on the biogenic preparation of silver nanoparticle from an arid plant *Capparis decidua* stem and their evaluation on antioxidant activity and human microbial pathogens.

## PLANT DESCRIPTION

*Capparis decidua* (Forsk.) Edgew (Capparidaceae), belongs to the medical plant of family Capparaceae locally known as Kair, is a drought resistant Plant, growing in dry and arid regions of Indian subcontinent (Gupta, 2010). It is a bushy shrub that grows with a height of 4-5m in length having spines on the twigs node (Singh and Singh, 2011). The plant specimen was deposited in the herbarium section for its authentication at the Siddha Central Research Institute, Chennai and the voucher number (C02022302D) was issued for the specimen (Rasheed and Jabeen, 2022).

## MATERIALS AND METHODS

### Preparation of aqueous extracts of the *Capparis decidua*.

Fresh, mature and healthy stem part of *Capparis decidua* plant was collected from the local region adjacent to Nims University Rajasthan campus. Further, the stem was completely washed and sterilized using deionized water and kept for drying at room temperature.

For the aqueous extract preparation, a known amount of stem sample has been taken and chopped into small uniform size then transferred into 250mL flask for boiling with 100mL of distilled water in water bath for about 10-15 min. Then the aqueous extract was cooled to room temperature and the supernatant was filtered through Whatman No.1 filter paper.

### Preparation of Silver Nanoparticle

The silver nanoparticle synthesis was done by preparing 1 mM stock solution of silver nitrate ( $\text{AgNO}_3$ ) dissolved in 100mL of deionized water and stored in amber color bottle which is covered in black plastic sheet to keep away from light. A 1:9 ratio of aqueous extract of leaf was mixed with Au (III) stock solution at normal temperature. The synthesis of nanoparticle began immediately with the appearance of reddish yellow color. Then this mixture was incubated at room temperature.

### UV-Vis Spectrophotometer

Preliminary characterization of the synthesized AgNPs was carried out using UV-Visible spectroscopy. The reduction of silver ions to the NPs was monitored by measuring the spectra. UV-Vis spectrograph of the colloidal solution of AgNPs was recorded as a function of time with water as reference and scanning the spectra between 200-600 nm (Ahlawat, 2017).

### Fourier transformation infrared (FTIR) Spectroscopy

The FT-IR absorption spectra provide valid information about the molecular environment of the organic molecules on the nanoparticle surface. FT-IR examination were used to identify the presence of potential biomolecules found in stem extract of *Capparis decidua* that could have played a role in the reduction of synthesized and measures the subsequent stabilization-capping agent of synthesized nanoparticle.

### In vitro antioxidant assays

#### DPPH<sup>•</sup> radical scavenging assay

The antioxidant activity of aqueous stem extract of *Capparis decidua* and AgNPs were measured on the basis of the scavenging activity of the stable 1, 1- diphenyl 2-picrylhydrazyl (DPPH) free radical. One mL of 0.1 mM DPPH solution in methanol was mixed with 1 ml of various concentrations (20-120  $\mu\text{g/mL}$ ) of leaf extract and nanoparticle. The mixture was then allowed to stand for 30 min incubation in dark. Distilled water was used as the reference standard. One mL methanol and 1 mL DPPH solution were used as the control (Blois, 1958). The decrease in absorbance was measured using UV-Vis Spectrophotometer at 517nm. The percentage of inhibition was calculated using the following formula:

$$\% \text{ of DPPH}^{\bullet} \text{ radical inhibition} = \left[ \frac{\text{Control} - \text{Sample} * 100}{\text{Control}} \right]$$

#### Phosphomolybdenum reduction assay

The antioxidant capacity of the aqueous stem extract of *Capparis decidua* and AgNPs were assessed as described by Prieto *et al.* The leaf extract and nanoparticle with concentrations ranging from 20 to 120  $\mu\text{g/mL}$  was combined with reagent solution containing ammonium molybdate (4 mM), sodium phosphate (28 mM) and sulphuric acid (600 mM). The reaction mixture was incubated in water bath at 90°C for 90 min. The absorbance of the colored complex was measured at 695 nm. Distilled water was used as standard reference (Prieto, 1999). The percentage of inhibition was calculated using the following formula:

$$\% \text{ of Phosphomolybdenum radical inhibition} = \left[ \frac{\text{Sample} - \text{Control} * 100}{\text{Sample}} \right]$$

#### Antibacterial activity

The AgNPs synthesized from stem extract of *Capparis decidua* was examined against various Gram-positive bacteria (*Bacillus subtilis*, *Micrococcus luteus*, and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*, *Proteus vulgaris*, *Shigella flexneri*) were used for the evaluation of antibacterial activity.

Tetracycline was chosen as the standard reference for bacteria. The controls consist of solidifying agar onto which was solvent, and the test compounds were soluble in it.

#### Nutrient broth agar medium

Nutrient broth agar medium was prepared according to the standard methods and was suspended in 200 mL of distilled water in a 500 mL conical flask, stirred, boiled to dissolve and then autoclaved at 121°C for 15 minutes. The hot medium

was poured in sterile petri plates which were kept in the aseptic laminar chamber and medium was allowed to solidify for 15 min (John *et al.*, 2017). Determination of antibacterial potential of the leaf blade extract was carried out using the agar well diffusion method. Five wells were created in each plate with the help of a sterile well-borer of 8 mm diameter. The control, extract and standard were then poured into each well of desirable concentrations. Tetracycline was used as the standard with the concentration of 20 µg. All the plates containing sample loaded wells were incubated for 24 h at 37°C. After the incubation period, zone of inhibition in each plate, for each concentration of extract and standard were measured by calculating the diameter of zone of inhibition.

### Antifungal Activity

The antifungal activity of AgNPs synthesized from stem extract of *Capparis decidua* was examined against fungal culture *Candida albicans* using agar well diffusion method with Potato Dextrose Agar medium (Potato 2gm and Dextrose 3gm). Fluconazole was used as the standard reference for fungal culture.

### Potato Dextrose Agar medium

Potato Dextrose Agar medium was prepared using standard procedure and suspended in 100mL distilled water. The sterilization was done by autoclaving for 20 minutes and hot medium was poured in sterile petri plates which were kept in the aseptic laminar chamber and medium was allowed to solidify. Determination of antifungal potential of the leaf blade extract was carried out using the agar well diffusion method against fluconazole and zone of inhibition was observed after incubation for 24-48hrs.

## RESULTS AND DISCUSSION

### UV-Vis Spectrophotometer

In this study, the bio-reduction of silver ions in the aqueous solution of silver nitrate reaction with the stem extract of *Capparis decidua* has been characterized by UV-Vis spectroscopy ranging from 200 to 600 nm.

The onset for the formation of silver nanoparticle can be visually demonstrated by the appearance of reddish brown during the reaction of metallic solution with stem extract. The UV-Vis spectrum for AgNPs clearly shows the presence of peak at 400 nm (Fig:1).

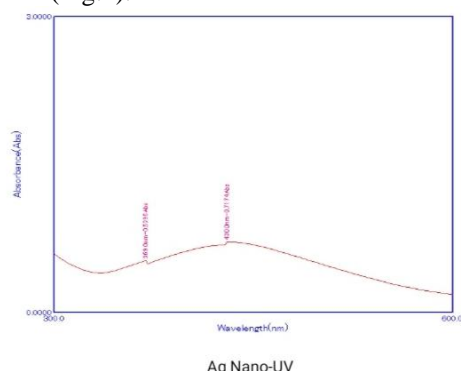


Fig 1: UV-Vis absorption spectrum for synthesized AgNPs

### SEM and EDAX Electron microscopic studies.

The SEM images provide the high resolution imaging of obtained AgNPs from reactant mixtures of stem extract, which revealed the presence of e-peak visible spectra, showed that the particle is 45-70nm in shape and having tetrahedron like structure. (Fig: 2,3)

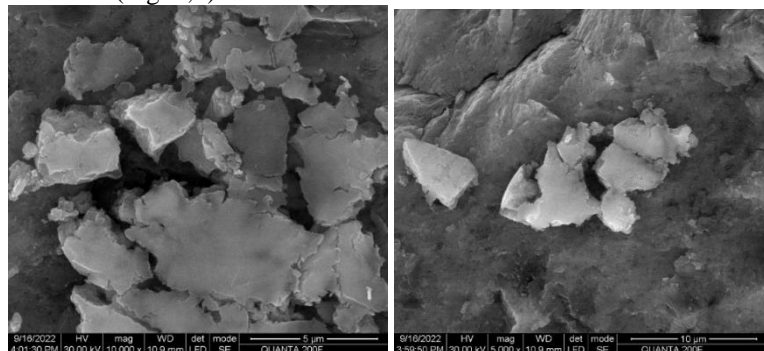


Fig 2: SEM micrograph of AgNPs at different magnification.

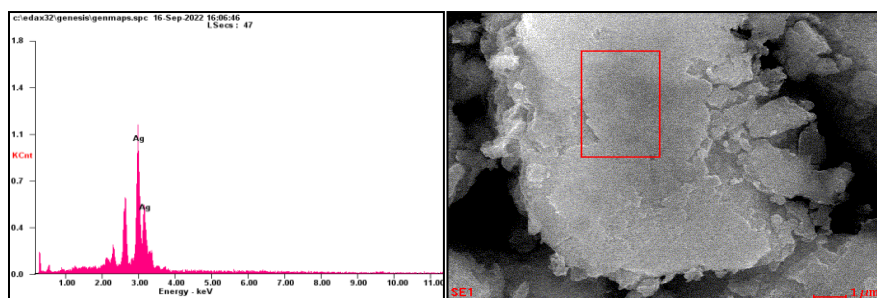


Fig 3: EDAX of synthesized AgNPs.

#### Fourier transformation infrared (FTIR) Spectroscopy

The results of AgNPs observed from FTIR analysis shows different stretches of bonds at different peaks; 3155- N-H stretch, 2909, 2843, 2328, 1884 etc. were observed. There is presence of strong absorption band at 1619–1380  $\text{Cm}^{-1}$  region and weaker signal in the 1195–1081  $\text{Cm}^{-1}$  region. (Fig: 4)

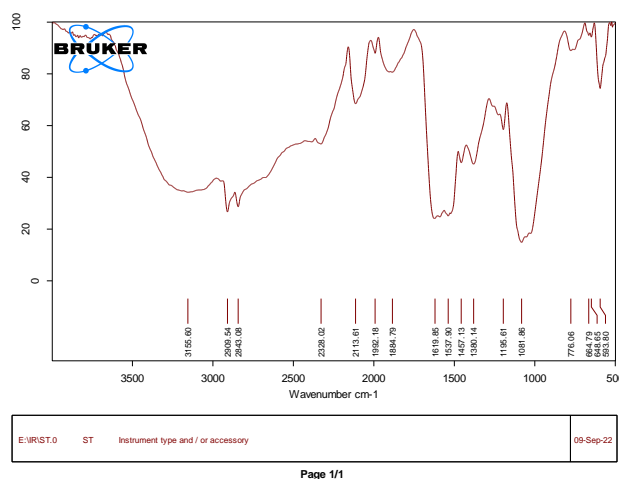
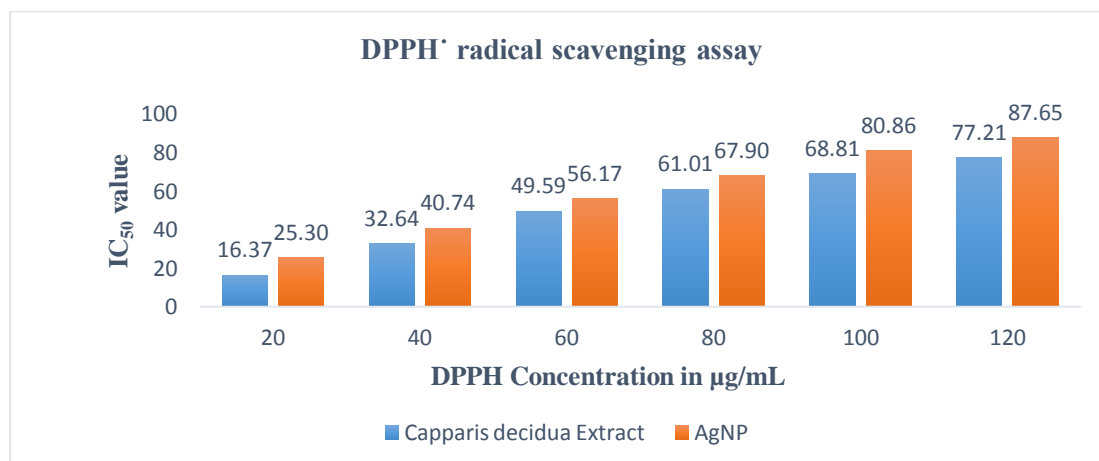


Fig 4: FTIR spectra of AgNP's synthesized by *Capparis decidua* stem extract.

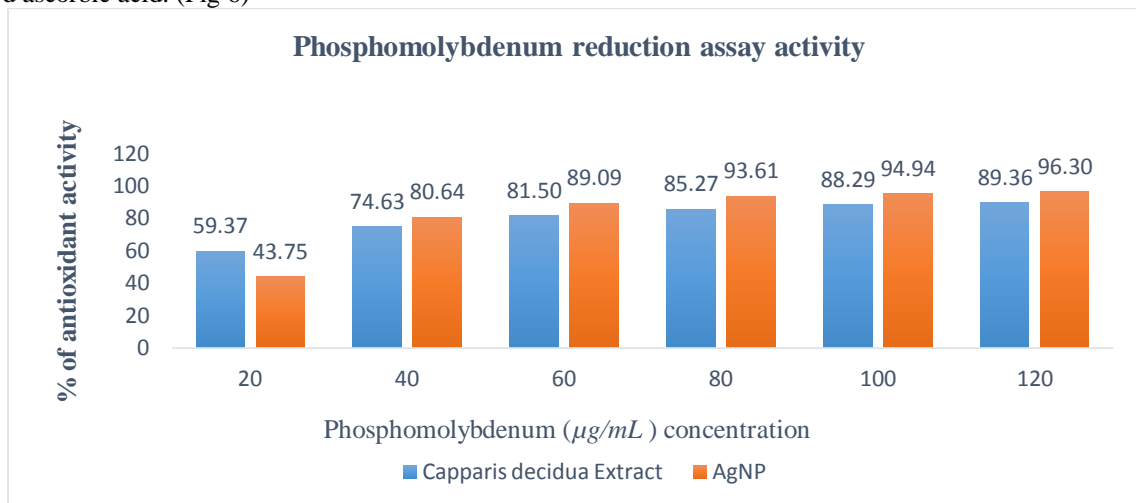
#### DPPH<sup>•</sup> radical scavenging assay

The ability of aqueous stem extract of *Capparis decidua* and AgNPs to scavenge the free radicals that are produced was assessed using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). The maximum DPPH<sup>•</sup> radical scavenging activity was **77.21%** for stem extract, **87.65%** for AgNPs at 120  $\mu\text{g}/\text{mL}$  concentration. Aqueous extract of *Capparis decidua* stem extract and AgNPs demonstrated high capacity for scavenging free radicals by reducing the stable DPPH (1,1-diphenyl-2-picrylhydrazyl) radical to the yellow colored 1,1-diphenyl-2-picrylhydrazine and the reducing capacity increased with increasing concentration of the extract and was compared with standard ascorbic acid. (Fig 5)



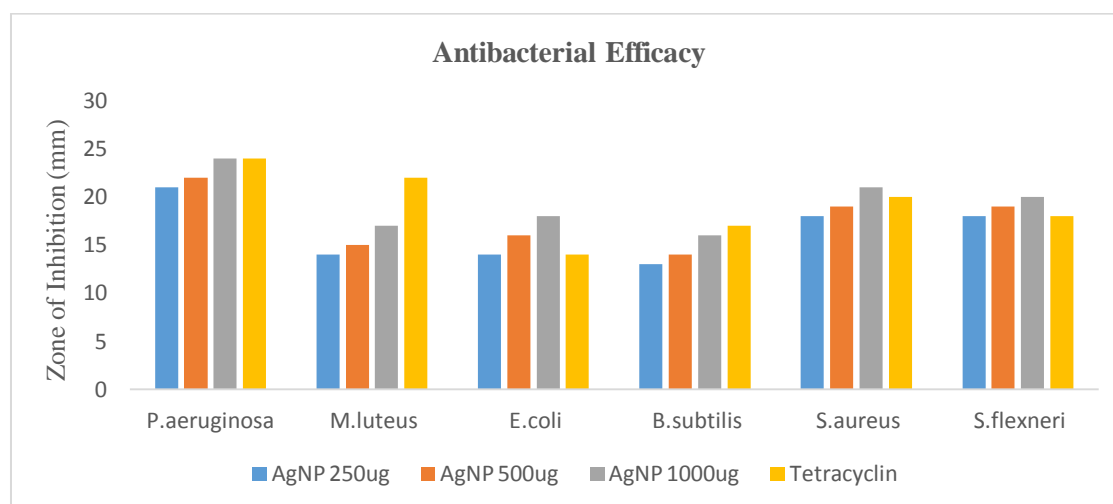
**Fig 5: DPPH radical scavenging activity of stem extract of *Capparis decidua* and AgNPs****Phosphomolybdenum reduction assay activity**

The total antioxidant activity of aqueous stem extract of *Capparis decidua* and AgNPs was measured by Phosphomolybdenum reduction method which is based on the reduction of Mo (VI) to Mo (V) by the formation of green phosphate/Mo (V) complex at acidic pH, with a maximum absorption at 695 nm. The maximum Phosphomolybdenum reduction was 89.36% for stem extract and 96.30% for AgNPs at 120  $\mu\text{g/mL}$  concentration and was compared with the standard ascorbic acid. (Fig 6)

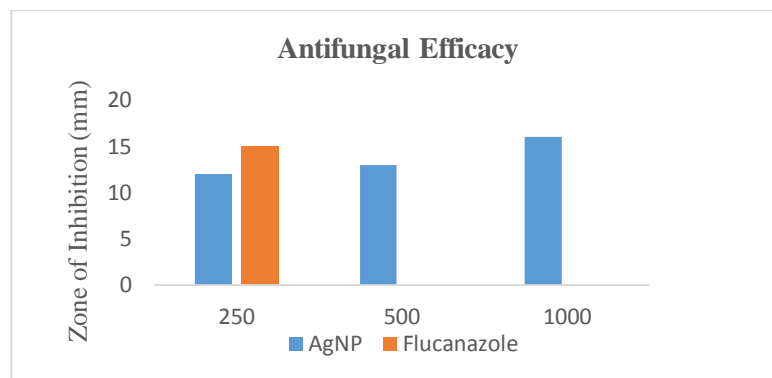
**Fig 6: Phosphomolybdenum reduction activity of stem extract of *Capparis decidua* and AgNPs****Antibacterial Analysis**

The AgNPs synthesized from stem extract of *Capparis decidua* was assayed for antibacterial activity against different microorganism including Gram-positive bacteria (*Bacillus subtilis*, *Micrococcus luteus*, and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*, *Proteus vulgaris*, *Shigella flexneri*). The antibacterial sensitivity of the stem extract and nanoparticles with respect to their potency were assessed quantitatively by measuring the diameter of clear zone produced by cultures in petriplates. (Fig: 7)

The antibacterial activity of the AgNPs at various concentration (250  $\mu\text{g/ml}$ , 500 $\mu\text{g/ml}$ , 1000  $\mu\text{g/ml}$ ) could be correlated due to the presence of secondary metabolites such as flavonoids, phenolic compounds, terpenoids, tannin and alkaloids that adversely affect the growth and metabolism of microbes.

**Fig 7: Antibacterial activity of synthesized AgNPs w.r.t standard.****Antifungal Analysis**

The AgNPs synthesized from stem extract of *Capparis decidua* was assayed for antifungal activity against *Candida albicans* microorganism at various concentration (250  $\mu\text{g/ml}$ , 500 $\mu\text{g/ml}$ , 1000  $\mu\text{g/ml}$ ). The antifungal sensitivity were assessed quantitatively by measuring the diameter of clear zone in cultures in petriplates. (Fig: 8)



**Fig 8: Antifungal activity of synthesized AgNPs w.r.t standard.**

### SUMMARY AND CONCLUSION

The present study reports the simplistic biogenic route for synthesizing silver nanoparticle by productively utilizing the arid plant *Capparis decidua*. The stem used for the experimentation gave rise to enormous quantities of biomass which has little or no existing economic value. We have exhibited, with various experiments described in this research article, that the stem extract of *Capparis decidua* can be brought into play for silver nanoparticle synthesis and their characterization (UV-Vis, FTIR, SEM) along with its antioxidant and antimicrobial assays were also determined. The morphology of the AgNPs was obtained through SEM characterization. DPPH and Phosphomolybdenum assay has been used to determine the free radical scavenging activity resulting in potent antioxidant activity. Equally significant is the fact that the process developed by us utilized the stem for silver nanoparticles synthesis provides us with substrates which have less cultivation experiment producing higher biomass growth, thereby reducing the harm they cause to the environment. Therefore, the present work is that it enables the synthesis of silver nanoparticles from the *Capparis decidua* plant part are environmentally safe, non-hazardous and inexpensive methods which can use in the medicinal field.

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