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# GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM THE EXTRACTS OF *RUBIA CARDIFOLIA* AND *MENTHA LONGIFOLIA*, AND THEIR ANTIBACTERIAL ACTIVITY STUDIES

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## ABSTRACT

Using water extracts of medicinal plants in the synthesis of silver nanoparticles, serves both the purposes of reducing agent and stabilizing agent, and hence is greener and economic. The present paper describes the synthesis of silver nanoparticles (AgNPs) from the water extracts of *Rubia cardifolia* and *Mentha longifolia* leaves. The particles were characterized using uv-visible extinction spectroscopy, scanning electron microscopy, transmission electron microscopy and powder X-ray diffraction studies. The average sizes of the AgNPs were found to be 28 nm and 24 nm respectively for AgNPs prepared from *Rubia cardifolia* and *Mentha longifolia*, and are of face centred cubic structure. Bactericidal effects of the nanoparticles against *Escherichia coli (E. coli)* and *Staphylococcuus aureus (S. aureus)* were checked. The AgNPs inhibit the spread of *E. coli* and *S. aureus* bacteria to different extents. Bactericidal effect of AgNPs synthesized from the extracts of *Rubia cardifolia* is found to be good against *E. coli* with a minimum inhibitory concentration of 70 µg/mL.

**Keywords:** Silver nanoparticls, *Rubia crdifolia*, *Mentha longifolia*, *Escherichia coli, Staphylococcus aureus.*

## 1. INTRODUCTION

Silver nanoparticles being not very much stable compared to gold nanoparticles, are studied to be applicable in photocatalysis [1], optical sensors [2], nanosphere lithography [3], optoelectronics [4], solar energy conversion devices [5] and as surface-enhanced Raman scattering (SERS) substrates [6]. In addition to technical applications, AgNPs exhibit variety of antimicrobial activities [7]. Thus the biomedicinal applications of AgNPs are being evaluated and the results were reviewed [8-10]. The antimicrobial property is also been explored in making materials for antibacterial water filter [11], activated carbon based antibacterial air filter [12] and in making silver nanoparticle embedded textile fabrics with antimicrobial activity [13-16].

Synthesis of AgNPs by wet chemical reduction method involves use of one or the other stabilizing agents. Garcia-Barrasa et al. [17] reviewed the use of stabilizing agents in the synthesis of AgNPs [17]. This indicates that silver nanoparticles are unstable without any molecular capping. AgNPs solutions obtained by chemical reduction methods using various reducing agents such as $NaBH_4$, $LiAlH_4$, $R_4N^+(Et_3BH^-)$ or hydrazine [18] might be contaminated with various reaction by-products such as borides, metal borates [19], $B_2H_6$, $NaNO_3$ etc., which may make the study of bactericidal effects complex. Stabilizing agents, used in the chemical reduction method of synthesizing AgNPs include trisodium citrate, 2,4,6-trimethylphenyl ring (Mes), pentafluoro-phenyl ring ($C_6F_5$), aminopropyltriethoxysilane (APS), polyvinylpyrrolidone (PVP), hexadecylamine (HAD), oleilamine (OLA), cetyltriethylammonium bromide (CTAB), sodium bis(2-ethylhexyl) sulfosuccinate (AOT), polyethyleneglycol (PEG) [17], and various polymers, and cationic polynorbornenes [20]. Achieving stability of AgNPs using capping agents makes the process expensive and multistep one as well. Reports on the efforts to develop green synthetic methods, using mixed-valence polyoxometallates, polysaccharide, Tollens irradiation, and biological methods have been reviewed in literature by Sharma et al. [21]. Using plant extracts is one of the greener approaches being explored in recent years [22-23].

Literature survey reveals that phytonutrient assisted synthesis of AgNPs using *Rubia cardifolia* is not been attempted. Though optimization of procedure for preparation of AgNPs using water extract of *Mentha longifolia* has been evidenced [24], complete characterization of the particles have not been realized.

Therefore, it was intended to undertake synthesis of AgNPs using water extracts of medicinal plants, *Rubia Cardifolia* and *Mentha longifolia*. The present study involves synthesis of AgNPs, characterization by uv-visible extinction spectroscopy, powder X-ray diffraction, SEM and TEM analysis, and the study of their antibacterial activities against *E. coli* and *S. aureus* bacteria.

## 2. MATERIAL AND METHODS

### 2.1. Material

All the chemicals are from Merck, Himedia or from S. D. Fine chemicals. Distilled water was used for all the experiments. Bacteria selected for the study were *E. coli* and *S. aureus*. R-8C laboratory centrifuge from Remi was used for isolation of particles for SEM and powder XRD analyses. Powder XRD spectra were recorded on a Rigacu Smartlab X-Ray diffractometer and the SEM and EDS were recorded on Ultra 55 scanning electron microscope from GEMINI technology. TEM imaging of the drop coated samples were done on Titan Themis 300kV from FEI. Systronics Uv-visible spectrophotometer 119 was used for recording the Uv-visible extinction spectra in the wavelength range of 300 nm to 700 nm.

### 2.2. Methods

#### *2.2.1. Extraction*

Weighed quantities of the fresh leaves, were taken in a mortar and crushed in to paste with a little amount of warm distilled water. The pastes were transferred in to a 250 mL beaker. The contents were added with around 100 mL water, stirred on a magnetic stirrer for about 30 minutes at 45-50 °C temperature, cooled to lab temperature and filtered through a pre-weighed piece of ordinary filter paper. The weight of the contents transferred to the extract was calculated by difference in weight method.

The well known routine method was adopted for the qualitative phytochemical analysis of the extracts [25].

#### *2.2.2. Synthesis of silver nanoparticles*

50 ml of the fresh extracts of the selected plants, containing approximately 0.04 ± 0.005 g of extracted substances were taken in a round bottomed flask. Contents were heated to 65 °C while stirring on a magnetic stirrer and 20 mL of 0.002 M $AgNO_3$ solution was added drop wise from a pressure equalizing dropping funnel for exactly 30 minutes. During addition of silver nitrate solution, temperature was maintained at 65 ± 5 °C. Contents were cooled to lab temperature and formation of nanoparticles was confirmed by recording the Uv-visible spectra.

2 liters of naoparticle solutions were centrifuged for isolation of the AgNPs, for powder XRD and SEM analyses. The samples were then dried in vacuum over anhydrous phosphorous pentoxide.

#### *2.2.3. Antibacterial activity*

The as prepared AgNPs solutions were used for antibacterial activity studies. The bacteria selected for the study were *E.coli* and *S. aureus*.

Petri dishes of 8 cm diameter and washed 50 mL beakers were sterilized in an autoclave. 28 grams of nutrient agar was suspended in 1000 mL of distilled water and dissolved by boiling. The contents were autoclaved. 20 mL aliquots of the nutrient agar media were then transferred in to sterilized 50 mL beakers and cooled to 60 – 65 °C. The media at this temperature were contaminated with various volumes of the AgNp solutions. Control experiments were done by contaminating the media with suspension of the standard substance ciprofloxacin, containing same amount of material as that of the AgNPs in their solutions. The media were then poured in to Petri dishes in a laminar air flow chamber. When the media in the Petri dishes were hardened in the flow chamber, upper surface of the media were inoculated with bacteria. The growth or spreading of bacteria was monitored with respect to time. Minimum inhibitory concentrations (MIC) were determined and expressed in μg/mL of media above which the inoculated bacteria will die or will not spread.

## 3. RESULTS AND DISCUSSION

The concentrations of the extracts used for phytochemical screening were three to four times higher than that used for the synthesis of nanoparticles. Extracts of both the plants showed the presence of phenolic compounds and flavonides.

Synthesis of AgNPs and their application in biology and medicine is becoming very important because of their biocompatibility in low concentration and antimicrobial activity [21]. However AgNPs synthesized by reducing $AgNO_3$ with sodium borohydride and without stabilizing agents are unstable.

In the present work, AgNPs are synthesized using water extracts of selected medicinal plants, without using any reducing agents, or stabilizing agents. The phytonutrients present in the plant extracts reduce the $AgNO_3$

in to metallic silver. The AgNPs prepared by this method are highly stable and the methods are fairly reproducible. The stability of the nanoparticles may be attributed to the formation an effective protective layer on the silver nanoparticles by the adsorption and formation of mono or multilayers of phytonutrient molecules present in the extracts [26].

### 3.1. Characterization

Silver nanoparticles are characteristic of having surface electrons and hence showing a surface plasmon resonance absorption band in the visible region [27-28]. Formation of the AgNPs is visually indicated by turning of the colour of the reaction mixture from pale green to reddish brown. However, presence of AgNPs was confirmed by recording a uv-visible absorption spectrum. AgNPs with 35-50 nm size, obtained by chemical reduction method give an absorption maximum at around 420 nm [26]. Fig. 1 shows uv-visible absorption spectrum of AgNPs prepared from the extracts of the selected plants for the study.

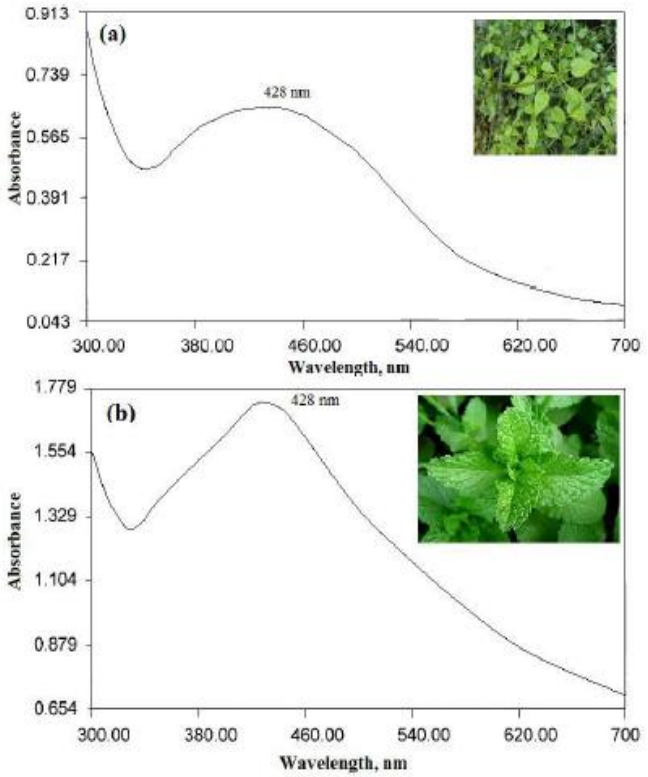


**Fig. 1: Uv-visible extinction spectrum of the AgNPs synthesized from the extracts of (a) *Rubia cardifolia* and (a) *Mentha longifolia*. The insets in the Fig.s show the pictures of the respective plants.**

The $\lambda_{max}$ values for the AgNPs solutions are 428 nm in both the cases. The Uv-visible absorption spectra recorded for the extracts, before treating with $AgNO_3$ solution shows no peaks throughout the radiation range of 300 to 700 nm, indicating that the extinction of radiation at 428 nm is because of the AgNPs formed when extracts were treated with $AgNO_3$ solution. Width of the absorption band represents approximate particle size distribution in the solution. Recording the uv-visible extinction spectrum of the same as prepared dilute AgNP solution repeatedly with regular intervals of one week, did not show either any shift in the $\lambda_{max}$ position or any decrease in its intensity up to 80 days. This observation realizes the remarkable stability of the AgNPs prepared by this method.

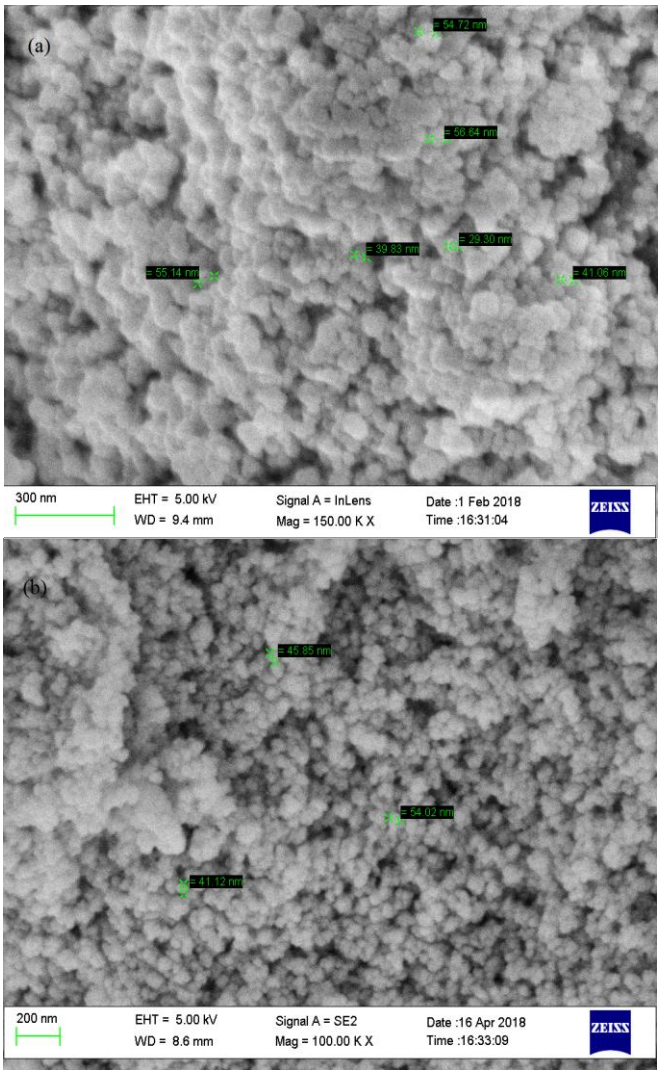


**Fig. 2: Scanning electron micrograph of AgNPs synthesized from the extracts of *Rubia cardifolia* (a) and *Mentha longifolia* (b).**

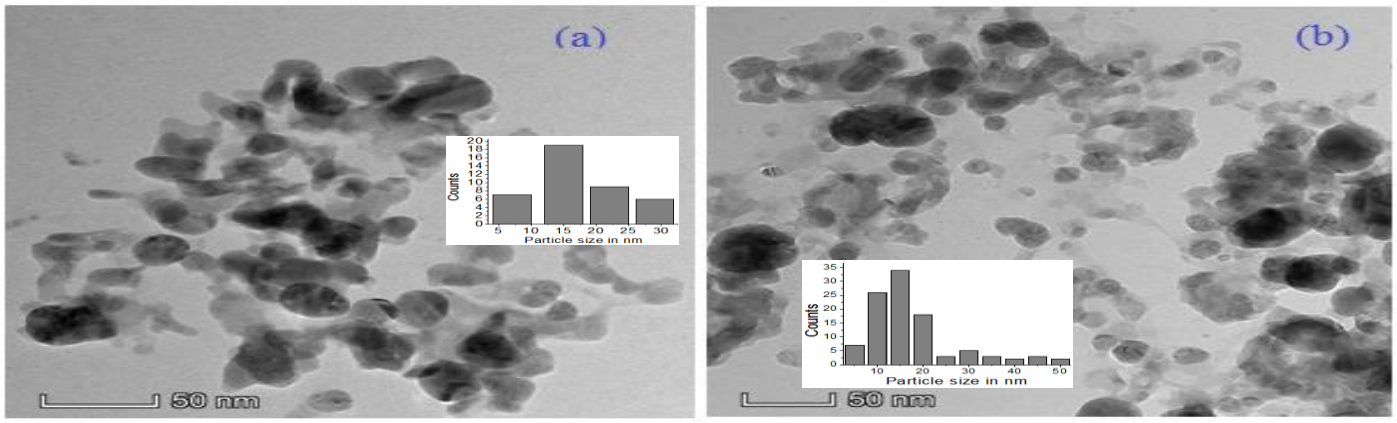


**Fig. 3: Transmission electron mocrographs of the AgNPs synthesized from the extracts of (a) *Rubia cardifolia* and (b) *Mentha longifolia*. Insets in the respective Fig.s represent the particle size distribution.**

Morphology and the elemental composition of the particles were understood by recording scanning electron microscopy and energy dispersive x-ray spectroscopy (EDS) respectively. SEM images of the AgNPs prepared using the selected plant extract and isolated by centrifugation at 3500 rpm are presented in Fig. 2.

It is clear from Fig. 2 that the AgNPs prepared from the extracts of the selected plants are spherical and their average sizes approximated to be 45 ± 2.5 nm. Elemental composition found by EDS studies shows that they are 92 % silver. The remaining 8 % composition consists of carbon, nitrogen, oxygen etc. The origin of the carbon, nitrogen and oxygen appeared in EDS spectrum may be the phytonutrient molecules that were coated upon the particles in order to stabilize them.

TEM images of the particles obtained are given in Fig. 3. The insets in Fig. 3 respectively indicates the averaged out particle size distribution. TEM images recorded with the drop coated samples indicate the spherical and quasi-spherical particles with the higher percentage of the particles with 15 nm in both the cases. The results are comparable with the literature reports and precedents are selectively mentioned here. Spherical AgNPs were reported to be synthesized using plant, *Terminalia bellirica* extract [29] and extracellular synthesis using Fungus, *Aspergillus niger* [30]. The quasi-spherical shaped AgNPs were synthesized using apiin as reducing agent and analyzed [31]. In the SEM analyses, particle size appears bigger and more uniform compared to TEM. It may be understood therefore that, when the AgNP solutions were centrifuged to isolate the solid and dried in vacuum over $P_2O_5$, the smaller particles with 10 - 15 nm size coated with organic molecules must have agglomerated by being adsorbed on bigger particles, to form aggregates of around 45 nm size. Hence the particle size appears to be bigger with respect to SEM analysis.

Powder X-ray diffraction spectra of the AgNPs were recorded to understand the crystal structure and particle size. Powder XRD patterns of the AgNPs synthesized from the selected plants are shown in Fig. 4.

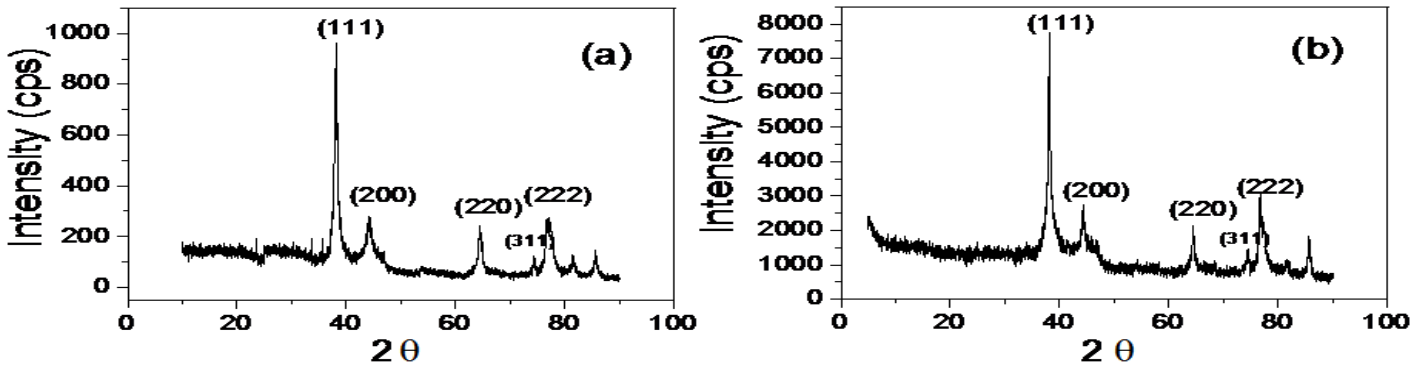


**Fig. 4: Powder XRD patterns of AgNPs synthesiszed from extract of (a) *Rubia cardifolia* and (b) *Mentha longifolia*.**

The identified crystallographic planes, (111), (200), (220), (311), (222) of the nanoparticles correspond to the face centered cubic (FCC) structure of the silver nanoparticles. Broad signals are characteristic of nanosized clusters of the particles. Sizes of the AgNPs prepared were calculated using Debye – Scherrer's formula $D = 0.94\ \lambda/\beta \cos\theta$, where D is the average crystalline size, $\lambda$ is the wavelength of X-ray, $\beta$ is full width at half maximum and $\theta$ is the angle of diffraction. The size of the particles calculated using the signal corresponding to (111) crystallographic plane at $2\theta$ value of 38.08, were found to be 28 nm in case of AgNPs synthesized from extract of *Rubia cardifolia* and 24 nm for those prepared from the extract of *Mentha longifolia*.

### 3.2. Antimicrobial activity studies

Silver nanoparticles are proved to exhibit antimicrobial activities [32] and the literature reports were extensively reviewed [33, 34].

In the present study, the antimicrobial properties were checked against the growth of the *E. coli* and *S. aureus* bacteria. Growth of the bacteria, in a nutrient agar media contaminated with AgNPs was monitored for a period of 20 hours and it was compared with that of the control. The control selected for the study was ciprofloxacin. Representative results of the AgNPs inhibiting the spread of *E. coli* bacteria are as shown in the following Fig.s. Fig. 5 shows the effects of the AgNPs prepared from water extract of *Rubia cardifolia* on the spreading of *E. coli* bacteria and Fig. 6 shows the effect AgNPs prepared from water extract of *Mentha longifolia* on the spreading of same bacteria.

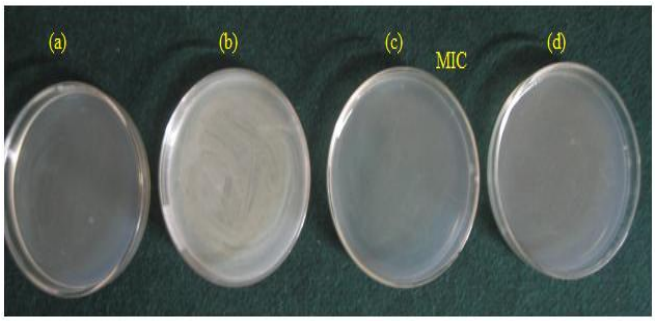


**Fig. 5: Growth of the *E-coli* bacteria in 20 ml nutrient agar media contaminated with (a) $2\times10^{-5}$ g/mL of ciprofloxacin, (b) 3.5 $\times10^{-5}$ g/mL, (c) 7 $\times10^{-5}$ g/mL, (d) 1.05 $\times10^{-4}$ g/mL of AgNPs prepared from extract of *Rubia cardifolia*.**

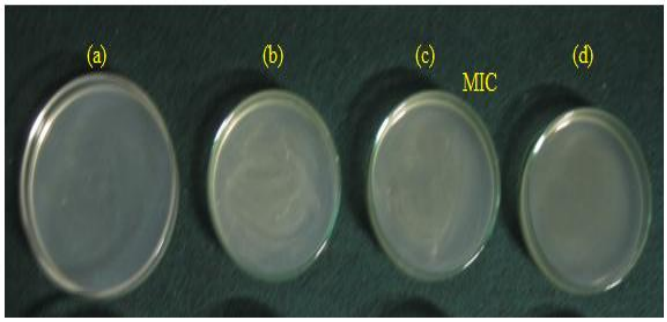


**Fig. 6: Growth of the *E-coli* bacteria in 20 ml nutrient agar media contaminated with (a) $2\times10^{-5}$ g/mL of ciprofloxacin, (b) 1.2 $\times10^{-4}$ g/mL, (c) 1.6 $\times10^{-4}$ g/mL, (d) 2 $\times10^{-4}$ g/mL of AgNPs prepared from extract of *Mentha longifolia*.**

The MIC indicated in Fig.s 5 and 6 is the minimum inhibitory concentration of the respective AgNPs at and above which the inoculated bacteria will not spread. The MIC against the growth of *E. coli* bacteria were found to be 70 µg/mL and 160 µg/mL respectively for the AgNPs synthesized from *Rubia cardifolia* and *Mentha longifolia*. The MIC of the reference selected for the study is 20 µg/mL in the laboratory conditions of the study. MIC of the AgNPs against *S. aureus* were 450 µg/mL and 160 µg/mL respectively in case of AgNPs synthesized from *Rubia cardifolia* and *Mentha longifolia*.

The results of the antibacterial activity studies reveal that the AgNPs prepared from the extracts of *Rubia cardifolia* inhibits the growth of *E. coli* bacteria to a maximum extent. However, the effect is not superior to that of the substance selected as reference. The bactericidal effect of the AgNPs synthesized from the extract of *Mentha longifolia* is same towards both the bacteria selected for the study.

## 4. CONCLUSIONS

Silver nanoparticles were prepared from the water extracts of leaf samples of *Rubia cardifolia* and *Mentha longifolia*, medicinal plants and the particles are very stable without any additional stabilizing agents. The particles were characterized to be of an average size of 28 nm and 24 nm respectively for those prepared from *Rubia cardifolia* and *Mentha longifolia*, and crystallize in to a face cantered cubic structure. The as prepared AgNPs exhibits antibacterial activity and inhibit the growth of *E. coli* and *S. aureus* bacteria. The MIC of AgNPs prepared from *Rubia cardifolia* against the growth of *E. coli* bacteria is found to be 70 µg / mL.

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