Green Synthesis of Silver Nanoparticles from Aegle Marmelos and Cissus Quadrangularis Leaf Extract and Evaluation of Its Anti-Inflammatory and Antioxidant Effect

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The principal motive of this research was to formulate AgNPs using Aegle marmelos and Cissus quadrangularis leaf extract. The biosynthesized nanoparticles are assessed for anti-inflammatory and antioxidant. The plant extract-mediated silver nanoparticles are evaluated for applications like anti-inflammatory and antioxidant. The antioxidant activity is analyzed using hydrogen peroxide assay, DPPH, FRAP, Nitric oxide assay, and ABTS Assay. The anti-inflammatory activity is estimated using Membrane stabilization assay, BSA denaturation assay, and Protein (Egg-albumin) Denaturation assay. The radical scavenging property of plant-mediated AgNPs shows that using DPPH Assay at the highest concentration of 50 μ l/ml 92.07 % was found, hydrogen peroxide assay exhibits 87.5%, the value of FRAP was 88.63, for nitric oxide assay the value is 87.47% and for ABTS assay, it is 87.47%. For anti-inflammatory activity, the value at the highest concentration for Protein (Egg-Albumin) Denaturation Assay is 78%, for BSA Denaturation Assay, it is 80%, and for membrane stabilization assay 86% is reported. In this present work, we can say that Aegle marmelos and Cissus quadrangularis mediated silver nanoparticles exhibit high potential as effective nanoparticles in the applied fields of anti-inflammatory and antioxidant activities and can be modified into products for therapeutic purposes.

Keywords: Anti-inflammatory, Antioxidant, bovine serum albumin, biomedical applications, green synthesis.

1. Introduction

Nanomedicine is seen as a field where both science and technology come together for the treatment and prevention of diseases. Nanomedicine holds great promise for the manufacture of nano-based products and nanotherapeutics. They are effective in the production of drugs because nanoparticles can give out a greater efficacy when compared to small molecule drugs because of large surface area, the flexibility included in surface modification and small size. The other features include enhanced pharmacokinetics, good therapeutic effect, less toxicity and good biocompatibility (1). Nano drugs incorporated with nanoparticles can be used as anticancer drugs. A nanomedicine was developed using copper ions and quercetin to target cancer cells and cause toxicity in breast cancer. Multiple nanomedicine drugs based on RNA and DNA are also applied when combined with chemotherapy. However, they are rendered not that ineffective because of their low drug-loading capacity and poor biocompatibility (2).

Nanoparticles are particles having sizes from 1-100 nm. Different techniques are used to determine the size of the nanoparticles like TEM, SEM, XRD, dynamic light scattering and AFM (3). For the synthesis of nanoparticles, two methods are carried out: the top-down method and the bottom-up method. Frequently methods used in top-down technique are ion etching, photolithography, anodization, lithography, milling processes and plasma etching. And the methods involved in bottom-up methods are sol-gel processing, chemical vapor deposition, laser pyrolysis, electrochemical nanostructural preparation, bio-based synthesis methods and flame or plasma spraying. The synthesis of nanoparticles is mainly divided into three ways: synthesis methods, physical methods and chemical methods (4). Green synthesis of nanoparticles is preferred more because of various features like the low cost involved, low chances of accidents, more economical, product developed is safe, can be used for pharmacy-based industries and other applications, protects human health and leftover waste is less (5).

In this research paper, the plant specimens used for the biosynthesis of AgNPs are Aegle marmelos and Cissus quadrangularis. Bael (Aegle marmelos) is considered an economically important tree species as it possesses multiple benefits: it can be used as an element for the medical preparations of herbs. They are popular when it comes to making pudding, jams and syrup. The parts of Aegle marmelos contain bioactive components like marceline, xanthotoxol, Angeline, coumarin, and imperatorin. These components of Aegle marmelos make it applicable to antifertility, insecticidal, immunogenic, and antidiabetic activities. The seeds of Aegle marmelos abode a specific fatty acid known as ricinoleic acid or 12-hydroxyoctadeccis-9-enoic acid which can be converted and used as biodiesel (6). Cissus quadrangularis is known to be a perennial herb belonging to the Vitaceae family. Various bioactive components like alkaloids, saponins, vitamins, steroids, glycosides, flavonoids, stillness and iridoids are present in the plant. It has been known for its antimicrobial, anticancer, anticonvulsant, anti-inflammatory, analgesic, and anti-osteoporotic applications (7). It can also be used in the treatment of antifungal, anthelmintic, antiulcer, anti-hemorrhoidal, and fracture healing (8).

Silver nanoparticles are considered nanomaterials having size in the range of 1-100 nm. They have a higher surface and greater capacity when compared to silver. Their specific catalytic, electrical, and optical features pave the way for these nanoparticles to be applied in diagnosis, imaging, detection, and targeted drug delivery (9). Silver nanoparticles can be utilized for applications like anticancer, antimicrobial, biosensing, bioimaging, and solar energy

harvesting. Multiple nanoparticles like Cu, Pt, Pd, and Au nanoparticles cannot take the place of Ag nanoparticles because they are capable of emitting the plasmonic band when it comes down to the vast range of wavelengths and they have the highest quality factor in the plasmonic region (10). Silver nanoparticles are portrayed as excellent antimicrobial agents as they can eliminate bacteria in both in vitro and in vivo. They have been found effective in multidrugresistant strains of bacteria. Antibacterial properties were observed to be found effective on Staphylococcus aureus and Escherichia coli when tested. The incorporation of silver nanoparticles has been used in clothing, catheters, dressings, surgical products, dressing, and dental products (11). Thereby in this paper silver nanoparticles synthesized from the plant extract Aegle marmelos and Cissus quadrangularis have been checked for multiple applications where the plant extract is prepared by green synthesis and with the incorporation of silver nitrate solution, the silver nanoparticles synthesized are applied for an antioxidant property using hydrogen peroxide assay and DPPH, antimicrobial activity is observed using Kirby–Bauer test and with the help of BSA and albumin denaturation assay, anti-inflammatory activity was assessed and at last cytotoxicity, analysis is done using brine shrimps.

2. Materials and Methods:

Plant extract preparation:

For the synthesis of plant samples, Aegle marmelos and Cissus quadrangularis leaves were collected and washed using tap water once and finally with distilled water. 5 grams of the samples were weighed using a weighing balance. The measured leaves were crushed using a mortar and pestle. About 100 ml of distilled water is introduced into the crushed plant sample. At 55° C for 15 -20 minutes, the solution was kept for boiling and later was cooled down. Using no 1 Whatman filter paper, the cooled sample was filtered and analyzed for further studies.

Synthesis of AgNPs:

2 millimolar of silver nitrate is added into 75ml of distilled water, which acts as a precursor. 25 ml of prepared aqueous extract (Aegle marmelos and Cissus quadrangularis round) is added with 75 ml of silver nitrate solution. The mixed solution was kept in an orbital shaker. With the help of UV–VIS, O. D values were estimated after 3 hours. After 24 hours, the values were calculated again with UV- VIS. The observation of color change indicates the biosynthesis of silver NPs. UV absorbance of biosynthesized silver NPs was observed under 250- 650 nm.

ANTIOXIDANT ACTIVITY

Hydrogen Peroxide Assay:

The antioxidant activity was determined using a hydrogen peroxide assay. Using a hot air oven, the washed test tubes are dried. Inside each test tube,1 ml of hydrogen peroxide was measured and added. The prepared silver nanoparticles C quadrangularis and A marmelos extract were added to the test tubes at different measurements ($10\mu g/ml$, $20\mu g/ml$, $30\mu g/ml$, $40\mu g/ml$ and $50\mu g/ml$). The test tubes were kept in the dark for 10 mins under incubation. Using a UV spectrometer at 530 nm, the samples were measured. The standard used in this experiment is Ascorbic acid (12).

DPPH Assay:

DPPH assay was also used to analyze antioxidant activity. Take 0.03g of DPPH powder and dissolve in 10 ml of methanol. Take 0.05 ml of stock solution and dissolve in 49.5 ml of methanol. This solution is known as the working standard solution. Add 1 ml of working standard solution to all test tubes. Samples are added at different measurements of 10 μ g/ml, 20 μ g/ml, 30 μ g/ml, 40 μ g/ml and 50 μ g/ml. Keep it in incubation for 10 minutes in a dark place. Observe the color changes. Take readings at 517 nm wavelength (13)

FRAP:

0.5 ml of ferric chloride was added into each test tube, along with nanoparticles of different concentrations. In each test tube, 2.5 ml of phosphate buffer and potassium ferricyanide was added. For 30 minutes, incubation was observed. In the test tubes, 2.5 ml of TCA was measured and added. Incubation was observed for 10 minutes. Then incubation for 10 minutes was done in a water bath. Extracts were centrifuged after incubation and 2-5 ml of the centrifuged supernatant was added. It is kept for 10 minutes incubation after the addition of 2.5 ml of distilled water. Reading was taken at 700 nm (14).

ABTS:

ABTS radical cation formation occurs by the chemical reaction of 2.45 mM potassium persulfate in distilled water with 7.0 mM ABTS in 50% ethanol. The prepared sample is kept in the refrigerator for 24 hours. Before use, the dilution of the prepared reagent occurs with the use of 50% ethanol till an absorbance range of 1.0 is observed at 734 nm. 20 μ l of silver nanoparticles and 50 μ l of ABTS are measured and taken in various concentration ranges of 10, 20, 30, 40, and 50 μ g/ml. They are measured and then carefully added together in a 96-well microplate. 10 minutes of dark incubation is observed for the samples. After incubation, the readings are taken at 734 nm (15).

Nitric oxide assay:

Analysis of the NO assay is done using Griess Ilosvay reaction. The addition of naphthyl ethylenediamine dihydrochloride (0.1% w/v) is what gives the modification in the reaction. The synthesized silver nanoparticles along with 0.5 ml of phosphate buffer and 2 ml of sodium nitroprusside were taken in 5 test tubes at various concentration ranges of 10, 20, 30, 40 and 50 µg/ml. It was then kept for 15 mins incubation at 25°C. For the process of diazotization, 0.5 ml of the reaction mixture is introduced to 1 ml of sulfanilic acid reagent and is kept for 5 minutes after incubation. Then 1 ml of naphthyl ethylenediamine dihydrochloride is added and observation is done at 25°C after 30 minutes. Under the diffused light, a pink-colored chromophore is reported. At 540 nm, the absorbance of the sample was recorded. The standard used in this experiment is rutin.

ANTI-INFLAMMATORY ACTIVITY

EA Assay:

With the help of an EA assay, the anti-inflammatory activity of the nanoparticles was analyzed. In this 10x phosphate buffer is converted into 1x. For the preparation of phosphate buffer, 9 ml of distilled water was measured and 1 ml of buffer was dissolved in it. 2.8 milliliters of prepared phosphate buffer is added to 5 test tubes. To each of the 5 test tubes, 0.2 ml of egg *Nanotechnology Perceptions* Vol. 20 No. S7 (2024)

white is added. Add the nanoparticles with the concentrations of $10~\mu g$, $20~\mu g$, $30\mu g$, $40\mu g$ and $50~\mu g$. Keep it for 10~minutes of incubation. After incubation, the samples are again incubated in a water bath at 50~C. The color change is reported. At 660~minute wavelengths, the readings are taken.

BSA Assay:

For the assessment of the anti-inflammatory activity of nanoparticles, a BSA assay was used. 50 ml of distilled water was measured and 0.05 of commercial bovine serum albumin was measured and dissolved in it. At different concentrations like 10 μ g, 20 μ g, 30 μ g, 40 μ g, and 50 μ g, 3 ml of the prepared solution is added to 5 test tubes. Keep it in incubation for 10 mins. Place it in a water bath and at 660 nm, readings should be taken.

Membrane stabilization assay:

In a sterile test tube containing anticoagulant, fresh human blood is collected. The collected blood is centrifuged at 1000~g for approximately 10~minutes at room temperature for the separation of RBCs from the other components of blood. The supernatant is removed and the RBCs are washed three times with PBS. The RBCs are resuspended in Tris-HCl buffer to obtain 10%(v/v) RBC suspension. 1 ml of the RBC suspension is introduced into each sterile centrifuge tube using a pipette. Different concentrations of the silver nanoparticles are added to each sterile centrifuge tube. Mix gently and incubation of the tubes is done for 30 minutes at 37 C. The tubes are centrifuged at 1000~g for 10~minutes at room temperature to pellet the RBCs. Using a UV-Vis spectrophotometer, the absorbance of the supernatant at 540~nm is measured.

3. Results:

Figure 1: Extraction of aqueous extract using Aegle marmelos and Cissus quadrangularis.







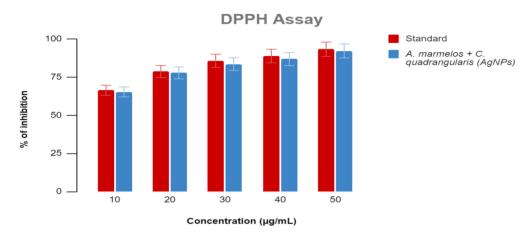
Figure 2: Overall Synthesis of AgNPs Aegle marmelos and Cissus quadrangularis.



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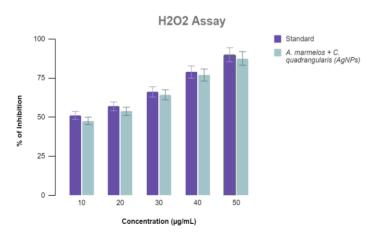
ANTIOXIDANT ACTIVITY:

Figure 3: Graph representing percentages of antioxidant action of biosynthesized AgNPs and standard using DPPH Assay.

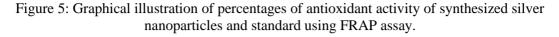


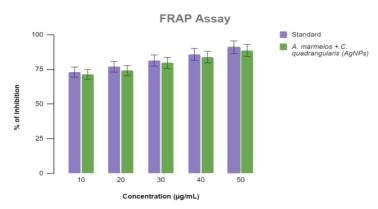
The silver nanoparticles synthesized using Aegle marmelos and Cissus quadrangularis analyzed for DPPH assay show the values 65.22, 77.69, 83.48, 86.72, and 92.07 at different concentrations. The highest inhibition percentage reported is at 50 μ l/ml for 92.07 and 93.15 was exhibited by the standard at the same concentration as shown in Figure 3.

Figure 4: Graph representing the percentages of antioxidant activity of synthesized silver nanoparticles and standard using hydrogen peroxide assay.



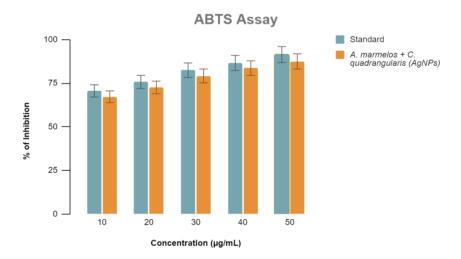
The green synthesized silver nanoparticles assessed by the hydrogen peroxide assay for antioxidant activity show the following inhibitory percentage values 47.6, 53.7, 64.3, 76.9, and 87.5 % at different concentrations. The highest value reported is 87.5 % at 50 μ g/ml and 89.9 % was shown by standard as shown in Figure 4.





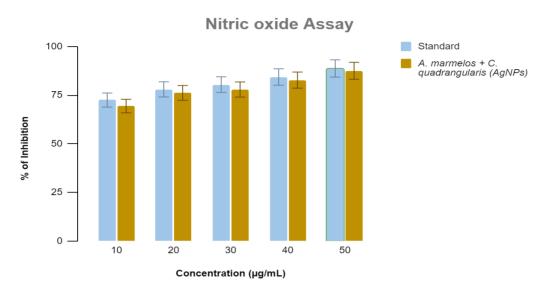
Aegle marmelos and Cissus quadrangularis mediated silver nanoparticles analyzed for antioxidant activity using FRAP Assay show the following values obtained at different concentrations. 71.31, 74.03, 79.62, 83.79, and 88.63 are the inhibitory percentages obtained for this assay. 88.63 % is the highest inhibitory percentage obtained at 50 μ l/ml and 90.89 % is the highest value for the standard as represented in Figure 5.

Figure 6: Graph representing the percentages of antioxidant activity of synthesized silver nanoparticles and standard using ABTS assay.



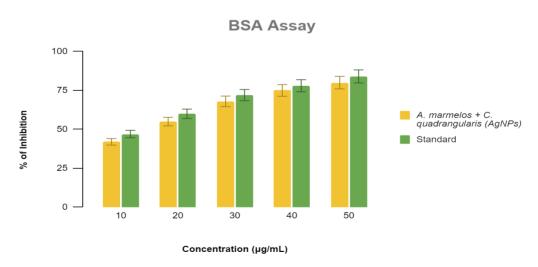
The silver nanoparticles synthesized on analyzing antioxidant activity using ABTS assay show the following values 67.21, 72.56, 79.18, 83.62 and 87.47 at different levels of concentrations. The highest value reported is 87.47 % at 50 μ l/ml and at the same concentration, the standard shows 91.39 % as shown in Figure 6.

Figure 7: Graph representing the percentages of antioxidant activity of synthesized silver nanoparticles and standard using Nitric oxide assay.



The Nitric oxide assay used for the evaluation of antioxidant activity shows the following values 69.36, 76.11, 77.85, 82.69 and 87.47 %. The highest value estimated is at 50 μ l/ml for 87.47 % and 88.67 % is seen for standard as represented in Figure 7.

Figure 8: Percentage of inhibition of anti-inflammatory action of biosynthesized AgNPs and standard with the help of BSA assay.

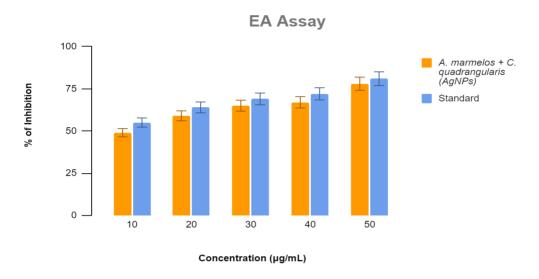


Using BSA assay for analyzing the anti-inflammatory action of AgNPs, the values obtained are 42%, 55%, 68%, 75% and 80 %. Out of this, the highest inhibition percentage is seen at 50 μ l/ml for 80%. The value for the standard is 84 % at the same level of concentration as

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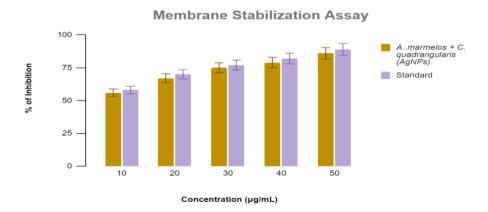
shown in Figure 8.

Figure 9: Graph representing percentages of anti-inflammatory action of biosynthesized AgNps and standard with the help of EA assay.



The values obtained from using an EA assay on the silver nanoparticles are 49%, 59%, 65%, 67%, and 78% at various levels of 10, 20, 30, 40 and 50 μ l/ml. 78% is the highest value of activity obtained at 50 μ l/ml and the standard value is 81% as shown in Figure 9.

Figure 10: The graph represents the percentages of anti-inflammatory action of biosynthesized AgNPs and the standard analyzed by membrane stabilization assay.



For the green synthesized silver nanoparticles, the values obtained using membrane stabilization assay are 56%, 67%, 75%, 79%, and 86% at different concentrations. The highest value obtained at maximum concentration is 86% while for standard it is 89% as shown in Figure 10.

4. Discussion:

The current research work explains the biosynthesis of AgNPs from Aegle marmelos and Cissus quadrangularis plant extract. When the values of the DPPH assay are assessed with the results of other papers, multiple outcomes of the activity are compared for further understanding. From (16), the highest DPPH activity was observed at 8 mg/ml with an inhibition percentage of 80 % which compared with our findings, the highest concentration of 50 mg/mL, 92.07 % of inhibition was seen. (17) shows that the highest scavenging activity was found to be 29.55 % whereas in our findings, the highest percentage of inhibition is 92.07 %. From the paper of (18), the synthesized silver nanoparticles exhibit 90 % scavenging activity at 100µg/ml whereas at only 50 mg/mL, a 92.07 % inhibition percentage is noted. In analyzing values for the hydrogen peroxide assay in this research paper, the highest inhibition percentage obtained by the hydrogen peroxide assay is 87.5 %. (19) shows that at 60 % of the inhibitory rate was reported at the highest level of 100µg/ml. The green synthesized silver nanoparticles from (20) show an inhibition percentage of 75.95 % at the highest concentration of 1 mg/mL. When the equal concentration of 50 µg/ml is assessed for their inhibition percentage, (21)) showcases that only 55 % inhibition is seen at 50 μg/ml whereas 87.5% is seen at the same concentration for the current research paper. When it comes down to ABTS antioxidant assay values, the highest value obtained in the research paper is 87.47 at 50 μg/ml whereas the highest antioxidant activity obtained at 100 µg/mL is 87.82% (22). In comparison, the research paper shows the highest antioxidant activity even at 50 µg/ml concentration. In the paper of (23). This shows that the silver nanoparticles synthesized from Aegle marmelos and Cissus quadrangularis are superior in antioxidant activity. On assessing the values of Nitric oxide assay, at the concentration of 50 µg ml, an inhibitory percentage of 87.47 % is reported from synthesized silver nanoparticles. In comparison with (24), only 78.46% is seen at the highest concentration of 100 μl/ml. In this paper (25), at the highest concentration of 200 μl/ml, only 45 % inhibition is seen. In analysis with the synthesized silver nanoparticles, 45 % at 200 μl/ml is very low in comparison with the value at 50 μg/ml. At the highest concentration of 200 μl/ml, an inhibitory percentage of 81 % is seen but for the silver nanoparticles synthesized from Aegle marmelos and Cissus quadrangularis even at 50 µl/ml, 87.47 inhibitory percentage is noted (26).

For egg albumin Assay, the highest inhibition percentage obtained is 78 % at 50 μ l/ml for the synthesized silver nanoparticles. In (27), the value of the inhibitory percentage for the AgNPs was 82% at the highest concentration of 500 μ g/mL. Maximum inhibition was seen at 500 μ g/mL at 81.13% which is more than diclofenac sodium drug which shows 73.58 % at the same concentration level (28). In this paper (29), 91.78% was the highest inhibition percentage value at the highest concentration of 500 μ g/mL and 51.73% was for the lowest concentration of 100 μ g/mL. For the BSA Assay, 80 % is obtained at the highest concentration of 50 μ g/mL. When the results are compared with (30) at the same concentration, a 92.9 % absorption percentage is reported. The silver nanoparticles from (31), Ch-AgNPs exhibited 85.3% at the maximum concentration of 500 μ g mL-1 and QE-AgNPs showed 100 % at 500 μ g mL-1. In, the highest inhibitory percentage is 92.9% at 50 μ L.

In comparison with the highest value of FRAP Assay, i.e. 88.63 % at $50 \mu g/mL$ along with (32) showing that the highest inhibition percentage is 73.98% at 1 mg/mL. In the paper of (33), the highest percentages are 79.0% and 73.7% at $500 \mu g mL-1$. In the Membrane stabilization

assay, the highest inhibition percentage reported is 86 % at 50 μ g/mL. At the highest concentration of silver nanoparticles, 82.5% is seen at 300 μ g/mL.

5. Conclusion:

In this paper, a brief idea about the antimicrobial, antioxidant, anti-inflammatory and cytotoxicity actions of Ag nanoparticles biosynthesized using Aegle marmelos and Cissus quadrangularis leaf extracts are explained. The following characterizations applied to the synthesized nanoparticles are XRD and FT-IR. In the tested concentrations, the nanoparticles exhibited efficient activities in comparison with the standard. Thus shortly, the various properties can be studied and observed by experimenting with other plant extracts.

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