

## Antioxidant and Antibacterial Potential of Silver Nanoparticles Mediated Through Aqueous Root Extract of *Rumex Acetosa*

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### Abstract

Silver nanoparticles (AgNPs) were synthesized using green chemistry concept with aqueous root extract of *Rumex acetosa* was evaluated for its antioxidant, antibacterial and photo catalytic efficiency. The AgNPs synthesized using *Rumex acetosa* (RA-AgNPs) with dark brown colour exhibited surface plasmon spectra centred at 410 nm. The RA-AgNPs had face-centred cubic (fcc) phase structure and the crystallinity of the synthesized AgNPs were determined with XRD analysis. The results of the present study indicated that RA-AgNPs exhibited significant antioxidant activity, inhibitory effects toward gram positive and gram negative bacteria. Hence it could be concluded that the root extract of *Rumex acetosa* could be utilized in the medicine for the development of therapeutic principles.

**Keywords:** *Rumex acetosa*, antioxidant, antibacterial, silver nanoparticles, Photocatalysis

### 1. Introduction

Engineered non-toxic bioactive materials with high degree of specificity have myriad applications in the field of medicine, pharmaceuticals, textiles and many other industries<sup>1-4</sup>. Metallic nanoparticles such as gold, silver etc, differ in their physical, chemical, optical and electromagnetic properties compared to their bulk counterparts<sup>1</sup>. Silver in its nanosize exhibit enhanced properties, which endow them with wide range of applications especially in the field of medicine and environment<sup>5-7</sup>. Several synthetic procedures have been used for the synthesis of nanoparticles for engineering the salient features including particle size, shape and morphology based on desired application. The conventional approach in the synthesis of nanoparticles utilizing strong chemicals is undesirable due to its disadvantage in causing environmental toxicity and hence recent researches focuses on exploring biological agents. Plant extracts offer enormous impetus in the synthesis of nanoparticles ascribing to their easy availability, eco-friendly and cost effectiveness. Previous literatures uncover the utilization of plants and plant products for the biogenic fabrication of silver nanoparticles. But the synthesis of silver nanoparticles from *Rumex acetosa* root extract is seldom reported. Hence the present study was aimed at synthesizing silver nanoparticles from aqueous root extract of *Rumex acetosa* for evaluating its prodigious potential as antioxidant, antibacterial and photocatalytic agent.

*Rumex acetosa* is a perennial dioecious herb, widely distributed in Europe, Asia, Africa and North America<sup>8</sup>. The whole plant is used in traditional medicinal practices for treating various diseases. Roots of this plant are used in folk medicine since ancient time. It is used to treat fever, ulcer, dysentery, scabies, skin itches and also reported to possess antioxidant,

astringent and haemostatic properties <sup>9-11</sup>. The leaves are rich source of macro and micronutrients and have a distinctive taste because of high content of oxalic acid. Leaf juice is used for treating high blood pressure <sup>12</sup>, jaundice and liver diseases <sup>13</sup>. The flowers are reported to possess antitumor activity <sup>11</sup>. Previous literature reported the antioxidant activity of ethanolic extract of *Rumex acetosa* leaves <sup>14</sup> and stem <sup>15</sup>. Similarly, silver nanoparticles synthesized using *Rumex acetosa* leaves exhibited antioxidant, antibacterial and cytotoxicity properties <sup>16</sup>. The present work is designed with the primary objective of evaluating the efficacy of *Rumex acetosa* root extract as a mediator in the synthesis of silver nanoparticles. In order to perceive this objective, characterization studies of RA-AgNPs were carried out with advanced instrumentations besides evaluating their potential as antioxidant, antibacterial and photocatalytic agents. The present investigation will be a pioneer report on the synthesis of silver nanoparticles from *Rumex acetosa* root extract.

## **2. Materials and Methods**

### **2.1 Chemicals and Plant material**

Chemicals used in this study are analytical grade, purchased from Sigma Aldrich chemicals, USA. Fresh and healthy roots of *W. fruticosa* were collected from Kodaikanal hills, Tamilnadu, India and identified by the Department of Plant Science, Bharathidasan University, Thiruchirapalli, Tamilnadu, India.

### **2.2. *R. acetosa* root extract**

The shadow dried roots of *R. acetosa* was rinsed with tap water and then washed thrice with sterile distilled water to remove all the dust particles. 20 grams of this dried root powder was added to 200 ml of sterile distilled water and boiled for 10 minutes in a hot plate with magnetic stirrer. The resulting solution was filtered using Whatman filter paper No.1 and stored at 4°C for further use.

### **2.3 Phytochemical analysis**

About 100g of dried root powder was added separately to different solvent (chloroform, methanol, hexane and water) for the extraction of phytochemicals using soxhlet apparatus for 24 h. The extract collected was filtered and concentrated using rotary evaporator. The solvent extracts were stored at 5°C for future experiments. The solvent extracts were subjected to phytochemical screening for the identification of alkaloids, flavonoids, saponins, tannins and glycosides as per the protocol of Kodota et al. <sup>17</sup>.

### **2.4 Gas chromatography Mass Spectrometry analysis**

The chemical composition of methanolic extract of *Rumex acetosa* roots was analysed using GC- MS (Agilen HP – 5973 Chromatograph equipped with Shimadzu QP – 500 mass spectrometer) as described by Abbasipour et al., (2011). Fused silica column coated with polydimethyl siloxane (30m/25nm and a film thickness of 0.25 µm) was used. Helium was used as the carrier gas at 1 ml/min flow rate. The temperature of the injector was fixed at 250 °C. The temperature of the oven was set at 60°C for 1 min, which was then increased to

225°C steadily at a rate of 2°C /min. After the steady rise in temperature of the column it was maintained for 5 min. The ionization voltage used was 70ev with 1:25 split rate. The composition of root extract was identified by comparing the retention time (or) its mass spectra with that of known compounds (or) from published data.

## **2.5 Biosynthesis of *R.acetosa* root extract mediated silver nanoparticles (RA-AgNPs)**

The nanoparticles was prepared as per the protocol of Ananth and Thangamathi <sup>18</sup>. Briefly, 10 ml of the root extract was added to 90 ml of 1mM AgNO<sub>3</sub> solution and mixed vigorously with magnetic stirrer for 30 minutes and allowed to rest at room temperature for the reduction of silver ions. The change in the colour of the reaction mixture indicates the synthesis of AgNPs. The synthesized nanoparticle solution was then subjected to centrifugation at 10,000 rpm for 10 minutes. Pellets obtained after decanting the supernatant was dispersed in deionized water thrice to remove the uncoordinated biological molecules. Finally the pellets were dried in lyophilizer.

## **2.6 Characterization of RA-AgNPs**

The characterization of synthesized nanoparticles was done according to Ananth and Thangamathi, (2018). A small aliquot of the synthesized nano solution was initially screened for the reduction of silver ions by UV-Vis spectrophotometer (Jasco UV-650, Japan) between wavelengths ranging from 300-800 nm at regular time intervals. The X-Ray powder diffraction (XRD) was carried out using X'Pert Pro X-ray diffractometer operated at a voltage of 40kv and a current of 30 mA with Cu K $\alpha$  radiation.

## **2.7 *In vitro* antioxidant assay**

The antioxidant property of the synthesized nanoparticles (*RA-AgNPs*) was determined with DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), nitric oxide scavenging assays and total reducing power assay. DPPH radical scavenging and total reducing power assay were followed as per the protocol of Syed et al <sup>19</sup>. The nitric oxide scavenging activity of *RA-AgNPs* was evaluated following the methodology of Hashemi and Ebrahimzadeh <sup>20</sup>. The inhibition of ABTS free radicals by *RA-AgNPs* was determined by the procedure of Hajebi et al., <sup>21</sup>.

## **2.8 Antibacterial activity of *R.acetosa* AgNPs**

Bacterial species *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumonia* and *Klebsiella pneumoniae* were obtained from IMTECH, India. Disc diffusion assay was performed to determine the antibacterial activity of *R.acetosa* crude flower extract and synthesized AgNPs following the method of Bauer et al., <sup>22</sup>. These bacterial strains were inoculated in the nutrient broth medium and cultures were adjusted to 0.5 McFarland standards ( $1 \times 10^8$  CFU mL<sup>-1</sup>) and spread on to sterilized Mueller Hinton agar plates. The plates were dried for 15 min before being used for sensitivity test. Discs were impregnated with different concentrations of AgNPs (40, 60, 80  $\mu$ g/ ml) and crude extract (20  $\mu$ L) respectively and placed on Mueller Hinton agar surface. Standard antibiotic Amoxicillin was

used as positive control. The plates were then incubated at 37°C for 24 h and the zone of inhibition was measured and recorded in triplicates.

## 2.9 Statistical Analysis

Experimental samples were run in triplicates. The values are given as mean ± standard deviation. The tool used was SPSS (Version 21). Variations among the experimental groups were determined by ANOVA followed by T-test. *p*-values were calculated for each data and interpretation was carried out for each sample. *p*-value < 0.05, 0.01 or 0.001 were considered statistically significant.

## 3. Result and Discussion

### 3.1 Phytochemical screening

The phytochemical analysis of the roots of *R. acetosa* was performed using hexane, chloroform, petroleum ether, methanol and water as solvents. Table (1) presents the bioactive compounds present in each extracts. The aqueous extract showed the presence of proteins and tannins. Flavonoids, steroids and tannins were observed with hexane extract. The chloroform extract showed the presence of almost all secondary metabolites except proteins and flavonoids. Tannins and terpenoids were extracted with acetone and methanol extract exhibited the presence of alkaloids, tannins and terpenoids. (Table 1).

**Table 1: Phytochemical analysis of *Rumex acetosa* root extracts**

| Phytochemicals | Solvent extracts |        |            |         |          |
|----------------|------------------|--------|------------|---------|----------|
|                | Water            | Hexane | Chloroform | Acetone | Methanol |
| Carbohydrates  | -                | -      | +          | -       | -        |
| Proteins       | +                | -      | -          | -       | -        |
| Saponins       | -                | -      | +          | -       | -        |
| Alkaloids      | -                | -      | +          | -       | +        |
| Glycosides     | -                | -      | +          | -       | -        |
| Flavonoids     | -                | +      | -          | -       | -        |
| Terpenoids     | -                | -      | +          | +       | +        |
| Steroids       | -                | +      | +          | -       | -        |
| Tannins        | +                | +      | +          | +       | +        |

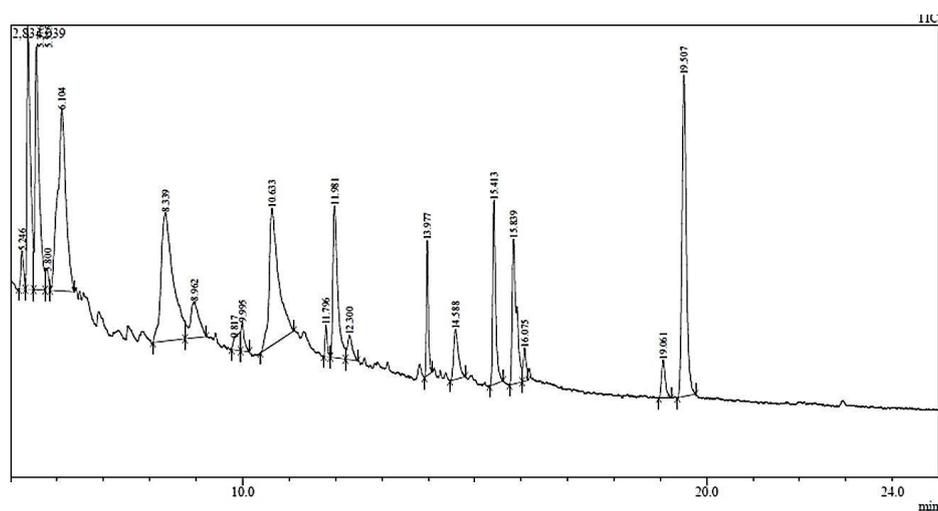
### 3.2 GC MS analysis of *R. acetosa* root extract

The methanolic extract of *Rumex acetosa* revealed the presence of various phytochemicals (Table 4 and Fig 18). The major compounds identified were 2-Furancarboxaldehyde, 5-(Hydroxymethyl)-, 1,2,3-Propanetriol, Monoacetate, 2,3-Dihydro-3,5-Dihydroxy-6-Methyl-4h-Pyran-4-One, Sucrose, Methyl,  $\alpha$ -D-Glucopyranoside, 1-Hydroxy-2-Acetyl-3-Metho naphthalene, Chrysophanol (Fig 19). The other compounds that were present in minute concentrations includes 5-Methyl-2,4-Hexanedione, 2-Methylbutyl Methylmalonate 1TMS, Levoglucosan, Ethyl Phthalate, Myristic Acid,  $\beta$ -D-Ribopyranoside, 3-O-Acetyl-Methyl, Palmitic

Acid, Ethyl 2-Hydroxycaproate Chrysophanic Acid, Anthranol.

**Table 2: Identification of bioactive compounds of *Rumex acetosa* roots**

| Peak | R.Time | Area % | M.formula  | Compound name                                     |
|------|--------|--------|--|---|
| 1    | 9.992  | 1.29   | C <sub>7</sub> H <sub>12</sub> O <sub>2</sub>                | 5-Methyl-2,4-Hexanedione                          |
| 2    | 10.233 | 0.10   | C <sub>6</sub> H <sub>5</sub> O <sub>3</sub>                 | 2-Furancarboxaldehyde, 5-(Hydroxymethyl)-         |
| 3    | 12.532 | 0.37   | C <sub>5</sub> H <sub>10</sub> O <sub>4</sub>                | 1,2,3-Propanetriol, Monoacetate                   |
| 4    | 13.123 | 0.19   | C <sub>12</sub> H <sub>24</sub> O <sub>4</sub> Si            | 2-Methylbutyl Methylmalonate 1TMS                 |
| 5    | 13.631 | 0.43   | C <sub>6</sub> H <sub>8</sub> FN <sub>5</sub> O <sub>4</sub> | 2,3-Dihydro-3,5-Dihydroxy-6-Methyl-4h-Pyran-4-One |
| 6    | 14.234 | 24.79  | C <sub>12</sub> H <sub>22</sub> O <sub>4</sub>               | Sucrose   |
| 7    | 15.873 | 0.51   | C <sub>6</sub> H <sub>10</sub> O <sub>5</sub>                | Levoglucofan                                      |
| 8    | 16.651 | 2.61   | C <sub>12</sub> H <sub>14</sub> O <sub>4</sub>               | Ethyl Phthalate                                   |
| 9    | 16.915 | 0.97   | C <sub>12</sub> H <sub>14</sub> O <sub>4</sub>               | Ethyl Phthalate                                   |
| 10   | 17.128 | 0.22   | C <sub>7</sub> H <sub>14</sub> O <sub>6</sub>                | $\alpha$ -D-Glucopyranoside, Methyl               |
| 11   | 17.404 | 1.60   | C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>               | Myristic Acid                                     |
| 12   | 18.225 | 0.50   | C <sub>7</sub> H <sub>14</sub> O <sub>6</sub>                | $\alpha$ -D-Glucopyranoside, Methyl               |
| 13   | 18.620 | 28.12  | C <sub>11</sub> H <sub>16</sub> O <sub>7</sub>               | $\beta$ -D-Ribopyranoside, 3-O-Acetyl-Methyl      |
| 14   | 19.301 | 0.36   | C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>               | Palmitic Acid                                     |
| 15   | 19.458 | 0.23   | C <sub>8</sub> H <sub>16</sub> O <sub>3</sub>                | Ethyl 2-Hydroxycaproate                           |
| 16   | 20.406 | 4.77   | C <sub>13</sub> H <sub>12</sub> O <sub>3</sub>               | Ethanone, 1-(1-Hydroxy-3-Methoxy-2-Naphthalenyl)- |
| 17   | 21.911 | 0.51   | C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>               | Linoleic Acid                                     |
| 18   | 22.295 | 0.38   | C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>               | Linoleic Acid                                     |
| 19   | 22.527 | 0.55   | C <sub>15</sub> H <sub>13</sub> O <sub>3</sub>               | 1,8,9-Anthracenetriol, 3-Methyl-                  |
| 20   | 24.196 | 31.93  | C <sub>15</sub> H <sub>10</sub> O <sub>4</sub>               | Chrysophanol                                      |



**Figure 1 GC MS chromatogram of *Rumex acetosa* root extract**

**3.3 Synthesis and characterization of Wf-AgNPs**

The green chemistry approach in the synthesis of nanoparticles, particularly employing plants is advantageous over other biogenic methods because plants can be used in large scale synthesis. Addition of *R. acetosa* root extract to aqueous silver nitrate solution (1mM) turned

the colour of the solution to dark brown. The change in the colour of the reaction mixture indicated the formation of AgNPs. The colour developed gradually intensified with increase in incubation time. After 180 min of incubation the colour of the solution remained stable indicating the saturation of AgNPs synthesis. The change in the colour of the synthesized nano solution was due to Surface plasmon resonance (SPR) of AgNPs.

### UV-vis spectrophotometry analysis

The absorbance spectrum revealed a broad peak at 410 nm. The optimal conditions for the synthesis of AgNPs was fixed at 70°C, pH= 7, 1mM AgNO<sub>3</sub> solution and 1: 9 ratio of extract and AgNO<sub>3</sub> and 180 min of incubation time. The UV spectral analysis exhibited SPR peaks at 410 nm measured at regular time intervals (Figure 2). Maximum absorbance was obtained after 180 min of incubation at room temperature. The excitation of surface plasmon resonance observed at 410 nm increased with increase in time (10 min to 180 min of incubation) but without any shift in the wavelength.

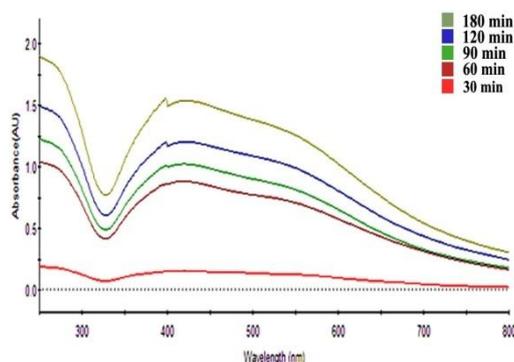


Figure 2 UV absorption spectrum of RA-AgNPs

### XRD analysis

The dry powder of *R.acetosa* root extract mediated AgNPs was used for XRD analysis. The aqueous extract and AgNPs solution that was used as control did not show any characteristics peaks. The diffracted intensities of AgNPs were recorded at  $2\theta$  angles from  $10^\circ$  to  $90^\circ$ . The XRD diffractogram presented in Fig (7) showed prominent De Braags reflection with a values of  $38^\circ$ ,  $44^\circ$ ,  $64^\circ$  and  $77^\circ$  corresponding to the crystalline plane index (111), (200), (220) and (311) respectively. These planes of index confirm the synthesis of faced centre cubic structure of silver nanoparticles. XRD pattern revealed that the silver nanoparticles synthesized were highly pure since there is no other peak indicating the presence of other substance. The peak broadening and noise visualized were probably due to nanosized particles and crystalline biological macromolecules. The results indicate that the Ag ions are reduced by the flower extract.

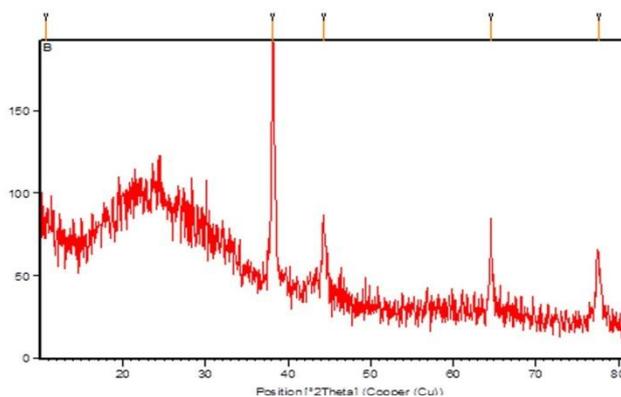


Figure 3 XRD spectrum of RA-AgNPs

### 3.4 Antioxidant assay

The antioxidant activity of RA-AgNPs was evaluated with DPPH, ABTS, Nitric oxide radical scavenging assay and total reducing power assays. The high unstable nature of free radicals lead to the generation of ROS that interact with other molecules in the biochemical reactions and causes cellular damage<sup>23</sup>. Quenching of these free radicals is instigated by the bio-reductive groups of phytochemicals present on the surface of the AgNPs<sup>24</sup>. Figure (4) describes the free radical scavenging property of synthesized AgNPs. Though the synthesized AgNPs exhibited significant scavenging activity, it was comparatively low with reference to standard ascorbic acid used in the present investigation. Results indicate that the antioxidant property of AgNPs was dose-dependent. The DPPH showed positive correlation with different concentrations of biogenic AgNPs that were used in the present study. High free radical scavenging activity (90.45%) was observed with maximum concentration of AgNPs (50µg/ml) used in the present study. The change in colour from purple to yellow when RA-AgNPs was treated with DPPH indicates the completion of scavenging activity, which was measured spectrophotometrically at 517 nm. The present study observed the DPPH free radical scavenging activity of Wf-AgNPs ranged from 35% to 90% with concentrations of 10 – 50 µg/ml. But the root extract of *Nepeta leucophylla* exhibited only 37.67% of DPPH radical scavenging activity at 50 µg/ml concentration<sup>25</sup>. Figure (4b) shows the Wf-AgNPs antioxidant activity against ABTS free radicals. The antioxidant activity increased with increase in concentration of AgNPs. 72% of ABTS scavenging activity was observed with 50 µg/ml of RA-AgNPs. Nitric oxide plays a vital role as regulatory molecules in immune, nervous and cardiovascular systems. The RA-AgNPs exerts NO scavenging activity by inhibiting the formation of nitrite and competing with oxygen and oxides of nitrogen. Due to very less stability in high electronegative environment, NO gets reduced by readily accepting electrons from RA-AgNPs. Figure (4c) displays the percentage of the nitric oxide inhibition by RA-AgNPs. 83% of inhibition was recorded at 50 µg/ml concentration.

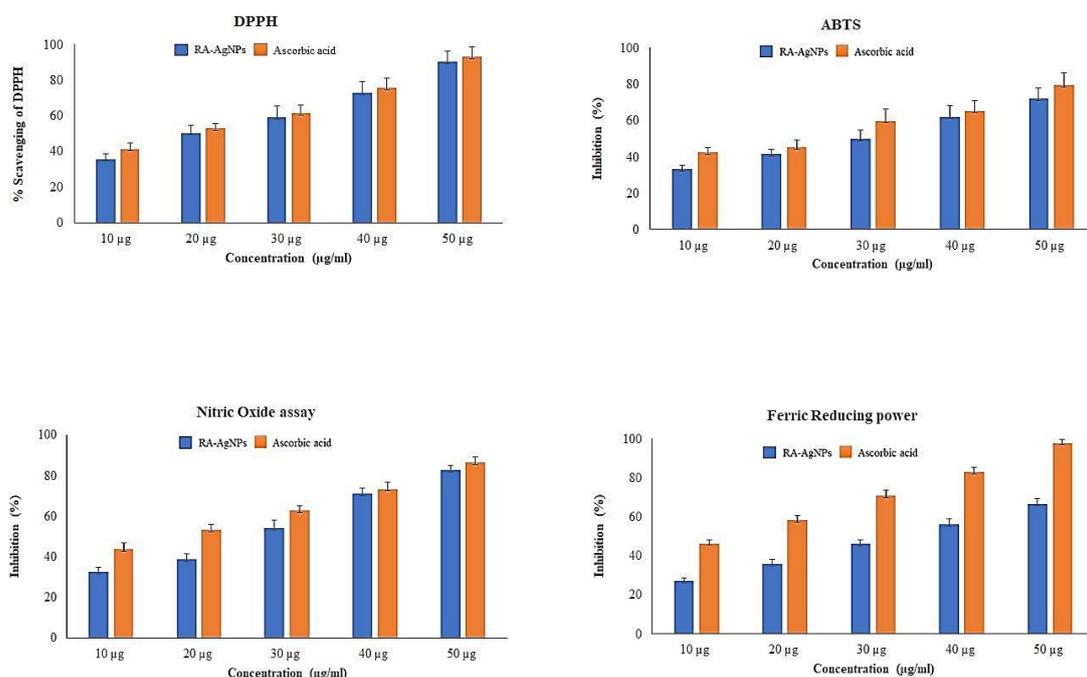


Figure 4 Antioxidant activity of RA-AgNPs

The total reducing power of RA-AgNPs and the reference compound ascorbic acid was presented in Figure (4d). Different concentrations of Wf-AgNPs (10, 20, 30, 40 and 50 µg/ml) were used for the reduction of Fe<sup>3+</sup> to Fe<sup>2+</sup>. The reduction of Fe ions was indicated by the formation of Perl blue colour that was measured using UV-visible spectrophotometer at 700 nm. Maximum percentage of reduction activity (66%) was observed with 50 µg/ml of RA-AgNPs. The antioxidant property of AgNPs was due to its hydrogen donating capacity, which breaks the free radical chain<sup>26</sup>. A dose dependent response for the reducing power of AgNPs synthesized using root extract of *Helicteres isora* was observed. These AgNPs exhibited better poor activity compared to standard BHT<sup>27</sup>.

### 3.5 Antibacterial activity

The antibacterial efficacy of RA-AgNPs was evaluated against *E.coli*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae* and *S.aureus*. The results indicated that different concentrations of RA-AgNPs (40, 50 and 60 µl) displayed effective zone of inhibition measuring 12 mm, 12.5 mm and 13 mm respectively against *E.coli* (Fig 5). Similarly, 14 mm, 16.5 mm and 17.5 mm inhibition zones were observed against *S.aureus*. *Klebsiella pneumoniae* exhibited 10 mm, 12 mm and 14 mm zone of inhibition with the biosynthesized AgNPs. Zone of inhibition observed with *Streptococcus pneumoniae* was found to be 10mm, 12 mm and 10 mm. The standard antibiotic, used in the study produced 16 mm, 18 mm, 25 mm and 18 mm respectively for *Klebsiella pneumoniae*, *Staphylococcus aureus*, *E.coli* and *Streptococcus pneumoniae*. The root extract of *Rumex hastatus*, belonging to same species *Rumex* was reported to possess antibacterial activity which inhibited the growth of gram negative bacteria *K.pneumoniae* exhibiting (5mm) zone of inhibition compared to other gram negative bacteria tested<sup>28</sup>.

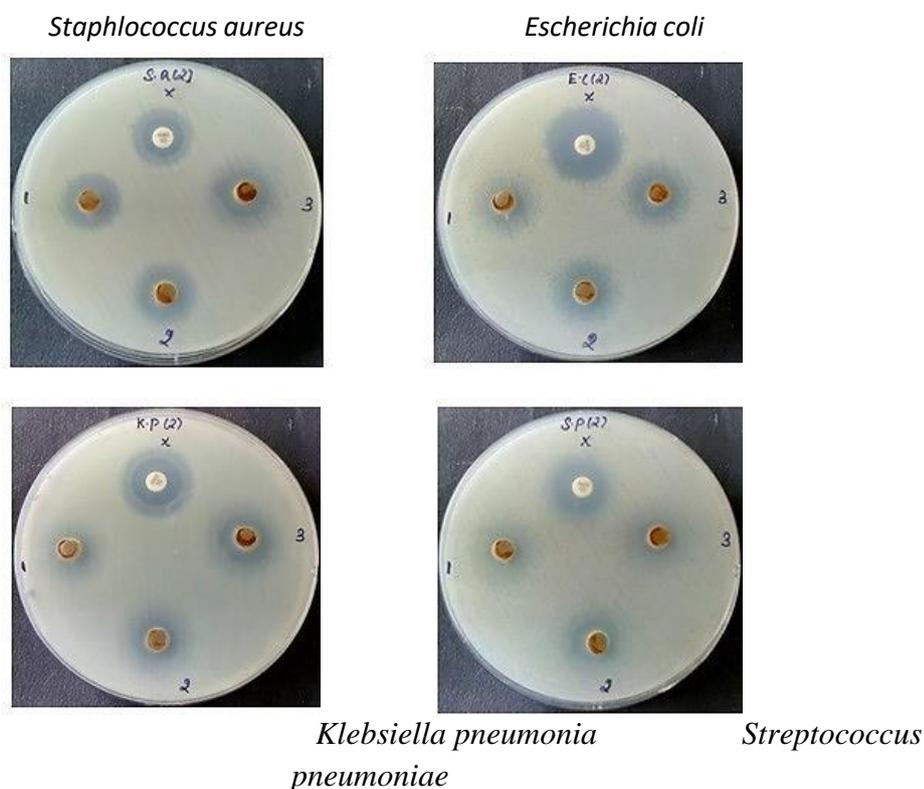


Figure 5 Antibacterial activity of RA-AgNPs

#### 4. Conclusion

The present study is a pioneer and a simple procedure in the synthesis of silver nanoparticles using *Racetosa* root extract. This bio reductive synthesis of AgNPs is eco-friendly, renewable and cost effective approach for the production of metallic nanoparticles in aqueous solution at room temperature. Characterization of synthesized nanoparticles revealed spherical shaped that are face-centred cubic phase structure and crystalline in nature. The synthesized RA-AgNPs exhibited good antioxidant and antibacterial activity. Hence the silver nanoparticles using *R.acetosa* can be effectively used in biomedical applications. Further studies can be carried out in determining the mechanism of antibacterial activity of AgNPs was whether due to inhibition of cell wall synthesis or disturbance in cell membrane integrity due the physical damage.

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