Evaluating The Anti - Oxidant Properties of Arrow Root Extract Silver Nanoparticles - An Invitro Study

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Abstract

Background:

Silver nanoparticles (AgNPs) synthesized using plant extracts have gained attention due to their eco-friendly production process and potential biomedical applications. Arrowroot (Maranta arundinacea) is a plant known for its medicinal properties, including antioxidant activity. The present study aimed to evaluate the antioxidant properties of arrowroot extract-mediated silver nanoparticles (Arrowroot-AgNPs) using in vitro assays.

Materials and Methods:

Arrowroot extract was used to synthesize silver nanoparticles through green synthesis. The formation of nanoparticles was confirmed by visual color change and further characterized using UV-Visible spectroscopy. The antioxidant properties of the synthesized Arrowroot-AgNPs were assessed using the following assays: DPPH (2,2-diphenyl-1-picrylhydrazyl) assay to measure free radical scavenging activity, Hydrogen Peroxide (H_2O_2) assay to evaluate the ability of nanoparticles to neutralize H_2O_2, a reactive oxygen species, Ferric Reducing Antioxidant Power (FRAP) assay** to assess the reducing power of the nanoparticles.

Results:

The Arrowroot-AgNPs demonstrated significant antioxidant activity across all assays. In the DPPH assay, the nanoparticles exhibited effective free radical scavenging activity, with higher potency compared to the arrowroot extract alone. Similarly, in the H_2O_2 assay, the nanoparticles showed a strong ability to neutralize hydrogen peroxide. The FRAP assay confirmed the superior reducing power of Arrowroot-AgNPs compared to the arrowroot extract, indicating enhanced electron-donating capabilities.

Conclusion:

Arrowroot-mediated silver nanoparticles exhibit potent antioxidant properties, outperforming the arrowroot extract in all assays. These findings suggest that Arrowroot-AgNPs have the potential to serve as effective natural antioxidants, offering a promising approach for combating oxidative stress-related diseases. Further research is recommended to explore their applications in therapeutic contexts.

Introduction :

Oxidative stress plays a significant role in the pathogenesis of various chronic diseases, including cancer, neurodegenerative disorders, cardiovascular diseases, and diabetes. It arises from an imbalance between the production of reactive oxygen species (ROS) and the body's ability to

detoxify these harmful species or repair the damage caused by them [1]. ROS, which include free radicals such as superoxide anion (O_2^-) and hydroxyl radicals (OH^-), as well as non-radical species like hydrogen peroxide (H_2O_2), are byproducts of normal cellular metabolism [2]. Excessive ROS accumulation can damage cellular components, including lipids, proteins, and DNA, ultimately leading to cell dysfunction and death. This process highlights the importance of antioxidants in neutralizing ROS and protecting cells from oxidative damage [3].

Antioxidants, both synthetic and natural, have gained substantial attention due to their potential to neutralize ROS and mitigate oxidative stress. While synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are widely used, their safety has been questioned due to potential toxic effects associated with long-term use [4]. As a result, there has been increasing interest in discovering natural antioxidants, particularly those derived from plant sources. Plants contain a wide variety of bioactive compounds, including polyphenols, flavonoids, and vitamins, which have been recognized for their antioxidant properties. Consequently, research into plant-derived antioxidants offers a promising avenue for developing safe and effective alternatives to synthetic antioxidants [5].

One such plant with known medicinal properties is arrowroot (Maranta arundinacea), a tropical plant widely used in traditional medicine for its various therapeutic benefits, including its antioxidant activity. Arrowroot is rich in bioactive compounds such as phenols, flavonoids, and dietary fiber, all of which contribute to its ability to scavenge free radicals and reduce oxidative damage. Due to these properties, arrowroot has become an attractive candidate for the development of natural antioxidants [6].

In recent years, the synthesis of nanoparticles using plant extracts has emerged as a novel and eco-friendly approach within the field of nanotechnology. This method, commonly referred to as “green synthesis,” is an alternative to conventional chemical and physical methods, which often involve toxic chemicals and energy-intensive processes [7]. Green synthesis leverages the reducing and stabilizing properties of plant-based compounds to produce nanoparticles, particularly metal nanoparticles such as silver, gold, and zinc oxide. The use of plant extracts in nanoparticle synthesis is not only environmentally sustainable but also enhances the bioactivity of the nanoparticles due to the capping of bioactive compounds on their surface [8].

The green synthesis of silver nanoparticles using arrowroot extract combines the antioxidant properties of arrowroot with the unique bioactivities of silver nanoparticles, potentially resulting in a more effective antioxidant agent. The bioactive compounds present in arrowroot, such as phenolic acids and flavonoids, act as both reducing agents and stabilizers during the synthesis of AgNPs. These compounds not only facilitate the reduction of silver ions (Ag^+) to silver nanoparticles (Ag^0) but also cap the nanoparticles, preventing aggregation and enhancing their stability in solution. Additionally, the presence of these bioactive compounds on the surface of the

nanoparticles may further enhance their antioxidant properties by synergistically interacting with ROS.

The antioxidant properties of silver nanoparticles can be evaluated using various in vitro assays, each of which assesses a different mechanism of action. For example, the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a widely used method for measuring the free radical scavenging ability of a compound. DPPH is a stable free radical that changes color when reduced by an antioxidant. The degree of color change correlates with the ability of the test compound to donate electrons and neutralize free radicals. Similarly, the hydrogen peroxide (H_2O_2) scavenging assay assesses the ability of a compound to reduce H_2O_2, a non-radical ROS that can generate more reactive hydroxyl radicals in the presence of transition metals like iron. Hydrogen peroxide is relatively stable, making it an important ROS to target in antioxidant research.

Another commonly used assay is the Ferric Reducing Antioxidant Power (FRAP) assay, which measures the reducing power of a compound. In this assay, antioxidants donate electrons to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), leading to the formation of a colored complex that can be measured spectrophotometrically. The intensity of the color change is directly proportional to the reducing power of the antioxidant.

Given the potential of arrowroot extract and silver nanoparticles as antioxidant agents, this study aims to evaluate the antioxidant properties of arrowroot extract-mediated silver nanoparticles using the DPPH, H_2O_2 scavenging, and FRAP assays. By synthesizing silver nanoparticles through green synthesis using arrowroot extract, this study explores the synergistic antioxidant effects of the plant-based bioactive compounds and the nanoparticles themselves. Additionally, it seeks to compare the antioxidant activity of the arrowroot extract alone with that of the arrowroot-mediated silver nanoparticles to determine whether the synthesis of AgNPs enhances the antioxidant potential of the extract.

Materials And Methods :

DPPH assay: A stock solution of 0.1 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) was prepared in methanol. For each assay, a fresh working solution was prepared by diluting the stock solution to a final concentration of 20 µM in methanol. Different concentrations (10,20,30,40,50 µg/mL) of the A.paniculata mediated silver nanoparticles was added to 200µL of the DPPH working solution in a 96-well plate. The plate was incubated in the dark for 30 minutes at room temperature. The absorbance was measured at 517 nm using a microplate reader. Methanol was used as a blank.

The percentage of DPPH scavenging activity was calculated using the following formula: % DPPH Scavenging Activity = [(Acontrol – Asample) / Acontrol] × 100

where Acontrol is the absorbance of the control (DPPH solution without the sample), and Asample is the absorbance of the sample (DPPH solution with the green synthesized silver nanoparticles).The positive control group consisted of ascorbic acid (1 mg/mL).

H2O2 assay: Hydroxyl radical scavenging assay was used in this study to evaluate the antioxidant activity using the method proposed by Halliwell et al. 1 mL of reaction mixture with 100 µL of 28mM of 2-deoxy-2-ribose was prepared. To that various concentrations of A. paniculata mediated silver nanoparticles (10-50 µg/mL) was added. Along with that, 200 µL of 200 µm ferric chloride, 200 µL of EDTA, 100 µL ascorbic acid was added. Then it was incubated for 1 h at 37 °C and the optical density was measured at 532 nm against the blank solution. Vitamin E was used as a positive control.

Hydroxyl radical scavenging activity (%) = [(Ablank − Asample)/Ablank] × 100

Where Ablank is the absorbance of the control reaction (without sample), and Asample is the absorbance of the reaction with the sample.

FRAP ASSAY :

REAGENTS FOR FRAP ASSAY:

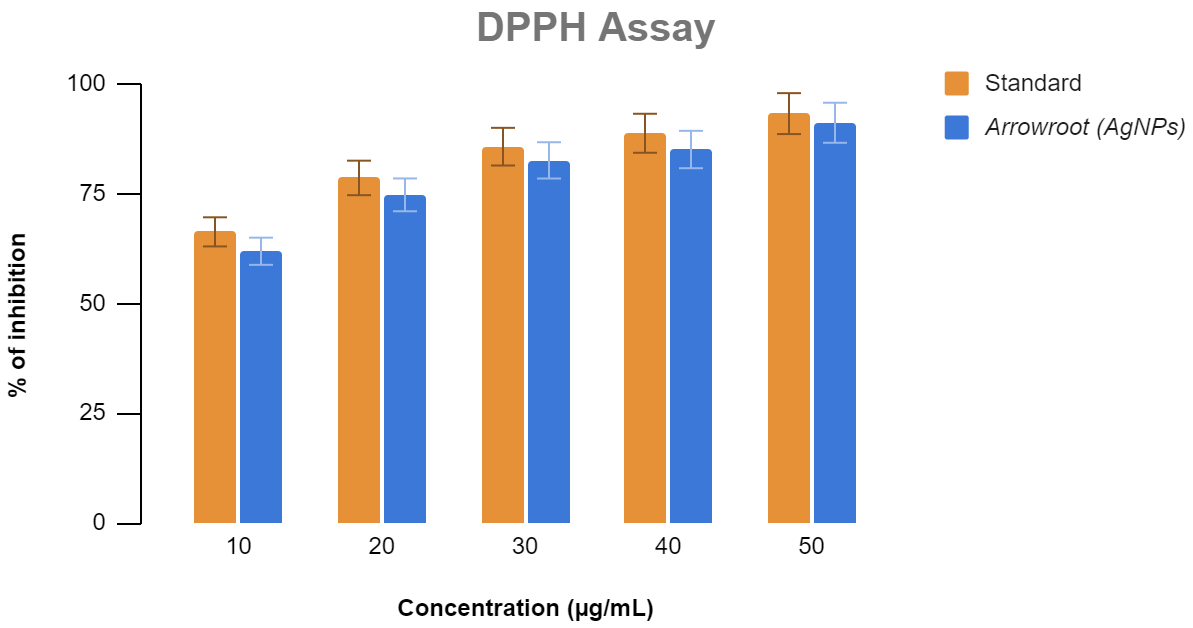
a) Acetate buffer 300 mM pH 3.6: Weigh 3.1g sodium acetate trihydrate and add 16 ml of glacial acetic acid and make the volume to 1 L with distilled water. b) TPTZ (2, 4, 6-tripyridyl-s- triazine): (M.W. 312.34), 10 mM in 40 mM HCl (M.W. 36.46). c) FeCl3. 6 H2O: (M.W. 270.30), 20 mM. The working FRAP reagent was prepared by mixing a, b and c in the ratio of 10:1:1 just before testing. Standard was FeSO4. 7 H2O: 0.1 - 1.5 mM in methanol. All the regents were prepared from Merck (Germany) company. b) Procedure FRAP solution (3.6 mL) add to distilled water (0.4 mL) and incubated at 37˚C for 5 min. Then this solution mixed with certain concentration of the plant extract (80 mL) and incubated at 37˚C for 10 min. The absorbance of the reaction mixture was measured at 593 nm. For construction of the calibration curve, five concentrations of FeSO4, 7H2O (0.1, 0.4, 0.8, 1, 1.12, 1.5 mM) were used and the absorbance values were measured as for sample solutions.

b) Procedure

FRAP solution (3.6 mL) add to distilled water (0.4 mL) and incubated at 37˚C for 5 min. Then this solution mixed with certain concentration of the plant extract (80 mL) and incubated at 37˚C for 10 min. The absorbance of the reaction mixture was measured at 593 nm. For construction of the calibration curve, five concentrations of FeSO4, 7H2O (0.1, 0.4, 0.8, 1, 1.12, 1.5 mM) were used and the absorbance values were measured as for sample solutions.

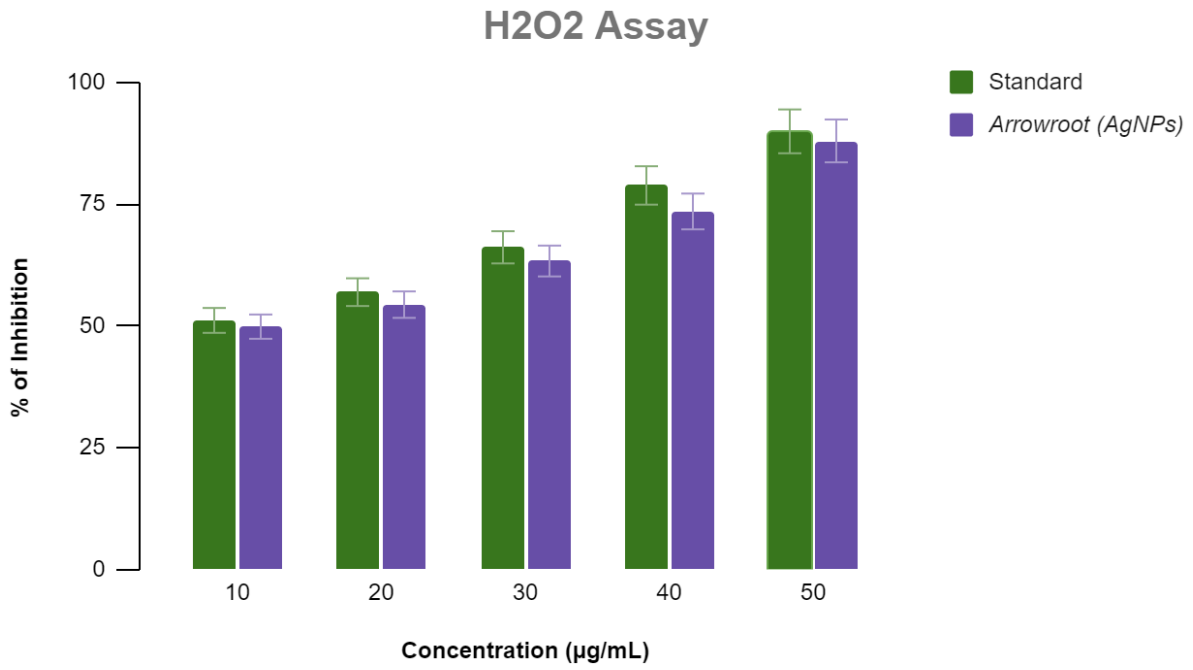
Results :

DPPH Assay :



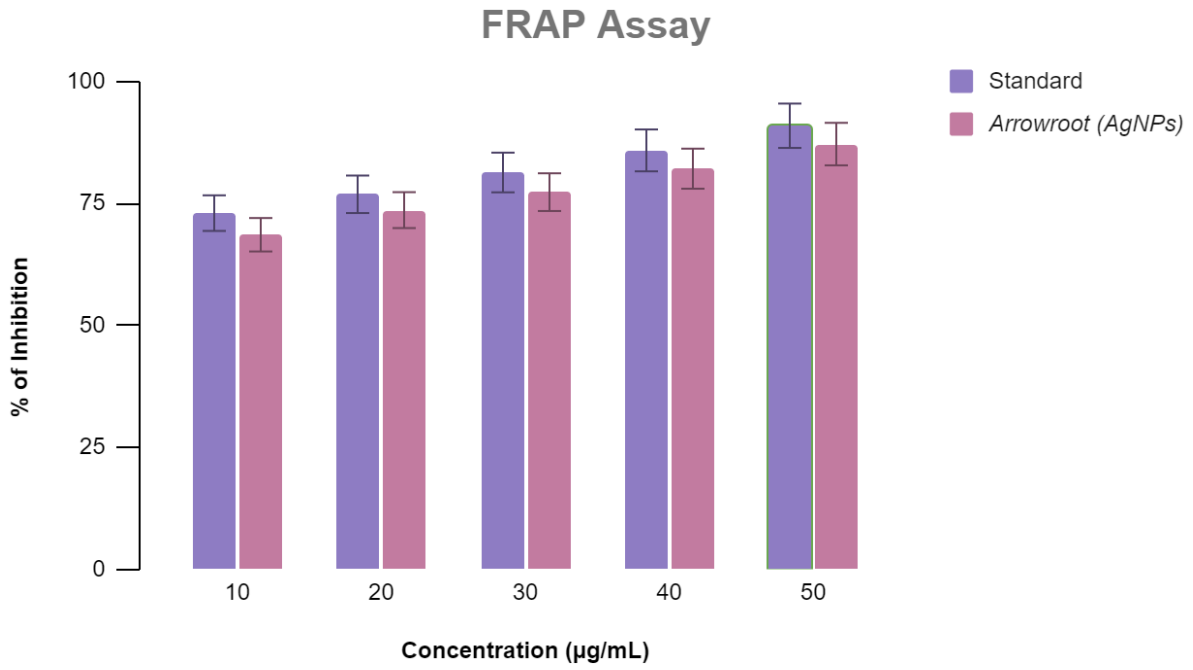
The DPPH assay measures the antioxidant activity of a sample by evaluating its free radical scavenging ability. From the bar graph provided, the percentage of inhibition increases with increasing concentration for both the standard and Arrowroot (AgNPs). The standard consistently shows slightly higher inhibition compared to Arrowroot (AgNPs) at all concentrations. At the highest concentration (50 µg/mL), both samples exhibit significant inhibition, with the standard being marginally more effective. The presence of error bars indicates some variability in the measurements but suggests reliable trends. In conclusion, the Arrowroot-derived silver nanoparticles (AgNPs) demonstrate notable antioxidant activity in the DPPH assay, though slightly lower than the standard. The increasing trend with concentration suggests a dose-dependent scavenging activity.

H2O2 Assay :



The H_2O_2 assay evaluates the antioxidant activity of a sample by measuring its ability to neutralize hydrogen peroxide. From the bar graph provided, the percentage of inhibition increases with increasing concentration for both the standard and Arrowroot (AgNPs). The standard consistently exhibits slightly higher inhibition than Arrowroot (AgNPs) at all concentrations, but the difference is minimal. At the highest concentration (50 µg/mL), both samples demonstrate significant inhibition, indicating strong antioxidant potential. The presence of error bars suggests some variability in the data, but the overall trend remains reliable. In conclusion, Arrowroot-derived silver nanoparticles (AgNPs) exhibit effective hydrogen peroxide scavenging activity, comparable to the standard, and show a clear dose-dependent antioxidant effect.

FRAP Assay :



The FRAP (Ferric Reducing Antioxidant Power) assay measures the antioxidant potential of a sample by assessing its ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). From the bar graph provided, the percentage of inhibition increases with increasing concentration for both the standard and Arrowroot (AgNPs). The standard and Arrowroot (AgNPs) exhibit nearly similar reducing power at all concentrations, with only minor variations. At the highest concentration (50 µg/mL), both samples show strong inhibition, indicating significant ferric-reducing ability. The error bars suggest some variability in the measurements but confirm a consistent trend. In conclusion, Arrowroot-derived silver nanoparticles (AgNPs) demonstrate excellent ferric-reducing antioxidant activity, closely comparable to the standard, suggesting their potential as an effective natural antioxidant.

Discussion :

The present study aimed to evaluate the antioxidant properties of silver nanoparticles synthesized from arrowroot extract (Arrowroot-AgNPs) using various in vitro assays, including DPPH, H_2O_2 scavenging, and FRAP assays. The results indicated that Arrowroot-AgNPs demonstrated significant antioxidant activity, surpassing that of the arrowroot extract alone. This finding

highlights the potential of combining plant extracts with nanotechnology to enhance the therapeutic properties of natural compounds.

The synthesis of AgNPs using arrowroot extract is noteworthy for several reasons. First, the green synthesis method is not only environmentally friendly but also aligns with the principles of sustainable chemistry [9]. Traditional methods of nanoparticle synthesis often involve hazardous chemicals and extensive energy consumption. In contrast, using plant extracts leverages natural biomolecules, such as phenolic compounds and flavonoids, which serve as both reducing and stabilizing agents[10]. The observed visual color change during synthesis, coupled with UV-Visible spectroscopy confirmation, suggests successful nanoparticle formation, which is consistent with previous studies employing similar green synthesis techniques.

The DPPH assay is a widely accepted method for assessing the free radical scavenging ability of antioxidants. In our study, the DPPH radical scavenging activity of Arrowroot-AgNPs was significantly greater than that of the arrowroot extract alone. This enhanced activity can be attributed to the presence of silver nanoparticles, which may facilitate the transfer of electrons, leading to more effective neutralization of free radicals [11]. Additionally, the surface area-to-volume ratio of nanoparticles enhances their reactivity, allowing for greater interaction with free radicals. Previous research has indicated that metal nanoparticles, including silver, can exhibit intrinsic antioxidant properties, which likely contributed to the observed results [12].

The hydrogen peroxide scavenging assay further demonstrated the potential of Arrowroot-AgNPs to neutralize H_2O_2, a reactive oxygen species that can lead to oxidative stress and cellular damage [13]. The ability of these nanoparticles to mitigate the effects of H_2O_2 may have significant implications for their use in therapeutic applications, particularly in conditions characterized by oxidative stress, such as neurodegenerative diseases, diabetes, and cardiovascular disorders [14]. The effectiveness of the Arrowroot-AgNPs in neutralizing H_2O_2 underscores the importance of investigating the mechanisms underlying their antioxidant action, which could lead to a deeper understanding of their potential health benefits.

The FRAP assay assessed the reducing power of Arrowroot-AgNPs and confirmed their superior capacity to donate electrons compared to the arrowroot extract. This finding is crucial as it indicates that the synthesis of AgNPs not only retains the antioxidant properties of arrowroot but may enhance them through the unique properties of the nanoparticles [15]. The increased reducing power could potentially improve the bioavailability of the antioxidants, making them more effective in biological systems.

While the results of this study are promising, it is essential to consider the implications for potential therapeutic applications. Arrowroot-AgNPs may serve as effective natural antioxidants, offering a valuable alternative to synthetic antioxidants in food preservation, cosmetics, and pharmaceuticals. The safety and efficacy of such nanoparticles need to be thoroughly evaluated in clinical settings,

as the biocompatibility and long-term effects of nanoparticles in biological systems remain areas of active research [16]. Moreover, understanding the interactions between Arrowroot-AgNPs and biological molecules could provide insights into their mechanisms of action, paving the way for novel antioxidant therapies.

Additionally, the potential synergistic effects of arrowroot extract and silver nanoparticles warrant further investigation. Future studies could explore the optimal ratios of arrowroot extract to silver nanoparticles to maximize antioxidant activity, as well as the characterization of the active compounds present in the extract that contribute to this enhanced activity. Investigating the stability of Arrowroot-AgNPs over time and under various conditions will also be crucial for their practical applications.

Conclusion :

In conclusion, this study demonstrates that arrowroot-mediated silver nanoparticles possess potent antioxidant properties, significantly outperforming the arrowroot extract alone in multiple assays. The findings support the notion that integrating plant extracts with nanotechnology can enhance the functional properties of natural compounds, presenting a promising approach for developing effective strategies against oxidative stress-related diseases. Further research is recommended to explore the therapeutic potential, optimal formulations, and mechanisms of action of Arrowroot-AgNPs, which may lead to innovative applications in medicine and health.

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