4.1 Ageratina adenophora

*Ageratina adenophora* is an ethnopharmaceutical medicinal plant. In this study, it was aimed to use *Ageratina adenophora* plant extract as a reducing agent in the reduction of silver ion to Ag\(^0\) (zero valent silver). Leaves of *Ageratina adenophora* were subjected to extraction at three different thermal conditions (room temperature, 60°C and 100°C) and at different pH conditions (4, 7 and 9.2). The extracts thus obtained were subjected to the reduction reaction.

The reaction between Ag ions and the extract of *Ageratina adenophora* obtained at room temperature, at 60°C, at 100°C and in all pH conditions studied did not show corresponding absorption signals in UV-Vis analysis (Fig.4.1.1). In addition, noticeable colour change was not observed in the reaction. It is inferred that the reduction process is unsuccessful.

**Fig.4.1.1.** UV-Vis spectra of silver ions with *Ageratina adenophora* extract obtained at (a) room temperature, (b) 60°C and (c) 100°C
Even though the reduction reaction was not properly recognised by SPR band, XRD characterization was performed to confirm it. The XRD result shows distinct diffraction peak at 32.8° (Fig. 4.1.2), which is indexed for the plane 110 of the body centered cubic silver nitrate. This data matches well with database of joint committee on powder diffraction standards (JCPDS) file No. 731860.

**Fig.4.1.2. XRD pattern of *Ageratina adenophora* leaf extract containing silver.**

Additional peaks observed at 29.09 and 38.7° are due to the bio-inorganic compounds and proteins present in the extract. All these facts confirm the presence of silver ion (Ag⁺) only, but not zero valent silver (Ag⁰).

*Ageratina adenophora* leaf extract was found to be unsuccessful in the reduction of silver ion to zero valent silver. Hence, further analysis was not carried out using this plant extract.
4.2 Acorus calamus

Acorus calamus is a medicinal plant. In this study, it was aimed to use Acorus calamus plant extract as a reducing agent in the reduction of silver ion to zero valent silver. Root of Acorus calamus was subjected to extraction at three different thermal conditions (room temperature, 60°C and 100°C) and at different pH conditions (4, 7 and 9.2). The extracts thus obtained were subjected to the reduction of AgNO₃.

The reaction between Ag ions and extract of Acorus calamus obtained at room temperature, at 60°C and at 100°C pH studied at all pH conditions did not show any corresponding UV-Vis absorption signals (Fig.4.2.1). And also, noticeable colour change was not observed in the reaction.

Fig.4.2.1. UV-Vis spectra of silver ions with Acorus calamus extract obtained at (a) room temperature, (b) 60°C and (c) 100°C

The XRD result shows distinct diffraction peak at 32.8°, which is indexed for the plane 110 of the body centered cubic silver nitrate (Fig.4.2.2). This data matches well with
database of JCPDS file No. 731860. The additional peak noticed at 29.09° is due to bio-
inorganic compounds and proteins present in the extract. All these facts confirm the absence
of zero valent silver.

Fig.4.2.2. XRD pattern of Acorus calamus extract containing silver

Acorus calamus root extract was found to be unsuccessful in the reduction of silver
ion to zero valent silver. Hence, further analysis was not carried out using this plant extract.
4.3 *Calotropis procera*

**Synthesis of Ag⁰**

Experiment was performed for the synthesis of Ag⁰ from the precursor AgNO₃ using *Calotropis procera* flower aqueous extract as reducing agent. For this, *Calotropis procera* flower was subjected to extraction at three different thermal conditions such as room temperature, at 60°C, 100°C and at different pH conditions (4, 7 and 9.2). Simple stirring as well as ultrasonic methods were employed.

The reduction reaction between Ag ions and the extract of *Calotropis procera* performed at 60°C only showed corresponding UV absorption signal (SPR band). Noticeable colour change (from pink to yellowish brown) was observed in the reaction mixture in both stirred and ultrasonic methods.

In the pH study, good result (change in colour) was observed at neutral pH for the extract obtained at 60°C (Fig. 4.3.1).

**Fig.4.3.1. Reaction mixture**

![Extract](image1)
![Synthesis by stirring method](image2)
![Synthesis by ultrasonic method](image3)

**UV-Visible analysis**

The reduction reaction was monitored by the UV-Vis spectra with characteristic SPR signal. Fig.4.3.2 shows the SPR signal obtained at pH 7 for the extract of *Calotropis*
*procera* obtained at 60°C. Other pH ranges (4 and 9.2) did not respond well. At pH 7 (after 1 h stir in the stirring method and after 15 minutes in the sonication method), the colour changed from pink to yellowish brown. The change in colour indicated the reduction of Ag\(^+\) to Ag\(^0\). Thus, a pH of 7 was considered as optimum pH.

In simple stirring method, as the reaction time was increased from 1 to 12 h, only broad band was appeared in the UV-Vis spectra, but further increase in the stirring time from 12 to 16 h yielded good absorbance peak due to plasmonic oscillations of silver.

Ultrasonic method showed broad absorbance band at 450 nm even at a reaction time of 30 min. In this method, the reduction of Ag\(^+\) to Ag\(^0\) was achieved for a reaction time up to 2 h. Further increase in reaction time did not produce any significant effect.

When compared between simple stirring method and ultrsonication method, SPR signal was appeared at 402 nm for the former and at 450 nm (longer wavelength) for the latter. This shift in wavelength might be due to the differences in size and shape in the formation of Ag\(^0\).

**Fig.4.3.2. UV-Vis spectra of Calotropis procera extract containing silver at pH 7**
XRD analysis

XRD analysis was carried out for Ag\textsuperscript{0} synthesized by both simple stirring and ultrasonic methods.

The XRD result of silver synthesized by stirring method shows four distinct diffraction peaks at 38°, 44°, 64° and 77°, which are indexed for the planes 111, 200, 220 and 311 respectively for the face centered cubic silver (Fig.4.3.3). This data matches well with JCPDS file No. 89.3722 for silver. Thus, the formation of zero valent silver is confirmed in this method.

Fig.4.3.3. XRD pattern of silver synthesized by simple stirring and ultrasonic methods

The XRD result of silver synthesized by ultrasonic method shows four distinct diffraction peaks at 38°, 44°, 64° and 77°, which are indexed for the planes 111, 200, 220 and 311 respectively of the face centered cubic silver. This data matches well with the database of JCPDS file No. 89.3722 for silver. Thus, the formation of zero valent silver is confirmed in this method.
Additional peaks obtained in XRD pattern could be due to the bio-inorganic compounds and protein matters present in the extract (as evidenced from the intensity of the Bragg reflections of strong X-ray scattering points in the crystalline phase arise from proteins in the nanoparticles synthesis) [1].

The average grain size of the silver particles synthesized by stirring as well as ultrasonic methods was calculated using Scherrer’s formula \[d=\frac{(0.89\lambda \times 180°)}{\beta\cos\theta}\] and found to be 40 and 35 nm respectively.

**FT-IR analysis**

FT-IR spectra were recorded individually for the crude extract as well as for the reaction mixtures obtained after selected duration of synthesis at pH 7 (Fig.4.3.4)

- For the crude extract, absorptions are noticed at 3400 cm\(^{-1}\), 1634 cm\(^{-1}\), 1414 cm\(^{-1}\) and 1399 cm\(^{-1}\). The band appeared at 3400 cm\(^{-1}\) is attributed to stretching of hydroxyl group, which is present as water in the reaction mixture. The prominent peak at 1634 cm\(^{-1}\) is attributed to C=O stretching possibly due to the presence of amide group, which might be responsible for the reduction of Ag ion to Ag\(_0\). The peak at 1414 cm\(^{-1}\) may be due to the stretching of C-O group. The peak at 1399 cm\(^{-1}\) is due to the bending vibration of C-H.

- For the extract containing Ag\(_0\) synthesized by simple stirring method, the absorption peak expected at 1414 cm\(^{-1}\) for the stretching of C-O group is absent in the reaction mixture that could be due to the stabilization of silver through C-O group (capping agent). The peak at 1399 cm\(^{-1}\) is due to the bending vibration of C-H [2]. This peak is shifted to lower frequency (1384 cm\(^{-1}\)) when compared to the response of crude extract only.
• For the extract containing Ag\(^0\) synthesized by ultrasonication, the C-O stretching peak as well as C-H bending peak are absent that could be due to stabilization of Ag\(^0\) through these groups. One distinct additional peak is present at 2380 cm\(^{-1}\), which is attributed NH\(_3^+\) group. From these results, it is inferred that amide group present in the crude extract is responsible for the reduction of silver ion and stabilization of Ag\(^0\).

Fig.4.3.4. FT-IR spectra of *Calotropis procera* crude extract and the extract containing silver synthesized by different methods.

SEM analysis
Silver particles synthesized by stirring as well as ultrasonication methods were subjected to SEM analysis. Cubic morphology of silver particles synthesized by stirring method is distinctly seen from the SEM image (Fig.4.3.5). The sizes of the silver particles are observed in the range from 200 to 1000 nm.

Silver particles synthesized by ultrasonication method shows uneven spherical morphology in the SEM image and the sizes of silver particles are observed in the range between 100 and 500 nm.

**Fig.4.3.5. SEM images of silver synthesized by (A) stirring method, (B) ultrasonication method**

**EDX analysis**

The energy dispersive spectrum (EDX) of silver synthesized by stirring and ultrasonication method reveals the presence of silver particles as evidenced from the peaks observed ~3 KeV (Fig.4.3.6), which is corresponding to the binding energy of AgL. There is also a strong peak for Cu in the EDX spectrum, which is due to the use of copper plate in the process of analysis. Other elemental signals observed in the spectrum are possibly due to the phytochemical elements present in the *Calotropis procera* extract.
The EDX result is used for the quantitative analysis of elements present. Quantitative analysis of sample shows good level of silver content (72% in stirring method and 30% in ultrasonication method). These results also confirm the presence of elemental silver.

**Fig.4.3.6. EDX spectra of silver (analyzed over Cu plate) synthesized by (A) stirring method and (B) ultrasonication method**

**Cytotoxicity analysis of Calotropis procera extract and the extract containing Ag⁰.**

Cytotoxicity experiments were conducted using HeLa cell line with aqueous extract of *Calotropis procera* to understand the potential application in biomedical field. Screening was performed using HeLa cell line with MTT assay. *Calotropis procera* extract containing Ag⁰ (ultrasonic method) showed higher cytotoxicity towards HeLa cancer cells than neat *Calotropis procera* extract and *Calotropis procera* extract containing Ag⁰ (stirring method). Extract containing Ag⁰ synthesized by ultrasonic method induced 99.73% death of HeLa cells at a sample concentration of 7.36 µg/mL (Table 4.3.1).
Table 4.3.1. Effect of sample concentration and % cell inhibition

<table>
<thead>
<tr>
<th>Sample Conc. (µg/mL)</th>
<th>Neat Extract</th>
<th>% Cell Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Extract containing Ag(^0) synthesized</td>
</tr>
<tr>
<td></td>
<td></td>
<td>By stirring method</td>
</tr>
<tr>
<td>0.4</td>
<td>2.13</td>
<td>0.44</td>
</tr>
<tr>
<td>0.9</td>
<td>17.95</td>
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</tr>
<tr>
<td>1.8</td>
<td>39.55</td>
<td>64.97</td>
</tr>
<tr>
<td>3.7</td>
<td>58.93</td>
<td>81.68</td>
</tr>
<tr>
<td>7.4</td>
<td>70.93</td>
<td>84.00</td>
</tr>
</tbody>
</table>

Noticeable morphological changes in HeLa cells were observed after 48 h of treatment with *Calotropis procera* aqueous extract, extract containing Ag\(^0\) synthesized by both simple stirring and ultrasonication methods. There is a reduction in the number of cells with distorted shapes and condensation of cytoplasm, which is significant with smaller cells. This phenomenon is utmost understandable when the concentration of the extract is increased up to 7.4 µg/mL (Fig. 4.3.7).

The cell inhibition effects are highly significant at sample (extract containing Ag\(^0\) synthesized by ultrasonication method) concentrations of 0.4 µg/mL as well as 7.4 µg/mL. The median effective concentration (IC50) of neat extract and the extract containing Ag\(^0\) synthesized by stirring as well as ultrasonication methods is obtained as 3.01, 1.54, and 0.02 µg/mL respectively.
Fig. 4.3.7. Micrographs on the effect of sample concentration and % cell inhibition

<table>
<thead>
<tr>
<th>Sample Conc. (µg/mL)</th>
<th>Neat extract</th>
<th>Cell inhibition</th>
<th>Extract containing Ag synthesized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>By stirring method</td>
</tr>
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<td><img src="https://via.placeholder.com/150" alt="Image" /></td>
<td><img src="https://via.placeholder.com/150" alt="Image" /></td>
</tr>
<tr>
<td>0.9</td>
<td><img src="https://via.placeholder.com/150" alt="Image" /></td>
<td><img src="https://via.placeholder.com/150" alt="Image" /></td>
<td><img src="https://via.placeholder.com/150" alt="Image" /></td>
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<tr>
<td>1.8</td>
<td><img src="https://via.placeholder.com/150" alt="Image" /></td>
<td><img src="https://via.placeholder.com/150" alt="Image" /></td>
<td><img src="https://via.placeholder.com/150" alt="Image" /></td>
</tr>
<tr>
<td>3.7</td>
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<td><img src="https://via.placeholder.com/150" alt="Image" /></td>
<td><img src="https://via.placeholder.com/150" alt="Image" /></td>
</tr>
<tr>
<td>7.4</td>
<td><img src="https://via.placeholder.com/150" alt="Image" /></td>
<td><img src="https://via.placeholder.com/150" alt="Image" /></td>
<td><img src="https://via.placeholder.com/150" alt="Image" /></td>
</tr>
</tbody>
</table>
Development of antibacterial finished polypropylene nonwoven material using neat Calotropis procera extract and the extract containing Ag⁰

Polypropylene nonwoven material was coated with neat Calotropis procera extract. And also, polypropylene nonwoven material was coated with the extract containing Ag⁰ to impart antibacterial effect. In order to understand the nature of coating / adhesion, the uncoated PP and coated PP were characterized by FT-IR and SEM analyses.

FT-IR analysis: The FT-IR spectra of PP, Calotropis procera extract coated PP, Calotropis procera extract containing Ag⁰ (stirring method) coated PP and Calotropis procera extract containing Ag⁰ (sonication method) coated PP are given in Fig.4.3.8.

Fig.4.3.8. FT-IR spectra of polypropylene nonwoven coated with Calotropis procera extract and extract containing Ag⁰.

When compared between the test samples, little changes are observed in the peak positions around 1600 and 3300 cm⁻¹. In addition, the intensity of peak around 1000 cm⁻¹ is
also altered. Thus, it is inferred that the coating of extract on the polypropylene nonwoven is significant. The band formed around 1613 cm\(^{-1}\) for the coated PP (which is absent in uncoated PP) is assigned to –NH bending resulting from the –NH group species present in the extract.

**SEM analysis:** SEM images of uncoated polypropylene and coated polypropylene are given in Fig.4.3.9. The coating of biomass on to polypropylene substrate is clearly observed. The bright spots representing Ag\(^0\) are clearly noticeable in SEM images (C and D), which are absent in uncoated polypropylene and neat extract coated polypropylene.

**Fig.4.3.9. SEM images of (A) uncoated PP, (B) *Calotropis procera* extract coated PP, (C) *Calotropis procera* extract containing Ag\(^0\) coated PP (stirring method) and (D) *Calotropis procera* extract containing Ag\(^0\) coated PP (ultrasonication method)**

EDX analysis: The uncoated and coated polypropylene (PP) materials were subjected to EDX analysis. Peaks are observed (Fig.4.3.10) for C, O and N, which are present in the PP matrix. The spectra representing the extract containing Ag\(^0\) (stirring
method) and the extract containing Ag\(^0\) (ultrasonication method) reveals the confirmation of elemental silver in the PP matrix with a weight % of 2.07 and 2.72 respectively. The peaks expected for P, K and Si present in the extract are not observed in this spectrum. This may be due to the suppression of the peaks due to lower concentration of extract when compared to the PP matrix.

Fig.4.3.10. EDX spectra of (A) uncoated PP, (B) *Calotropis procera* extract coated PP, (C) extract containing Ag\(^0\) coated PP (stirring method) and (D) extract containing Ag\(^0\) coated PP (ultrasonication method)

Evaluation of Antibacterial efficiency

Antibacterial efficiency of uncoated PP and coated PP was investigated by estimating the number of viable bacteria cells in the *S. aureus* and *E. coli* suspension after
being contact with substrate for two different time durations of 24 h and 48 h (Fig.4.3.11). Significant antibacterial efficiency is observed for all substrates. The difference in the percentage of inhibition is significant at 24 h and almost saturated at 48 h. Antibacterial efficiency of *Calotropis procera* extract containing Ag\(^0\) (synthesized by ultrasonication method) coated PP is higher than others.

**Fig.4.3.11. Comparison of Antibacterial efficiency of uncoated PP and coated PP**

<table>
<thead>
<tr>
<th></th>
<th>E. coli 24h</th>
<th>E. coli 48h</th>
<th>S. aureus 24h</th>
<th>S. aureus 48h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncoated PP</td>
<td>21.8</td>
<td>50</td>
<td>20.5</td>
<td>46.3</td>
</tr>
<tr>
<td>Neat extract coated PP</td>
<td>37.5</td>
<td>50</td>
<td>32.3</td>
<td>46.3</td>
</tr>
<tr>
<td>Extract containing Ag coated PP (stirring method)</td>
<td>50</td>
<td>60.5</td>
<td>35.1</td>
<td>56</td>
</tr>
<tr>
<td>Extract containing Ag coated PP (sonication method)</td>
<td></td>
<td>59.3</td>
<td>71</td>
<td>60.9</td>
</tr>
</tbody>
</table>

**Summary of findings**

- *Calotropis procera* flower has been tested for an effective reducing as well as stabilizing agent in the eco-friendly synthesis of Ag\(^0\) from the precursor AgNO\(_3\). The formation of Ag\(^0\) is observed by visible colour change of reaction mixture from pink to brown and confirmed by UV–visible (surface plasmon resonance) and XRD.
analyses. From the XRD result, the average grain size of $\text{Ag}^0$ is calculated as 40 and 35 nm for product obtained by stirring and ultrasonication methods respectively. FT-IR spectrum reveals the presence of reducing phytochemicals in the crude plant extract.

- *Calotropis procera* extract containing $\text{Ag}^0$ synthesized by ultrasonication method shows the cytotoxicity level of 99.73 % death of HeLa cells at a sample concentration of 7.29 µg/mL.

- The antibacterial results of coated PP show the improved inhibition efficiency against *E.Coli* for the *Calotropis procera* extract containing $\text{Ag}^0$ synthesized by ultrasonication method (71 %) than *Calotropis procera* extract containing $\text{Ag}^0$ synthesized by stirring method (60.5 %). The crude extract coated PP shows an inhibition efficiency of 50 %. In a similar analysis against *S.aureus*, crude extract showed an inhibition efficiency of 46.3 %. The extract containing $\text{Ag}^0$ synthesized by ultrasonication method shows higher inhibition efficiency (60.9 %) than the extract containing $\text{Ag}^0$ synthesized by stirring method (56 %). The role of ultrasound is significant in the size, shape and efficiency of $\text{Ag}^0$. 
4.4 Manihot esculenta

Synthesis of Ag$^0$

AgNO$_3$ was used as precursor in the synthesis. It was reduced to Ag$^0$ using aqueous extract of *Manihot esculenta* leaves as reducing agent as well as capping agent. *Manihot esculenta* leaves were subjected to extraction at three different thermal conditions such as room temperature, at 60°C and at 100°C, and at three different pH conditions (4, 7 and 9.2). Two different (simple stirring and ultrasonic) methods were employed in the reduction process.

In both methods, when the reaction was initiated, the colour of the extract changed from dark green. In the stirring method, brown colour was formed (Fig.4.4.1) after a stirring time of 15 min, but in the sonication method, the mixture turned to brown colour within 1 min of reaction time. The change in colour from dark green to brown was considered as the reduction reaction of silver ion to Ag$^0$.

Fig.4.4.1. Reaction mixture

![Extract](image1) ![Synthesis by stirring method](image2) ![Synthesis by ultrasonic method](image3)

UV-Visible analysis

The reaction mixture was monitored by UV-Vis analysis with the characteristic SPR signal for silver particles. The reactions carried out with the extracts obtained at 60°C and at 100°C did not show UV-Vis absorption signals at pH 4 and 9.2 conditions. Characteristic
absorption signal was observed at neutral pH only for the extract obtained at room
temperature. Hence further experiments were carried out with this pH only.

The UV-Vis spectra of the reaction between Ag ions and extract of *Manihot
esculenta* is given in Fig.4.4.2. Until a reaction time of 15 min, it did not display the
characteristic signal for the reduction process; but for a reaction time of 30 min, distinct
peak was observed at 440 nm indicating the formation of Ag$^0$.

**Fig. 4.4.2. UV-Vis spectra of *Manihot esculenta* extract containing silver ions**

When the reaction mixture was subjected to ultrasonication for 1 min, no
representative peak for silver was observed, but the characteristic signal of Ag$^0$ was
observed at 380 nm after an ultrasonication time of 5 min.

The SPR peak position was not identical between stirring and ultrasonication
methods adopted. This could be due to the variation of size and shape of Ag$^0$ synthesized.

**XRD analysis**

XRD profile of Ag$^0$ synthesized by stirring and ultrasonication method is displayed
in Fig.4.4.3. The diffraction peaks obtained at 38°, 44°, 64° and 77° are assigned to 111,
200, 220 and 311 planes respectively of the face centered cubic lattice of silver. This data is matched with the JCPDS file No. 89.3722 for silver.

The average grain size of the silver particles synthesized was calculated using Scherrer’s formula and found to be 45 and 25 nm for the experiments carried out under simple stirring and ultrasonication methods respectively.

Absence of additional diffraction peaks indicates the absence of crystallographic impurities in the sample. All the observed diffractions are related to silver with face centered cubic (fcc) symmetry. The high intense peak for fcc is generally 111 diffraction, which is observed in the sample of analysis. The high intensity of peak reflects the high degree of crystallinity of the silver particles.

Fig. 4.4.3. XRD pattern of silver synthesized by stirring and ultrasonic methods

FT-IR analysis
FT-IR spectra were recorded for the crude extract (Fig.4.4.4) and also for the reaction mixture after 30 min in simple stirring method and after 5 min in ultrasonication method.

For the neat *Manihot esculenta* extract, IR absorptions were observed at 3500 cm\(^{-1}\), 2072 cm\(^{-1}\), 1637 cm\(^{-1}\) and 1060 cm\(^{-1}\). The band at 3500 cm\(^{-1}\) is attributed to the stretching of hydroxyl group. The band at 2072 cm\(^{-1}\) may be attributed to the terminal alkene. The prominent peak at 1637 cm\(^{-1}\) is attributed to C=O stretching possibly due to the presence of amide group that is responsible for the reduction of Ag ion to Ag\(^0\). The peak at 1060 cm\(^{-1}\) may be due to alcoholic C-O stretching absorption.

**Fig. 4.4.4.** FT-IR spectra of *Manihot esculenta* extract and extract containing Ag\(^0\).
For the *Manihot esculenta* extract containing Ag\(^0\) synthesized by stirring method, the 2072 cm\(^{-1}\) peak intensity decreased when compared to that obtained with neat extract. In addition, the band expected at 1060 cm\(^{-1}\) was observed at 1042 cm\(^{-1}\) only, which could be due to the capping action by O-H group in the synthesis [2].

For the *Manihot esculenta* extract containing Ag\(^0\) synthesized by ultrasonication method also, the 2072 cm\(^{-1}\) peak intensity decreased when compared to that obtained with neat extract. In addition, the absence of band at 1042 cm\(^{-1}\) is noticed when compared with the neat extract.

From the above results, it is observed that biomolecules present in the crude extract are responsible for the reduction of Ag ion and stabilization of Ag\(^0\).

**SEM analysis**

Ag\(^0\) particles synthesized by stirring as well as ultrasonic methods were subjected to SEM analysis. SEM micrographs show clusters of spheres for Ag\(^0\) synthesized by stirring method (Fig.4.4.5) and lump like shape for the Ag\(^0\) synthesized by ultrasonication method. The grain sizes of the silver particles obtained are in the range from 200 to 1000 nm.

**Fig.4.4.5. SEM image of silver synthesized by (A) stirring method, (B) ultrasonication method**
EDX analysis

The EDX spectra of Ag\(^0\) in the biomass synthesized by stirring and ultrasonication methods reveal the presence of silver (\(~3\text{KeV}\) (Fig.4.4.6). The spectra indicate the formation of Ag\(^0\). Peak of Cu is due to the use of copper substrate in sample preparation. Peaks of P, K, Cl and Si are due to the presence of phytochemical elements present in the biomass. The silver content in the sample is 63 % for the synthesis by stirring method and 54 % for the synthesis by ultrasonication method.

Fig. 4.4.6. EDX spectra of *Manihot esculenta* extract containing Ag\(^0\) synthesized by (A) stirring method, (B) ultrasonication method

HR-TEM analysis

Ag\(^0\) synthesized by stirring as well as ultrasonic methods were subjected to HR-TEM analysis.
The HR-TEM micrographs depict different sizes and shapes of the silver particles synthesized. The sizes of Ag\textsuperscript{0} synthesized by stirring method vary from small (10 nm) to large (150 nm) particles and the average particle size is observed as 30 nm (Fig.4.4.7).

The Ag\textsuperscript{0} synthesized by ultrasonication method shows variation in their size from 5 nm to 100 nm and the average particle size is observed as 30 nm (Fig.4.4.8).

Well detached uneven spherical morphology is noticed for Ag\textsuperscript{0} synthesized by stirring as well as ultrasonic methods.

**Fig.4.4.7.** HR-TEM (original and magnified) images of Ag\textsuperscript{0} synthesized by stirring method

![HR-TEM images](image1)

**Fig.4.4.8.** HR-TEM (original and magnified) images of Ag\textsuperscript{0} synthesized by ultrasonication method

![HR-TEM images](image2)
Selected Area Electron Diffraction (SAED) analysis

Ag\textsuperscript{0} synthesized by stirring as well as ultrasonic methods were subjected to SAED analysis. The representative high resolution images (Fig.4.4.9) with clear lattice fringes reveal the presence of silver favourably in the 111 plane. The interplanar distance of silver 111 plane is in good agreement with the 111 d-spacing of bulk Ag. This observation is comparable with the SAED pattern reported by Krishnaraj et al. [3].

**Fig. 4.4.9. SAED pattern of Ag\textsuperscript{0} synthesized by (A) stirring method, (B) ultrasonication method**

![SAED pattern images](image)

**Cytotoxicity analysis of Manihot esculenta extract and the extract containing Ag\textsuperscript{0}**

Cytotoxicity analysis of neat extract and the extract containing Ag\textsuperscript{0} was tested against the HeLa cell line with the MTT assay.

Ag\textsuperscript{0} in the extract synthesized by both stirring and ultrasonication methods show significant cytotoxicity, which is higher than that obtained with neat *Manihot esculenta* extract only. The activity is depending on the dose of extract containing Ag\textsuperscript{0} (Table.4.4.1). It shows 100 % inhibition of HeLa cells at a sample concentration of 3.6 µg/mL.
Table 4.4.1. Effect of concentration and % cell inhibition

<table>
<thead>
<tr>
<th>Sample conc. (µg/mL)</th>
<th>% Cell Inhibition</th>
<th>Neat extract</th>
<th>By stirring method</th>
<th>By ultrasonication method</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td>0.44</td>
<td>2.4</td>
<td>8.26</td>
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</tr>
<tr>
<td>0.9</td>
<td>6.66</td>
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</tbody>
</table>

Noticeable morphological changes in HeLa cells are observed after 48 h of treatment with *Manihot esculenta* aqueous extract as well as extract containing Ag\(^0\) synthesized by stirring and ultrasonication methods. This is clearly understandable with the increase in sample concentration of the sample upto 7.3 µg/mL (Fig. 4.4.10).

The change in cell population is significant for the extract containing Ag\(^0\) synthesized by both stirring and ultrasound methods at a concentration of 3.6 µg/mL. The IC50 values of neat extract, the extract containing Ag\(^0\) synthesized by stirring method and the extract containing Ag\(^0\) synthesized by ultrasonication method are calculated as 2.2, 0.99 and 0.99 µg/mL respectively.
Fig. 4.4.10. Micrographs on the effect of sample concentration and cell inhibition

<table>
<thead>
<tr>
<th>Sample Conc. (µg/mL)</th>
<th>Cell Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
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<tr>
<td>0.4</td>
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<tr>
<td>0.9</td>
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<tr>
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</tr>
<tr>
<td>3.6</td>
<td><img src="image10" alt="Image" /></td>
</tr>
</tbody>
</table>

*Development of antibacterial finished polypropylene nonwoven material using neat Manihot esculenta extract and the extract containing Ag⁰*
PP nonwoven materials were pad-coated with neat *Manihot esculenta* extract and the extract containing Ag\(^0\) separately to impart antibacterial activity. The coated PP materials were characterized by FT-IR and SEM analyses to understand the nature of coating.

**FT-IR analysis:** FT-IR spectra of uncoated PP, *Manihot esculenta* extract coated PP and extract containing Ag\(^0\) coated PP are given in Fig.4.4.11. When compared between them, changes are observed in the peak intensities around 1000 cm\(^{-1}\), 1400 cm\(^{-1}\), 1600 cm\(^{-1}\) and 3300 cm\(^{-1}\).

**Fig.4.4.11. FT-IR spectra of PP nonwoven and coated PP with *Manihot esculenta* extract and the extract containing Ag\(^0\)**

From the above changes, it is inferred that the coating is significant. The band around 1613 cm\(^{-1}\) for the coated PP is corresponding to N-H bending due to the presence of N-H bonds in the species of the extract. This band is absent in uncoated PP.
**SEM analysis:** SEM images of uncoated PP and coated PP are given in Fig.4.4.12. Effect of coating (image B) and the presence of Ag⁰ (images C and D) are clearly observed. It is inferred that silver particles are embedded on PP matrix.

Fig.4.4.12. SEM images of (A) uncoated PP, (B) *Manihot esculenta* extract coated PP, (C) *Manihot esculenta* extract containing Ag⁰ coated PP (stirring method) and (D) *Manihot esculenta* extract containing Ag⁰ coated PP (ultrasonication method)

**EDX analysis:** Uncoated PP, neat extract coated PP and extract containing Ag⁰ coated PP materials were subjected to EDX analysis (Fig 4.4.13). Peaks are observed for C, O and N, which are present in the PP matrix. The presence of Ag⁰ on PP matrix is confirmed and the weight % is noted as 1.71 (stirring method) and 6.30 (ultrasonic method).
The peaks expected for P, K and Si (present in the neat extract) are not observed in this spectrum. This may be due to the suppression of the peaks at lower concentration of extract when compared to the PP matrix.

**Fig.4.4.13.** EDX spectra of (A) uncoated PP, (B) *Manihot esculenta* extract coated PP, (C) *Manihot esculenta* extract containing Ag$^0$ coated PP (stirring method) and (D) *Manihot esculenta* extract containing Ag$^0$ coated PP (ultrasonication method)

**Evaluation of Antibacterial efficiency**

*Manihot esculenta* extract and the extract containing Ag$^0$ coated PP were tested for antibacterial efficiency by estimating the number of viable bacteria cells against *S. aureus* and *E. coli* suspension for a contact time of 24 h and 48 h (Fig.4.4.14).
Fig. 4.4.14. Comparison of Antibacterial efficiency of uncoated PP and coated PP

The difference in the inhibition percentage is significant at different time of contact for 24 h as well as for 48 h. Neat extract coated PP shows good antibacterial activity against *S. aureus*. PP coated with extract containing Ag⁰ (ultrasonication method) with a contact time of 48 h shows the highest (92.1 %) activity against *E. coli*.

Even though the neat extract exhibited good antibacterial activity, the presence of Ag⁰ in the extract improved the desired activity by synergistic effect against both microorganisms studied. It is assumed that the size of the silver particles play a vital role in the antibacterial activity.

**Summary of finding:**
• Ag\textsuperscript{0} was successfully synthesized from AgNO\textsubscript{3} using a natural plant \textit{Manihot esculanta} extract by simple stirring and ultrasonication methods. Extract is tested for effective reducing as well as stabilizing agent and found to be successful. The formation of Ag\textsuperscript{0} in the reaction mixture is observed with visible colour change from green to brown and confirmed by UV–Visible (surface plasmon resonance) analysis. The presence of Ag\textsuperscript{0} is again confirmed by XRD and EDX analyses. From the XRD result, the average grain size of Ag\textsuperscript{0} synthesized by simple stirring and ultrasonication methods is calculated to be in the range from 25 to 55 nm. FT-IR spectrum reveals the presence of reducing phytochemicals in the crude extract.

• \textit{Manihot esculanta} extract containing Ag\textsuperscript{0} synthesized by stirring as well as ultrasonication methods show the cytotoxicity 100% proliferation of HeLa cells at a treatment concentration of 3 µg/mL.

• The antibacterial activity of coated PP against \textit{E. coli} for 48 h is found to be in the order of 71% (neat extract) < 75% (extract containing Ag\textsuperscript{0} synthesized by stirring method) < 92.1% (extract containing Ag\textsuperscript{0} synthesized by ultrasonication method). Similarly, the antibacterial activity of coated PP against \textit{S.aureus} is found to be in the order of 48.7% (neat extract) < 63.4% (extract containing Ag\textsuperscript{0} synthesized by stirring method) < 68.2% (extract containing Ag\textsuperscript{0} synthesized by ultrasonication method).

4.5 \textit{Coleus aromaticus}

\textbf{Synthesis of Ag\textsuperscript{0}}

Ag\textsuperscript{0} was synthesized using the extract of \textit{Coleus aromaticus} (as reducing agent) and AgNO\textsubscript{3} (as precursor). Extraction was done at three different thermal conditions such as room temperature, 60°C, 100°C, and at three different pH conditions (4, 7 and 9.2). Simple stirring and ultrasonication methods were employed. After the reduction process, colour of
the reaction mixture changed from yellow to blackish yellow in both stirring and ultrasonication methods (Fig.4.5.1).

**Fig.4.5.1. Reaction mixture**

![Extract](image1) ![Synthesis by stirring method](image2) ![Synthesis by ultrasonic method](image3)

**UV-Visible analysis**

UV-Vis spectra of the reaction mixture containing Ag$^0$ synthesized by stirring and ultrasonication methods are presented in Fig.4.5.2.

The reaction between Ag ions and the extract of *Coleus aromaticus* obtained at room temperature as well as at 100°C did not show corresponding UV-Vis absorption signals at pH 4 and pH 9.2, but good representative absorption signals were observed at pH 7 for the extract obtained at 60°C only. Hence this condition was considered as optimum.

UV-Vis spectra depicts that, Ag$^0$ is formed at longer time (1 h) in stirring method and shorter time (10 min) in ultrasonication method. The characteristic SPR bands of Ag$^0$ synthesized by stirring and ultrasonication methods are seen at 430 and 380 nm respectively. This variation in reaction time could lead to changes in the shape and size of the synthesized Ag$^0$ particles. The absorbance observed in the spectra (Fig.4.5.2) is due to plasmonic oscillations exhibited by the Ag$^0$.

**Fig.4.5.2. UV-Vis spectra of *Coleus aromaticus* extract containing Ag$^0$**
XRD analysis

XRD analyses were carried out for the Ag⁰ synthesized by stirring as well as ultrasonication methods (Fig.4.5.3). They show four distinct peaks at 38°, 44°, 64° and 77° corresponding to the 111, 200, 220 and 311 planes respectively for the fcc silver. This matches good with the JCPDS file No. 89.3722. Thus, formation of silver is confirmed.

The average grain size of silver particle is calculated using Scherrer’s formula and observed to be 34 nm in stirring method and 24 nm in ultrasonication method. No spurious diffractions were observed in the XRD pattern indicating the absence of crystallographic impurities in the test sample.

Fig.4.5.3. XRD pattern of silver synthesized by stirring and ultrasonication methods
FT-IR analysis

FT-IR analysis was performed to predict the role of stabilizing capability of *Coleus aromaticus* extract. Individual FT-IR spectrum was recorded for the neat extract, reaction mixture obtained by stirring method and ultrasonication methods.

For the neat extract, absorption peaks are observed at 1635 cm\(^{-1}\), 1417 cm\(^{-1}\) and 1386 cm\(^{-1}\). The prominent peak at 1635 cm\(^{-1}\) is attributed to the C=O stretching of amide group that is responsible for the reduction of Ag ion to Ag\(^0\). The peak at 1417 cm\(^{-1}\) may be due to the stretching of C-O group. The band at 1386 cm\(^{-1}\) is due to C-H bending vibration [2].

The reaction mixture (both stirring and ultrasonication methods) shows peaks at 1635 cm\(^{-1}\), 1414 cm\(^{-1}\) and 1384 cm\(^{-1}\) (Fig.4.5.4). When compared to the neat extract, a blue shift (from 1386 to 1384 cm\(^{-1}\)) is noticed in C-H bending. Another blue shift (from 1417
cm\(^{-1}\) to 1414 cm\(^{-1}\) \) is also observed for C-O stretching. These shifts may be due to the capping action by C-O group in the synthesis of Ag\(^0\). From these results, it is observed that the crude extract is effective enough for the synthesis and stabilization of Ag\(^0\).

**Fig.4.5.4. FT-IR spectra of *Coleus aromaticus* extract and the extract containing Ag\(^0\)**

**SEM analysis**

Synthesized silver particles were subjected to SEM analysis. Fig.4.5.5 displays SEM images. Polygonal shaped Ag\(^0\) particles (Fig.4.5.5A) and nanowire Ag\(^0\) (Fig.4.5.5B) are distinctly noticed. The sizes of the silver particles obtained are in the range from 60 to 500 nm and Ag nanowire is in 30 nm width. Thus, the reaction condition is playing a vital role in size and shape of the silver.

**Fig.4.5.5. SEM image of silver particles synthesized by (A) stirring method, (B) ultrasonication method**
EDX analysis

Ag\(^0\) synthesized by stirring and ultrasonication methods were subjected to energy dispersive spectrum (EDX) analysis.

**Fig.4.5.6.** EDX spectra of *Coleus aromaticus* extract containing Ag\(^0\) synthesized by (A) stirring method and (B) ultrasonication method

The EDX (Fig.4.5.6) confirms the presence of Ag\(^0\) (peak at ~3KeV). A strong peak at ~8KeV is for copper used in the preparation of the sample for SEM analysis. Other elemental signals reported in the spectrum are possibly due to the phytochemical elements present in the *Coleus aromaticus* extract.
Cytotoxicity activity analysis of Coleus aromaticus extract and the extract containing Ag$^0$

The cytotoxicity activity of neat extract and the extract containing Ag$^0$ was performed against HeLa cell line with the MTT assay.

Neat extract shows 28% activity towards HeLa cancer cells at a concentration of 7.26 µg/mL (Table.4.5.1).

Table.4.5.1. Effect of sample concentration and cell inhibition

<table>
<thead>
<tr>
<th>Sample Conc. (µg/mL)</th>
<th>% Cell Inhibition</th>
</tr>
</thead>
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<td>Neat extract</td>
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</table>

Extract containing Ag$^0$ synthesized by stirring as well as ultrasonication methods show excellent cytotoxicity. Significant activity (99% death) is noticed towards HeLa cancer cells (Fig.4.5.7) at sample concentration of 3.6 µg/mL. From these results, it is inferred that biomass containing Ag$^0$ shows better cytotoxicity activity than with neat extract. The silver particles are responsible for cell inhibition.
### Fig. 4.5.7. Micrographs on the effect of sample concentration and cell inhibition

<table>
<thead>
<tr>
<th>Sample conc. (µg/mL)</th>
<th>Neat extract</th>
<th>Extract containing Ag synthesized</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>By stirring method</td>
<td>By sonication method</td>
</tr>
<tr>
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<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
<tr>
<td>0.9</td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
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<td>3.6</td>
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<tr>
<td>7.2</td>
<td><img src="image13.png" alt="Image" /></td>
<td><img src="image14.png" alt="Image" /></td>
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</table>
Morphological changes in HeLa cells are noticed after 48 h treatment with the neat extract and the extract containing Ag\(^0\). This phenomenon is well understood when the concentration of the extract increased up to 7.2 µg/mL.

The changes are significant for the extract containing Ag\(^0\) synthesized by stirring as well as ultrasonication methods at a concentration of 3.6 µg/mL. The IC50 values of the neat extract, the extract containing Ag\(^0\) synthesized by stirring and ultrasonication methods are noticed as 14.77, 0.42, and 1.05 µg/mL respectively.

**Development of antibacterial finished polypropylene nonwoven material coated with neat Coleus aromaticus extract and extract containing Ag.**

Polypropylene nonwoven materials were coated with neat Coleus aromaticus extract and the extract containing Ag\(^0\) separately to impart antibacterial activity. In order to understand the nature of coating, the uncoated PP and coated PP were characterized by FT-IR, SEM and EDX analyses.

**FT-IR analysis:** FT-IR spectra of uncoated PP and coated PP clearly demonstrate the effect of coating (Fig.4.5.8).

When compared between them, changes are observed in the peak intensities around 1000 cm\(^{-1}\) and 1400 cm\(^{-1}\). From these results, it is noticed that coated PP attained significant improvement when compared to uncoated PP.

![Fig.4.5.8. FT-IR spectra of PP nonwoven and coated with Coleus aromaticus extract and extract containing Ag.](image-url)
**SEM analysis:** SEM images of uncoated PP and coated PP are shown in Fig.4.5.9. The effect of coating can be visibly observed from the images. The presence of silver particles on PP matrix can be viewed bright spots in the coated material, which is absent in both neat extract coated and uncoated PP. SEM images reveal that the silver particles are embedded on the PP matrix.
EDX analysis: The uncoated PP and coated PP were subjected to EDX analysis (Fig.4.5.10). Peaks are observed in all spectra for C, O and N, which are present in the PP matrix. The EDX spectra depict the presence and confirmation of silver on the PP matrix. The weight % of silver is observed as 1.71 and 3.23 for the Ag\(^0\) synthesized by stirring and ultrasonication method respectively.
Evaluation of Antibacterial efficiency

Antibacterial efficiency of neat extract and the extract containing Ag\(^0\) coated PP was investigated against *S. aureus* and *E. coli* suspension (24 and 48 h). The antibacterial activity of Ag\(^0\) coated PP is higher than the neat extract coated and uncoated PP. The difference in the percentage of inhibition is significant at 24 h as well as at 48 h (Fig.4.5.11). This may be due to the presence of silver and its effect.
Summary of findings

- *Coleus aromaticus* leaves extract was employed as an effective reducing as well as stabilizing agent for the synthesis of $\text{Ag}^0$. Ag nanowires with a width of $\sim 30$ nm were successfully synthesized by an ultrasonication method. The characterization techniques for the analysis of reaction product confirmed the formation of $\text{Ag}^0$. The presence of $\text{Ag}^0$ is confirmed by XRD and EDX analyses. From the XRD pattern, the sizes of silver particles are calculated as 34 and 24 nm for stirring method and ultrasonication method respectively. The size and shape of silver formed are dependent on the experimental conditions employed. FT-IR spectrum revealed the presence of reducing phytochemicals in the crude extract.
• *Coleus aromaticus* extract with Ag\(^0\) synthesized by simple stirring and ultrasonication shows the cytotoxicity (99 %) proliferation of HeLa cells at a treatment concentration of 3 µg/mL.

• The antibacterial activity of coated PP against *E. coli* is ordered as *Coleus aromaticus* extract containing Ag\(^0\) by ultrasonication method (71 %) > extract containing Ag\(^0\) by stirring method (65.7 %) > neat extract (57.8 %). In a similar analysis, the antibacterial activity against *S.aureus* is ordered as *Coleus aromaticus* extract containing Ag\(^0\) by ultrasonication method (68.2) > extract containing Ag\(^0\) by stirring method (60.9 %) > neat extract (51.2 %).

• The special finding in this study is the formation of nanowire silver by a simple reaction between Ag ion and *Coleus aromaticus* extract under ultrasonication method.
4.6 *Tragia ramosa*

**Synthesis of Ag⁰**

Ag⁰ was synthesized using *Tragia ramosa* extract as a reducing agent. AgNO₃ was used as precursor. *Tragia ramosa* leaves were subjected to extraction by three different thermal conditions such as room temperature, 60°C, 100°C and at different pH conditions (4, 7 and 9.2). Ag⁰ synthesis was performed by two different methods such as simple stirring and ultrasonication methods.

The extract obtained at 60°C and 100°C did not show the corresponding UV-Vis absorption signals at pH 4 and pH 9.2. The characteristic UV-Vis signals were observed only for the extract obtained at room temperature and at pH 7. Hence these conditions were chosen as optimum.

The colour of the reaction mixture changed from light brown to blackish brown that indicated the reduction reaction of Ag ion to Ag⁰ (Fig.4.6.1).

**Fig.4.6.1. Reaction mixture**

Extract | Synthesis by stirring method | Synthesis by ultrasonic method
---|---|---

**UV-Visible analysis**

The reduction reaction was regularly monitored by UV-Vis absorption at different time intervals. The UV-Visible spectrum of the extract containing silver particles is shown in Fig.4.6.2. In general, the formation and nature of SPR signal depends on the composition, shape and size of the silver particles formed.
Characteristic SPR absorption bands are observed at 420 and 374 nm for Ag\(^0\) synthesized by stirring and ultrasonication methods respectively. The position of SPR band is different for stirring and ultrasonication methods. A blue shift is observed from 420 to 374 nm, which may be due to the change in size and shape of Ag\(^0\).

From the UV-Vis analysis, it is inferred that reduction is complete in 1 h by stirring method, but complete within 15 min in ultrasonication. Hence, the ultrasonication synthesis method is considered as a quick method.

**Fig.4.6.2. UV-Vis spectra of *Tragia ramosa* extract containing silver**

**XRD analysis**

XRD analysis was performed for the extract containing Ag\(^0\) synthesized by both stirring and ultrasonication methods. The XRD patterns obtained are presented in Fig.4.6.3.

The Ag\(^0\) synthesized by stirring as well as sonication methods show four distinct diffraction peaks at 38\(^\circ\), 44\(^\circ\), 64\(^\circ\) and 77\(^\circ\), which are indexed for the planes 111, 200, 220 and 311 respectively for the fcc cubic silver (JCPDS file No. 89.3722). Thus, the formation of silver is confirmed.

The additional peak obtained at 42\(^\circ\) for Ag\(^0\) synthesized in sonication method may be due to the bioinorganic compounds and protein matters present in the extract. The
average grain size of the particles is calculated as 62 and 47 nm for Ag\(^0\) obtained in stirring and sonication method respectively.

**Fig.4.6.3. XRD pattern of silver synthesized by stirring and ultrasonication methods**

![XRD pattern of silver synthesized by stirring and ultrasonication methods](image)

**FT-IR analysis**

Individual FT-IR spectrum was recorded for the neat extract, reaction mixture obtained by stirring method and ultrasonication methods (Fig.4.6.4).

- FT-IR absorption peaks of *Tragia ramosa extract* are observed at 3300 cm\(^{-1}\), 2365 cm\(^{-1}\), 1639 cm\(^{-1}\), 1360 cm\(^{-1}\) and 1221 cm\(^{-1}\). The band at 3300 cm\(^{-1}\) is attributed to the stretching of hydroxyl group. The peak at 2365 cm\(^{-1}\) attributes to the nitrile group. The prominent peak at 1639 cm\(^{-1}\) is attributed to the C=O stretching mode of amide group, which could be responsible for the reduction of Ag ion to Ag\(^0\). The peak at 1360 cm\(^{-1}\) may be attributed to the tertiary amine and the peak at 1221 cm\(^{-1}\) is attributed to the aromatic C-H bending.
• In the spectrum of Ag\textsuperscript{0} synthesized by stirring method, the peak expected at 1360 cm\textsuperscript{-1} and 1221 cm\textsuperscript{-1} are shifted towards lower wavenumber of 1345 cm\textsuperscript{-1} and 1190 cm\textsuperscript{-1} respectively. This may be due to the stabilization of the Ag\textsuperscript{0} particles.

• In the spectrum of Ag\textsuperscript{0} synthesized by ultrasonication method, the peak at 1345 cm\textsuperscript{-1} is shifted to higher wavenumber of 1388 cm\textsuperscript{-1}, which may be due to the interaction of Ag\textsuperscript{0}, phytochemical fragments associated with heterooxy groups like nitrates (any inorganic compound that forms bonds of a covalent nature within a molecular ion fragment cation or anion may produce a characteristic absorption spectrum with associated group frequencies) [4]. The peak expected at 1221 cm\textsuperscript{-1} is shifted towards lower wavenumber of 1198 cm\textsuperscript{-1} that may be attributed to the stabilization of Ag\textsuperscript{0} particles through C-H group.

From the above results, it is observed that phytochemicals of \textit{Tragia ramosa} extract are responsible for the reduction of silver ion and stabilization of Ag\textsuperscript{0}.

\textbf{Fig.4.6.4. FT-IR spectra of \textit{Tragia ramosa} extract and the extract containing Ag\textsuperscript{0}}
SEM analysis

Synthesized silver particles were subjected to SEM analysis. SEM micrographs of Ag\(^0\) synthesized by stirring as well as ultrasonication methods are shown in Fig.4.6.5.

Cluster like morphology with the grain size of 200 nm range is observed for sample obtained by stirring method. The grain sizes of ultrasonic synthesized Ag\(^0\) particles are in the range from 80 to 200 nm with uneven spherical morphology.

EDX analysis

Synthesized silver particles were subjected to EDX analysis. The EDX spectrum of Ag\(^0\) synthesized by stirring as well as ultrasonication methods show strong peak at \(~3.0\) KeV (Fig.4.6.6). Thus, the presence of silver is confirmed. There is a strong peak for Cu in the EDX spectrum, which is due to the use of copper plate in SEM analysis. Elemental silver composition is observed as 72 % in stirring and 30 % in ultrasonication method.
Fig. 4.6.6. EDX spectra of *Tragia ramosa* extract containing silver; (A) stirring method, (B) ultrasonication method

*Cytotoxicity analysis of Tragia ramosa extract and the extract containing Ag\(^0\)*

Cytotoxic activity of neat extract and the extract containing Ag\(^0\) was tested against HeLa cell line with MTT assay in the dose dependent manner.

Neat *Tragia ramosa* extract and the extract containing Ag\(^0\) synthesized by stirring method as well as ultrasonication methods show potent cytotoxicity (Table 4.6.1) towards HeLa cancer cells. Extract containing Ag\(^0\) induced 100% death of HeLa cells at a sample concentration of 7.5 µg/mL.

**Table 4.6.1. Effect of sample concentration and cell inhibition**

<table>
<thead>
<tr>
<th>Sample Conc. (µg/mL)</th>
<th>% Cell Inhibition</th>
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Fig. 4.6.7. Micrographs on the effect of sample concentration and cell inhibition

<table>
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<tr>
<th>Sample Conc. (µg/mL)</th>
<th>Neat extract</th>
<th>Cell Inhibition</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Extract containing Ag synthesized</td>
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<tr>
<td>0.4</td>
<td>![Image]</td>
<td>![Image]</td>
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<tr>
<td>0.9</td>
<td>![Image]</td>
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<td>7.5</td>
<td>![Image]</td>
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</tbody>
</table>
Recognizable morphological changes in HeLa cells are observed after 48 h (Fig.4.6.7). The % cell inhibition is significant for the extract containing Ag⁰ (synthesized by stirring as well as ultrasonication methods) at sample concentration of 3.7 µg/mL. The IC50 values obtained are 4.67, 2.04 and 2.13 µg/mL for neat extract, the extract containing Ag⁰ synthesized by stirring method and the extract containing Ag⁰ synthesized by ultrasonication methods respectively.

**Development of antibacterial finished polypropylene nonwoven material using neat Tragia ramosa extract and the extract containing Ag⁰**

**FT-IR analysis**: FT-IR spectra of uncoated PP, neat extract coated PP and extract containing Ag⁰ coated PP are shown in Fig.4.6.8.

Fig.4.6.8. FT-IR spectra of PP nonwoven coated with *Tragia ramosa* extract and extract containing Ag.
There are changes in the peak intensity (around 3300 cm\(^{-1}\)) for coated PP when compared to uncoated PP. The band at 1613 cm\(^{-1}\) for coated PP is corresponding to –NH bending vibrations (which is absent in uncoated PP). From the FT-IR results, it is confirmed that the coating might be responsible for the improved antibacterial activity.

**SEM analysis:** The uncoated PP, neat extract coated PP and the extract containing Ag\(^0\) coated PP samples were subjected to SEM analysis (Fig.4.6.9). The coating of extract on to PP is clearly observed. Ag\(^0\) containing extract coated PP sample images are found to be brighter than others. This suggests that silver particles are successfully embedded on PP matrix.

Fig.4.6.9. SEM images of (A) uncoated PP, (B) *Tragia ramosa* extract coated PP, (C) extract containing Ag coated PP (stirring method) and (D) extract containing Ag coated PP (ultrasonication method)
**EDX analysis:** Uncoated and coated PP samples were subjected to EDX analysis (Fig.4.6.10). In all spectra, peaks are observed for C, O and N, which are present in the PP matrix. The spectrum C and D reveal the presence and confirmation of elemental silver on the PP matrix. The silver content of the PP is 1.01 and 1.04 wt% in stirring and ultrasonication sample coatings respectively.

**Fig.4.6.10.** EDX spectrum of (A) uncoated PP, (B) *Tragia ramosa* extract coated PP, (C) *Tragia ramosa* extract containing Ag coated PP (stirring method) and (D) *Tragia ramosa* extract containing Ag coated PP (ultrasonication method)
Evaluation of Antibacterial efficiency

The antibacterial activity of uncoated PP, *Tragia ramosa* extract coated PP and the extract containing Ag$^0$ coated PP was investigated by estimating the number of viable bacteria cells in the *S. aureus* and *E. coli* suspension after being contact with substrate for two different time durations of 24 h and 48 h (Fig.4.6.11).

**Fig.4.6.11. Comparison of Antibacterial efficiency of uncoated PP and coated PP**

![Graph showing antibacterial efficiency](image)

<table>
<thead>
<tr>
<th></th>
<th>Uncoated PP</th>
<th>Neat extract coated PP</th>
<th>Extract containing Ag coated PP (stirring method)</th>
<th>Extract containing Ag coated PP (sonication method)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. coli 24h</td>
<td>21.8</td>
<td>50</td>
<td>55.3</td>
<td>56.2</td>
</tr>
<tr>
<td>F. coli 48h</td>
<td>50</td>
<td>65.7</td>
<td>68.3</td>
<td>71.1</td>
</tr>
<tr>
<td>S. aureus 24h</td>
<td>20.5</td>
<td>29.4</td>
<td>44.1</td>
<td>47</td>
</tr>
<tr>
<td>S. aureus 48h</td>
<td>46.3</td>
<td>41.4</td>
<td>56</td>
<td>56</td>
</tr>
</tbody>
</table>

The *Tragia ramosa* extract containing Ag$^0$ synthesized by ultrasonication method coated PP has higher antibacterial activity than others. The difference in the percentage inhibition is significant at 24 h and 48 h. This may be due to the reduced size of silver particles.

**Summary of findings:**
• This part of study has introduced a new reducing agent for the preparation of Ag\(^0\) from AgNO\(_3\). Extract of *Tragia ramosa* leaves has been tested as reducing as well as stabilizing agent in the synthesis of Ag\(^0\) by stirring and ultrasonication methods. The formation of Ag\(^0\) is observed by the visible colour change from light brown to blackish brown. The presence of Ag\(^0\) is confirmed by XRD and EDX analyses. From the XRD result, the size of Ag\(^0\) synthesized by simple stirring and ultrasonication is calculated as 62 and 47 nm respectively. FT-IR spectrum revealed the presence of reducing phytochemicals in the crude extract.

• *Tragia ramosa* extract containing Ag\(^0\) synthesized by simple stirring and ultrasonication methods show cytotoxicity of 100% death of HeLa cells at a sample treatment concentration of 7 µg/mL.

• The antibacterial activity of coated PP against *E. coli* is ordered as *Tragia ramosa* extract containing Ag\(^0\) by ultrasonication method (71 %) > extract containing Ag\(^0\) by stirring method (68.3 %) > neat extract (65.7 %). In a similar analysis, the antibacterial activity against *S.aureus* is ordered as *Tragia ramosa* extract containing Ag\(^0\) by ultrasonication method (56.0 %) = extract containing Ag\(^0\) by stirring method (56.0 %) > neat extract (41.4 %).
4.7 Overall comparative summary of findings

The comparative summary regarding the plant material used in the synthesis of Ag\(^0\), method of synthesis, size and shape of Ag\(^0\) particles obtained and its application to cytotoxicity and antibacterial efficiency is presented below;

Six medicinal plants were selected for this study. Out of 6 plant extracts studied, 2 plant extracts (Ageratina adenophora and Acorus calamus) were found to be not successful in the synthesis of Ag\(^0\). Other 4 plant extracts were found to be useful in the synthesis of Ag\(^0\) in both simple stirring and ultrasonication methods.

From the literature, it is observed that the natural plants contain reducing agents like citric acid, ascorbic acid, flavonoids, reductases, dehydrogenases and extracellular electron shuttlers that influence the biosynthesis of metal nanoparticles [5]. In addition, carbonyl group(s) present in plant constituents is also responsible for the reduction reaction of converting Ag ion to Ag\(^0\). Perusal of literature reveals the presence of following chemical constituents in the plant extracts mentioned.

**Ageratina adenophora leaves**: Literature survey reveals that *Ageratina adenophora* consists of 66% of monoterpenes and 28% of sesquiterpenes. 64 constituents are identified by their \(^1\)H-NMR spectra and GC/MS. Amorphene derivatives (10%) are the main constituents of the sesquiterpene part. The presence and structure of three natural products, amorpha-4,7(11)-diene, 5,8-epoxyamorpha-3,7(11)-diene and amorpha-4,7-dien-11-ol, is confirmed by their \(^1\)H- and \(^{13}\)C-NMR data [6]. GC and GC/MS analysis of essential oils from the aerial parts of *Eupatorium adenophorum* collected from different localities of Kumaun and Garhwal revealed the dominant presence of bornyl acetate (7.6-15.9%), p-cymene (0.1-16.6%), 3-acetoxyamorpha-4,7(11)-dien-8-one (0.3-16.3%), \(\alpha\)-phellandrene (1.5-9.6%), camphene (<0.1-8.9%), \(\alpha\)-bisabolol (1.7-7.8%), \(\alpha\)-cadinol (0.6-
6.2%) and amorph-4,7(11)-dien-8-one (3.2-5.7%). Amorphene derivatives (19.8-41.4%) may be considered as characteristic constituents [7,8].

<table>
<thead>
<tr>
<th>Chemical constituent</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amorpha-4,7(11)-diene</td>
<td><img src="image1" alt="Structure" /></td>
</tr>
<tr>
<td>5,8-epoxyamorpha-3,7(11)-diene</td>
<td><img src="image2" alt="Structure" /></td>
</tr>
<tr>
<td>Amorpha-4,7-dien-11-ol</td>
<td><img src="image3" alt="Structure" /></td>
</tr>
<tr>
<td>3-acetoxyamorpha-4,7(11)-dien-8-one</td>
<td><img src="image4" alt="Structure" /></td>
</tr>
<tr>
<td>Bornyl acetate</td>
<td><img src="image5" alt="Structure" /></td>
</tr>
<tr>
<td>α-phellandrene</td>
<td><img src="image6" alt="Structure" /></td>
</tr>
<tr>
<td>p-cymene</td>
<td><img src="image7" alt="Structure" /></td>
</tr>
<tr>
<td>α-bisabolol</td>
<td><img src="image8" alt="Structure" /></td>
</tr>
<tr>
<td>α-cadinol</td>
<td><img src="image9" alt="Structure" /></td>
</tr>
</tbody>
</table>
*Ageratina adenophora* plant extract contains major fraction as amorphene derivatives that contains low level of carbonyl groups, the overall reducing efficiency is assumed to be poor. This may be the reason for unsuccessful utility of this extract in the synthesis of Ag\(^0\).

**Acorus calamus** root: Literature survey reveals that major compounds present in *Acorus calamus* are 1-\(\beta\), 4-\(\beta\), 7-\(\alpha\)-trihydroxyeudesmane bullatantriol, teuclatriol, threo-1',2'-dihydroxyasarone, erythro-1',2'-dihydroxyasarone, (+)-de-4'-O-methyleudesmin, (+)-de-4'-0-methylmagnolin, (+)-eudesmin, (+)-magnolin, beta-sitosterol [9], glucoside acorin, methyl isoeugenol [10].

<table>
<thead>
<tr>
<th>Chemical constituent</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bullatantriol</td>
<td><img src="image" alt="Bullatantriol" /></td>
</tr>
<tr>
<td>Teuclatriol</td>
<td><img src="image" alt="Teuclatriol" /></td>
</tr>
<tr>
<td>(+)-Eudesmin</td>
<td><img src="image" alt="(+)-Eudesmin" /></td>
</tr>
<tr>
<td>Magnolin</td>
<td><img src="image" alt="Magnolin" /></td>
</tr>
<tr>
<td>Beta-sitosterol</td>
<td><img src="image" alt="Beta-sitosterol" /></td>
</tr>
<tr>
<td>Methyl isoeugenol</td>
<td><img src="image" alt="Methyl isoeugenol" /></td>
</tr>
</tbody>
</table>
Major fractions of *Acorus calamus* plant extract contain poor carbonyl groups. Thus, the reducing efficiency is assumed to be poor. This may be the reason for unsuccessful utility of this plant extract in the synthesis of Ag$^0$.

**Calotropis procera flower:** Literature survey says that *Calotropis procera* flower contains the flavonoids, queretin-3-ratinoside, sterol, calactin, calotoxin, calotropagenin, calotropin, polysaccharides with D-arabinose, glucose, glucosamine and L-rhamnose. Flowers also contain enzymes 3-proteinase and calotropain (protease). Other chemical constituents of *Calotropis procera* flowers are lupeol, uscharin, proceroside, proceragenin (cardenolide), syriogenin, taraxast-20(30)-en-3-(4-methyl-3-pentenoate), 3-thiazoline cardenolide, gigantin, giganteol, isogiganteol, uscharidin, uzarigenin voruscharin a-calotropeol, 3-epimoretenol, a-lactuceryl acetate and a-lactuceryl isovalerate [11], 5-hydroxy-3,7-dimethoxyflavone-4′-O-β-glucopyranoside, 2β,19-epoxy-3β,14β-dihydroxy-19-methoxy-5α-card-20(22)-enolide and β-anhydroepidigitoxigenin-3β-O-glucopyranoside [12].

**Chemical constituent**

<table>
<thead>
<tr>
<th>Chemical constituent</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rutin</td>
<td><img src="image1" alt="Rutin Structure" /></td>
</tr>
<tr>
<td>Calotropin</td>
<td><img src="image2" alt="Calotropin Structure" /></td>
</tr>
<tr>
<td>Calactin</td>
<td><img src="image3" alt="Calactin Structure" /></td>
</tr>
</tbody>
</table>
Calotoxin

5-hydroxy-3,7-dimethoxyflavone-4′-O-β-glucopyranoside

2β,19-epoxy-3β,14β-dihydroxy-19-methoxy-5α-card-20(22)-enolide

B-anhydroepidigitoxigenin-3β-O-glucopyranoside

Since the major fractions of *Calotropis procera* plant extract contain more number of reducing carbonyl groups, the reducing efficiency is expected to be good. This may be the reason for the successful utility of this plant extract in the synthesis of Ag⁰.

**Manihot esculenta leaf:** Literature survey reveals the major phenolic constituents as quercetin and luteolin glycoside, chlorogenic acid, *p*-coumaric, caffeic, ferulic, sinapic acids, glycosides of caffeic and ferulic acid [13], glycosides including cyanogenic glycosides, hydroxycoumarins, terpenoids, flavan-3-ols, fatty acids and esters [14] present in the extract.

<table>
<thead>
<tr>
<th>Chemical constituent</th>
<th>Structure</th>
</tr>
</thead>
</table>
Since the major fractions of *Manihot esculenta* plant extract contain more number of carbonyl groups, the reduction reaction is expected to be good. This may be the reason for the successful utility of this extract in the synthesis of Ag⁰.

**Coleus aromaticus** leaves: Literature reveals that leaves of *Coleus aromaticus* contain salvigenin, genkwanin, quercetin, chrysoeriol, luteolin and apigenin, flavanone eriodictol, flavanol taxifolin, triterpenic acids; oleanolic acid, 2,3-dihydroxyoleanolic acid, crategolic acid, ursolic acid, pomolic acid, euscaphic acid, tormentic acid and 2,3,19,23-tetrahydroxyursolic acid [15], rosmarinic acid [16].
Since the major fractions of *Coleus aromaticus* plant extract contain more number of carbonyl groups, the reduction reaction possibility is expected to be good. This may be the reason for the successful reducing efficiency of this plant extract in the synthesis of $\text{Ag}^0$.

*Tragia ramosa leaves*: Literature says that oil derived from *Tragia ramosa* contains about 62% linoleic acid ($\text{CH}_2=\text{CH}-\text{CH}=\text{CH}-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$) and enzymes. They are also rich in proteins [17].
The major fraction of *Tragia ramosa* plant extract contains carbonyl group. Hence, the reduction reaction is expected to be good. This may be the reason for the successful use of this plant extract in the synthesis of Ag$^0$.

The comparative summary regarding the method of synthesis, size (as per XRD result) and shape (as per SEM analysis) of Ag$^0$ particles and its application to cytotoxicity and antibacterial efficiency is given below;

<table>
<thead>
<tr>
<th>Plant material used</th>
<th>Method of synthesis</th>
<th>Average size of Ag$^0$ (nm)</th>
<th>Shape of Ag$^0$</th>
<th>Effect of Cytotoxicity (%) / Conc (µg/mL)</th>
<th>Antibacterial efficiency of coated PP at 48 h (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>XRD</td>
<td>SEM</td>
<td></td>
<td>E. coli</td>
</tr>
<tr>
<td><em>Ageratina adenophora</em></td>
<td>Stirring</td>
<td>40</td>
<td>200 - 1000</td>
<td>Cube</td>
<td>99 / 7</td>
</tr>
<tr>
<td></td>
<td>Sonication</td>
<td>35</td>
<td>200 - 1000</td>
<td>Cluster</td>
<td>84 / 7</td>
</tr>
<tr>
<td><em>Acorus calamus</em></td>
<td>Stirring</td>
<td>45</td>
<td>200 - 1000</td>
<td>Uneven Spherical</td>
<td>100 / 3</td>
</tr>
<tr>
<td></td>
<td>Sonication</td>
<td>25</td>
<td>200 - 1000</td>
<td>Uneven Spherical</td>
<td>100 / 3</td>
</tr>
<tr>
<td><em>Calotropis procerfa</em></td>
<td>Stirring</td>
<td>34</td>
<td>50 - 200</td>
<td>Polygon</td>
<td>99 / 7</td>
</tr>
<tr>
<td></td>
<td>Sonication</td>
<td>24</td>
<td>30 - 50</td>
<td>Wire</td>
<td>99 / 7</td>
</tr>
<tr>
<td><em>Manihot esculenta</em></td>
<td>Stirring</td>
<td>62</td>
<td>100 - 500</td>
<td>Cluster</td>
<td>100 / 7</td>
</tr>
<tr>
<td></td>
<td>Sonication</td>
<td>47</td>
<td>80 - 200</td>
<td>Uneven Spherical</td>
<td>100 / 7</td>
</tr>
</tbody>
</table>

Out of the four successful plant extracts studied extensively, *Manihot esculenta* is found to be very effective in the application areas such as cytotoxicity (100 % for 3µg/mL) and antibacterial (92.1 %). Based on these application results, this plant extract is further utilized for the development of facemask material and presented in the next chapter.

**References**