BIOCATALYTIC EFFECT OF *Simarouba glauca* LEAF PHYTOCHEMICALS ON BIOLOGICALLY ACTIVE SILVER NANOPARTICLES YIELD AND ABTS ANTIOXIDANT ACTIVITY: GREEN SYNTHESIS

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**ABSTRACT**

Green synthesis of gold, copper, and silver metal nanoparticles is currently growing research due to different medical applications. Necessary natural human health-protecting components can be derived using nanobiotechnology using plant extracts in biodiversity enriched countries. Silver nanoparticles have shown various biological applications such as antimicrobial, anticancer, and antidiabetic activities. Hence, this work studied the yield and antioxidant activity of silver nanoparticles derived from various solvent extracts of *Simarouba glauca* leaf-like methanol, ethanol, chloroform, ethyl acetate, and water. The derived silver nanoparticles of various extracts were characterized by absorption and vibrational spectra. Out of the five extracts, high yield silver nanoparticles surface morphology was inspected by scanning electron microscope. Also, the prepared silver nanoparticle’s antioxidant activity was examined using the 2, 2’-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid assay protocol, and the results were compared for future work. From the results, this work observed that the silver nanoparticles of ethanol crude exposed 72% yield and 39% yield obtained from chloroform extract. The silver nanoparticles of methanol crude showed good antioxidant activity (IC\(_{50}\) = 16.59μg/ml) when compared to the other extract nanoparticles due to the phytochemicals concentration difference.

**Keywords**: Silver Nanoparticles, Bio-reduction, Yield, Characterization, Antioxidant Activity.

**INTRODUCTION**

Traditional therapeutic plants are providing useful medicinal products in developing and developed countries owing to the lower side effects except for heavy metal issues.\(^1\) Most of the bioactive compounds are isolated by various techniques such as column chromatography, precipitation techniques, and solvent extraction methods for the biological efficacy investigations.\(^2-4\) these medicinal plant parts contain various useful phytochemicals such as polyphenols, flavonoids, anthocyanidins, phytoestrogens, glucosinolates, terpenoids, carotenoids, phytosterols, limonoids, fibers, and alkaloids.\(^5\) These chemical constituents are showing medicinal and catalytic properties. In recent days, phytochemicals of medicinal plants are used as vital catalysts in green synthesis which includes nanoparticle preparation.\(^6\) Hence, the environmentally safe low-cost, nontoxic secondary metabolite-based nanoparticles preparation research works are receiving great interest from the researchers.\(^7\) Numerous investigation results have stated that the metals like Ag, Cu, and Au-based nanoparticles are killing both drug-resistant and nonresistant pathogens.\(^8\)

Instead of various nanoparticles, silver nanoparticles prepared by a green synthesis approach using several plant extracts and microbes were reported in a short period due to their wide spectrum of applications.\(^9-1\) Similarly, this work examined the reports on *Simarouba glauca* (Lakshmi taru) and its medicinal applications in various fields.\(^12,13\) So, this work investigated the silver nanoparticles yield using various

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extracts of *Simarouba glauca* plant leaf and analyzed the nanoparticles yield with the concentrations of phytochemicals present in each extract.\(^{14,15}\) The derived silver nanoparticles (AgNAP) were characterized by UV and FTIR. The spectral results were compared with the reported data.\(^{16}\) This work extended the investigation to measure the antioxidant character of each crude AgNAP product by 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid assay (ABTSA). Then, the effective antioxidant result produced nanoparticle surface morphology was examined by scanning electron microscope (SEM).

**EXPERIMENTAL**

**Chemicals and Plant Collection**
All Sigma Aldrich grade solvents and Silver nitrate were received from bio corporals, Chennai, Tamil Nadu. *Simarouba glauca* plant leaves were collected from Kolli hills, Namakkal district, Central Tamil Nadu. Professor P. Jayaraman, Plant Anatomy Research Institute, West Tambram, Chennai, Tamil Nadu, India, verified and authenticated the plant leaves of *Simarouba glauca* L. (authentication no. PARC/2020/4260). The ABTSA, K\(_2\)S\(_2\)O\(_8\) (Potassium persulfate), and myoglobulin were also received from Sigma Aldrich, India.

**Instruments**
The prepared silver nanoparticles were characterized by Shimadzu making UV-Vis 2600 in a water solvent. FTIR vibrational spectrum was recorded using the KBr pellet method in Shimadzu IR affinity IS connected with the DLATGS detector. Silver nanoparticle surface morphology was analyzed by HITACHI SEM Model S-3000H.

**Dressing and Preparation of *Simarouba glauca* Leaf Extract**
Collected *Simarouba glauca* leaves were washed, dried, and ground well about 1kg for further process. Then, it was sieved and carried for further process. 5 X 200 g of ground powder was weighed and treated with the selected solvents like ethanol (C\(_2\)H\(_5\)OH), methanol (CH\(_3\)OH), ethyl acetate (CH\(_3\)-COOCH\(_3\)), chloroform (CHCl\(_3\)), and water (H\(_2\)O) for the maceration extraction.\(^{17}\) The solvents with leaf were kept for four days and filtered. The filtered extracts were concentrated in Rota vapor-R-100 and concentrated crudes were carried out for the preparation of further nanoparticles.

**Phytochemicals by Spot Test Methods**
Each crude extract phytoconstituents were examined using known spot test reagents.\(^{18-20}\) The polar solvent extracts were investigated with each spot test reagent and the results were recorded based on the intensity.

**Synthesis of Silver Nanoparticles**
5 sets of 500 mL round bottom three neck flasks were fitted with a condenser under reflux conditions. 5 mM of 100 ml (0.85g) Silver nitrate solution was placed in all 500 ml round bottom flasks. Then, 0.5 g of the prepared crudes (CHCl\(_3\), CH\(_3\)-COOCH\(_3\), CH\(_3\)OH, C\(_2\)H\(_5\)OH, and H\(_2\)O) were dissolved in 100 ml ethanol and added drop-wise over the period of 30 min with constant stirring in dark conditions. All reaction mixtures were stirred at 90°C for 4 hours.\(^{21, 22}\) This work observed the immediate color changes while the addition of silver nitrate solution. After four hours, the mixture temperature was brought to room temperature and centrifuged at the speed of 8000-9000rpm. All the isolated well-dried residues were carried out for further characterization and antioxidant assay. Bio-reduction reaction of silver ion has been shown in Fig.-1.

![Bio-reduction reaction of silver ion](image)

Fig.-1: Silver Nanoparticles Preparation Bio-Reduction Mechanism
Characterization
Absorption and Vibrational Spectra
The prepared five various crudes used silver nanoparticles absorption spectrum between the range of 190 nm and 1100 nm were recorded in a water solvent. Recorded outcomes were processed using origin Pro software. Similarly, the prepared five biologically reduced silver nanoparticles vibrational spectra were recorded between 400 cm\(^{-1}\) and 4000 cm\(^{-1}\) using KBr pellets.

SEM
The surface structure and morphology of the highest yield Ag nanoparticles were examined for the size and shape of the nanoparticles.

ABTS Assay
A ferryl myoglobin radical is prepared by treating metmyoglobin with hydrogen peroxide for the ABTSA radical formation. The green ABTSA cation free radical was measured by absorbance at 734 nm. The supplied test compounds or materials inhibited ABTSA radical formation (Figure-2) and were measured using the same calorimetry technique. The antioxidant concentration is inversely proportional to the ABTSA radical formation and 734 nm absorbance.\(^{23-25}\) The standard solutions such as 7 mm ABTSA and 2.4 mm \(\text{K}_2\text{S}_2\text{O}_8\) were used for the analysis. The test solution was prepared by treating the two standard solutions in equal amounts and was permitted to react for 14 h at room temperature and was kept in dark conditions. ABTSA (1 ml) was diluted using 60 ml methanol to get an absorbance of 0.706 ± 0.01 units in a spectrophotometer at 734 nm. Serially diluted AgNAPs samples (100, 90, 80, 70, 60, 50, 40, 30, 20, and 10 µg/ml) were treated with freshly prepared ABTSA (1ml) solution and the absorbance was examined at 734 nm after 7 min. The ABTSA scavenging tendency of the test samples was compared with ascorbic acid inhibition percentage using the following formula (1) and IC\(_{50}\) was measured using formula (2),

\[
\text{ABTSA free radical control} = \left(\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}}\right) \times 100 \quad (1)
\]
\[
Y = \text{Min} + \left\{\frac{\text{Max} - \text{Min}}{1 + \left(\frac{X}{\text{IC}_{50}}\right)^{\text{Hill coefficient}}}\right\} \quad (2)
\]

Where, Abs\(_{\text{control}}\)-absorbance of ABTSA radical in methanol, Abs\(_{\text{sample}}\)-absorbance of the ABTSA radical solution treated with sample extract/standard. Ascorbic acid exposed the OD of 0.87 at 734 nm.

RESULTS AND DISCUSSION
Biologically active \textit{Simarouba glauca} leaves phytochemicals were successfully extracted using medium polar solvents (CHCl\(_3\), CH\(_3\)-COOCH\(_3\)) and polar solvents (CH\(_3\)OH, C\(_2\)H\(_5\)OH, and H\(_2\)O). The extracted crude phytochemicals were identified using reported spot test reagents.\(^{19}\) The results exposed that the phytochemicals differed based on the polarity of the solvents and have been displayed in Table-1. From the outcomes, this research observed that the chloroform extract contains fewer concentrations of alkaloids, sterols, and lignin-like constituents. Likewise, the ethyl acetate extract contains higher concentrations of Flavonoids, Glycosides, Phenols, and Lignins except for Sterols. But, the remaining polar solvents exhibited all phytochemicals which were
involved in bioreduction reactions. After the phytochemical analysis, the extracts were carried out for biologically active silver nanoparticles preparation to measure the yield and to investigate the effect of the phytochemicals in bioreduction.

Table-1: Different Simarouba glauca Leaf Extracts and Their Phytochemical Analysis Results

| Constituent     | Test          | CHCl₃ | CH₃-COOCH₃ | CH₂OH | C₂H₅OH | H₂O |
|-----------------|---------------|-------|------------|-------|--------|-----|
| Alkaloids       | Iodine        | -     | -          | -     | -      | -   |
|                 | Wagner’s      | ++    | -          | ++    | ++     | +   |
|                 | Dragendorff’s | +     | -          | ++    | ++     | -   |
| Glycosides      | Keller-Killani| +     | ++         | ++    | ++     | +   |
|                 | Glycosides    | -     | ++         | -     | ++     | +   |
|                 | Conc. H₂SO₄   | ++    | ++         | ++    | ++     | +   |
|                 | Molisch’s     | ++    | ++         | ++    | ++     | +   |
| Flavonoids      | Pew’s         | -     | -          | +     | +      | -   |
|                 | Shinoda       | -     | -          | +     | ++     | +   |
|                 | NaOH          | -     | +          | -     | ++     | +   |
| Phenols         | Ellagic acid  | -     | ++         | ++    | ++     | +   |
|                 | Phenol        | -     | ++         | ++    | ++     | +   |
| Lignins         | Labat         | +     | -          | +     | +      | -   |
| Saponins        | Foam test     | -     | -          | +     | -      | +   |
| Sterols         | Salkowski’s   | +     | +          | ++    | ++     | +   |
| Tannins         | Gelatine      | +     | -          | -     | +      | +   |
|                 | Lead acetate  | -     | -          | ++    | ++     | +   |
|                 | Bomtrager’s   | -     | -          | -     | ++     | +   |
| Anthraquinone   | -             | +     | +          | ++    | +      | +   |
| Phlobatannins   | -             | +     | ++         | +     | -      | +   |
| Reducing sugar  | -             | +     | +          | ++    | +      | +   |
| Volatile oil    | -             | +     | +          | -     | +      | -   |

The reactions were conducted with all crudes (0.5g) in ethanol and the yields of each reaction were measured. The calculated yields are shown in Table-2. The outcomes of silver nanoparticles (Fig.-3) exposed the efficiency of the phytochemicals in bioreduction. Also, this work observed moderate yields in medium polar solvents which exist between 39% and 54%. But, the polar crudes have produced higher yields between 67% and 72%. Apart from the five extracts, ethanol extract has produced higher yields (72%) due to the presence of secondary metabolites concentration and water miscibility, which enhanced the reduction reaction of silver ions. In addition to this, polyphenols present in extracts also enhanced the reduction and yield of the silver nanoparticles. The reports of the poly-phenols exposed that they are the secondary metabolites in plant extracts and are a good biocatalyst for the preparation of silver nanoparticles. But in chloroform extract, phenolic compounds are absent and the resultant yield is also lower. The remaining solvents exhibit maximum phytoconstituents and hence they have shown good yield.

Table-2: Simarouba glauca Leaf Extracts Catalysed Silver Nanoparticles Yield

| Crude extract source | Solvent | Crude quantity in g | Input AgNO₃ in g (5mm) | Actual Yield in g Ag⁰ | Obtained Yield in g | Yield AgNAP in ≈% | Condition   |
|----------------------|---------|---------------------|------------------------|------------------------|---------------------|-------------------|-------------|
| Chloroform           | 50ml    | 0.5                 | 0.85                   | 0.54                   | 0.21                | 39                | Reflux /4h   |
| Ethyl acetate        | 50ml    | 0.5                 | 0.85                   | 0.54                   | 0.29                | 54                | Reflux/4h    |
| Methanol             | 50ml    | 0.5                 | 0.85                   | 0.54                   | 0.37                | 68                | Reflux/4h    |
| Ethanol              | 50ml    | 0.5                 | 0.85                   | 0.54                   | 0.39                | 72                | Reflux/4h    |
| Water                | 50ml    | 0.5                 | 0.85                   | 0.54                   | 0.36                | 67                | Reflux/4h    |
The prepared nanoparticles of different crude were carried out for characterization to analyze the variation among the products based on the intensities of the phytochemicals. The UV-vis spectra of the five different silver nanoparticles are shown in Fig.-4. The comparison of the five absorption spectra discloses the part of the phytochemicals in silver nanoparticle synthesis. All AgNAP exhibit peaks at 250 nm and 420 nm. The maximum absorption peak at 420 nm exposes the silver nanoparticle’s surface plasmon resonance nature and coincidence with the reported results. The vibrational spectrum of the silver nanoparticles has presented in Fig.-5. FT-IR spectra of all AgNAP show the peaks at 3281 cm\(^{-1}\), 1610 cm\(^{-1}\), 1387 cm\(^{-1}\) and 1115 cm\(^{-1}\) due to O–H stretching and C–H stretching of the phytochemicals such as phenols, sugars, and sterols. In addition to these, polar solvents have shown one weak peak at 2931 cm\(^{-1}\). The vibrational spectra of the silver nanoparticles outcomes (Fig.-4) are in coincidence with the reported values.

Both UV and FTIR spectra confirmed the formation of the silver nanoparticles of all crude and coincidence with the reported results. But, the yield varied based on the phytochemicals present in the crude. After the characterization, all AgNAP were carried out for the ABTSA assay for the antioxidant tendency investigation. As per the reported protocol, an ABTSA assay was carried out for the derived five silver nanoparticles using ascorbic acid as standard. The entire five silver nanoparticles’ antioxidant tendency was measured after the conversion of the triplicated trials outcomes mean value. The triplicated trials’ mean values have shown in Table-3. The resultant absorbance values were subjected to online inhibition concentration for 50% of the radicals (IC\(_{50}\)) calculator and plotted the graphs (Fig.-5). The calculated IC\(_{50}\) values exist between 16.59 μg/ml and 26.14μg/ml. Also, this work observed slight variation among the five different nanoparticles based on their phytochemicals except for the methanol extract. The remaining silver nanoparticles are showing a good and almost nearby difference in inhibition concentrations. The methanol extract crude used silver nanoparticles showed inhibition at lower concentrations (IC\(_{50}\) = 16.59 μg/ml) may be presence of the saponins-like constituents.
After the antioxidant assay, the higher active silver nanoparticles of methanolic extract were investigated for the nature of nanoparticle identifications (Fig.-6). The scanning electron microscope image exposed the formation of the capsule-like nanoparticles, which is almost like the reported surface morphology of the silver nanoparticles. The size and the morphology may be the reason for the good radical inhibition when compared to other prepared silver nanoparticles. These nanoparticles of methanol crude have exposed good surface and which may be involved in free radical suppression.

| Tested sample concentrations (μg/ml) | OD Value at 734 nm of different solvent bio reduced silver nanoparticles (triplicated trials mean value) |
|-------------------------------------|-------------------------------------------------------------------------------------------------------|
|                                    | CHCl₃ | CH₃-COOCH₃ | CH₃OH | C₂H₅OH | H₂O |
| 100                                | 79.54 | 85.52      | 90.69 | 93.33   | 89.89 |
| 90                                 | 78.85 | 84.83      | 89.43 | 91.34   | 89.20 |
| 80                                 | 78.74 | 84.71      | 88.62 | 90.92   | 89.08 |
| 70                                 | 77.59 | 83.56      | 88.39 | 87.77   | 87.93 |
| 60                                 | 77.47 | 83.45      | 87.82 | 89.66   | 87.82 |
| 50                                 | 77.01 | 82.99      | 87.82 | 89.20   | 87.36 |
| 40                                 | 76.78 | 82.76      | 86.78 | 88.97   | 87.13 |
| 30                                 | 74.14 | 80.11      | 85.06 | 86.32   | 84.48 |
| 20                                 | 70.57 | 76.55      | 82.53 | 82.76   | 80.92 |
| 10                                 | 67.70 | 73.68      | 79.31 | 79.89   | 78.05 |
| IC₅₀ (μg/ml)                       | 26.12 | 26.13      | 16.59 | 25.71   | 26.14 |

Ascorbic acid (Control) 0.87=100%
CONCLUSION
From the results, this work observed that phytochemical compositions are acting as an important component in the green synthesis of silver nanoparticles. Also, the solvents used for the extraction have also exposed to the impact on AgNAP yield. Finally, this work concluded that the phytochemicals with phenolic group concentrations are playing a vital role in nanoparticle yield and nanoparticle dimensions in green synthesis.

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