

Green Synthesis of Silver Nanoparticles from *Nyctanthes arbor tristis* L. Leaf Extract and Evaluation of Its Antidiabetic and Antioxidant Properties

Ashok Kumar Tripathi

Institute of Pharmacy V.B.S. Purvanchal University

Alok Kumar Dash

Institute of Pharmacy V.B.S. Purvanchal University

Jhansee Mishra (✉ alokjina@gmail.com)

Institute of Pharmacy V.B.S. Purvanchal University

Research Article

Keywords: *Nyctanthes arbor-tristis*, Silver nanoparticle, SEM, TEM, DPPH, XRD

Posted Date: February 9th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-3917633/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

In *Nyctanthes arbor-tristis*, green production of silver nanoparticles has been described. After combining 1% aqueous leaf extract with 1 mM silver nitrate solution and letting it sit for 10 minutes at room temperature in the light, nanoparticles were effectively created. The brown reaction mixture's UV–Vis spectra revealed a strong surface plasmon resonance at 460 nm. It verified that silver nanoparticles are biosynthesised. SEM and TEM were used to characterize the nanoparticles. According to the data, produced nanoparticles have a diameter between 10 and 50 nm. The polycrystalline nature of the biosynthesized nanoparticles was verified by XRD measurements. Compared to extract, silver nanoparticles demonstrated a stronger antidiabetic effect. Every clinical isolate with antidiabetic properties was sensitive to nanoparticles. Additionally, the silver nanoparticles showed strong free radical scavenging capabilities.

1 Introduction

Synthesis of nanoparticles with a variety of applications in human life is the field of nanotechnology, which is still in its infancy. Because of its extensive use in the fields of physiochemical and biological processes, the emphasis on the production of nanomaterials has steadily increased. For the purpose of creating nanoparticles, a number of unique metals, including copper [4], gold [2], palladium [3], and silver [1], were employed. Silver is one of these metals that is frequently used to create nanoparticles [5]. There are several ways to create silver nanoparticles, including biological and physico-chemical techniques. Because it is non-toxic and environmentally benign, the biological route of synthesis is better appropriate for this technology [6]. Due to their unique characteristics, including optical behavior, catalytic activity, electrical conductivity, and chemical stability, silver nanoparticles (AgNPs) have drawn a lot of attention [7]. Biochemistry, medicine, and the material sciences all employ green synthetic nanoparticles [8]. The green produced silver nanoparticle's anticancer [9], antidiabetic [10], and antioxidant [11] properties make it useful in a variety of medical applications. The microorganisms are poisonous to the compounds based on silver as well as the silver ions. They investigated their biocidal efficacies against a number of harmful microorganisms [12]. Commercial applications for silver nanoparticles include medication administration, wound treatment, biomolecular detection, and diagnostic research [13–14]. The need for herbal-based nanomedicines in therapeutics is currently growing rapidly [15]. For the manufacture of nanoparticles, several plant extracts from the stem, leaf, root, latex, and seeds were employed [16]. Because green synthesis of herbal-based nanoparticles has a deep penetration capability and is affordable, environmentally friendly, and cost-effective, it offers advantages over the conventional medical system [17]. Different phytoconstituents with a variety of functional groups can be found in plant extracts [18]. Without the need for artificial chemicals, these functional groups operate as a reducing agent to produce stable nanoparticles [19]. Recently, extracts from *Capparis spinosa* [20], *Syzygium aromaticum* [21], and *Calliandra homalocephala* [22] have been used to create phyto-silver nanoparticles that can be used for a variety of biological purposes. A significant medicinal plant in the Oleaceae family, *Nyctanthes arbor-tristis* L. demonstrates pharmacological properties such as antioxidant [23] and antidiabetic [24] effects. No reports of photocatalyzed green silver nanoparticle synthesis utilizing *Nyctanthes arbor-tristis* aqueous leaf extract exist. The goal of the current work was to characterize the silver nanoparticles using various approaches and to optimize the various parameters for an effective green production. In order to evaluate these nanoparticles' potential as an anti-diabetic agent, they were evaluated against green synthetic nanoparticles' diabetic action.

2 Materials and Methods

2.1 Preparation of Leaf Extract

Fresh *Nyctanthes arbor-tristis* leaves were collected and thoroughly cleaned with double-distilled water from the Pharmacy Department of Veer Bahadur Singh Purvanchal University, formerly known as the Purvanchal University Garden. Plant materials were extracted from both of them. It was mixed with 10g of powdered mint leaves and 150 ml of distilled water. The solution was heated to 100°C for two minutes, cooled to room temperature, and then filtered through Whatman 1 filterpaper to extract the components of the mint leaves. When the extracted solution reached 4 degrees Celsius, it was kept

2.2 Synthesis of Silver Nanoparticles

We used the *Nyctanthes arbor-tristis* aqueous leaf extract that was made in the previous stage to create silver nanoparticles. This involved boiling 90 mL of a 1 mM aqueous silver nitrate solution for three hours at 80 °C while continuously stirring in 10 mL of leaf extract. The color shift from yellow to dark brown served as a preliminary indicator of the AgNPs' development. Centrifugation was used to separate the green-produced nanoparticles for 20 minutes at 15,000 × g. To remove the free silver linked to Cp-AgNPs, this procedure was carried out three times. Cp-AgNPs, the final green-synthesised silver nanoparticles, were freeze-dried and kept at 4 °C until needed again.

2.4 Green Synthesized Silver Nanoparticle Characterization

2.4.1 Spectroscopy in UV-Vis

Using a Shimadzu UV-1800 double beam UV–Vis spectrophotometer, the optical density of the reaction mixture was measured at various time intervals against Milli Q water as a blank to track the synthesis of silver nanoparticles. The reaction mixture's absorbance was measured between 300 and 700 nm.

2.4.2 Silver Nanoparticle SEM Analysis

Analyses using scanning electron microscopy (SEM) were carried out on an EVOSEM MA 15/18 outfitted with an EDS device. A copper grid was used to make a thin film of the sample by simply dropping a very little amount of the sample onto the grid. The size and shape of the nanoparticles were examined using a scanning electron microscope to ascertain whether elemental silver was present in AgNPs (Nova Nano SEM 450, USA)

2.4.3 Analysis of TEM

To prepare the sample for analysis using transmission electron microscopy (TEM; Tecnai G2-F20 TWIN, FEI USA), the generated AgNPs colloidal solution is centrifuged at 10,000 rpm for 15 minutes at 25 °C to remove the non-covalently attached molecules from their surfaces. For the nanoparticles to be properly dispersed, it was further sonicated. To create a thin film, a few drops of the solution were applied to a copper grid and evenly distributed across the grid. The grid was dried for 120 minutes at 50 °C in a hot air oven (NSW-India-143). The nanoparticles' size was also examined at various magnifications. (25)

2.4.4 XRD Utilizing an X-ray diffractometer (Rigaku Minifex 600) fitted with a Cu K α radiation source and a nickel monochromator filter in the 2 θ range of 20°–80° at a scanning rate of 2° min⁻¹, the X-ray diffraction pattern (XRD) of AgNPs was examined. A glass microscope slide with a nanoparticle solution was used for this analysis. To create a layer on the glass slide, the operation was repeated. The glass slide was completely dried at 60 °C in a hot air oven. The dehydrated AgNPs were thrown out for additional analysis. The Debye-Scherrer equation was used to determine the AgNPs' average particle size. where D is the average crystallite domain size, which is perpendicular to the reflecting planes; K is the Scherrer's constant, with a value between 0.9 and 1; λ is the X-ray source's wavelength (0.1541), which is used in XRD; β is the diffraction angle; and together these values indicate the full width at the half maximum of the diffraction peak. Selected area electron diffraction (SEAD) was examined in order to verify that AgNPs are crystalline.

2.4.5 Examination of Fourier Transform Infrared Spectroscopy (FT IR)

Using an FTIR spectrophotometer (Bruker, Germany), the Fourier transform infrared (FTIR) spectra of the dried extract and nanoparticle were recorded in order to characterize the surface structure of the silver nanoparticle in the wavelength range of 4000–400 cm⁻¹.

2.4.6 DPPH Assay in Vitro

Using a slightly modified Brand-W technique, the DPPH scavenging activity of plant extracts against stable 2, 2 diphenyl 2 picryl hydroxyl hydrate (DPPH) was ascertained. When DPPH interacts with an antioxidant, it can contribute hydrogen and be reduced. Using a BMG Fluostar (GERMANY), the color change (from deep violet to light yellow) was observed at 517 nm. The MARS software from BMG (Germany) was used to evaluate the percentage inhibition data. Before UV measurements, a fresh solution of DPPH in methanol 6 × 10⁻⁵ M was made every day. A 100 microgram/ml concentration of separate plant extracts and a herbal preparation were combined with three milliliters of this solution. After allowing the samples to sit at room temperature in the dark for fifteen minutes, the absorbance dropped. (26) The percentage of DPPH free radical scavenging activity = [(Control absorbance - sample absorbance) - control absorbance] × 100

2.4.7 Reduced Glutathione

The Reduced Glutathione (GSH) Assay Kit relies on an enzymatic cycling technique with a chromophore and GSH present. A stable product is produced by the reduction of the chromophore, and this can be monitored kinetically by measuring the absorbance at 450 nm (A450). As a result, the absorbance and GSH content of the sample are directly correlated. The lack of interference from GSSG in the assay makes the kit extremely sensitive and specific. 5-Sulfosalicylic acid (SSA) is included in the kit to safeguard the samples' endogenous GSH level. (SSA functions as an antioxidant and deproteinizing agent.) In a 100 L reaction, the assay can precisely detect reduced glutathione (GSH) as low as 50 pmol/well and is straightforward and repeatable. (Table-2) In short, 1ul of enzyme mix B, 2ul of anzyme mix C, 2ul of substrate mix C, and 55ul of GSH assay buffer are combined with 10ul of substrate mix A. The OD is then determined at 450 nm. GSH from standard curve/(reaction volume added to sample X sample concentration in mg protein/ml) X sample dilution factor is expressed in nmol/mg for computations.(Fig. 7)

2.4.8 Assay for Lipid Peroxidase TBARS (MDA)

Put 200 µL of sample and 600 µL of TBA reagent into each well. Incubate for 60 minutes at 95°C. Spend ten minutes cooling in an ice bath to room temperature. For analysis, transfer 200 µL of the reaction mixture—which contains the MDA-TBA adduct—into a 96-well microplate. For a colorimetric assay, determine absorbance right away using a microplate reader set to OD 532 nm.

2.4.9 Studies on Acute Toxicity

Test samples were appropriately weighed and freshly triturated with water and 1% CMC as needed. For 15 days, test samples were given orally via gavage at a dose of 100 mg/g. Animal urine samples were taken at the conclusion, and blood samples were obtained by heart puncture and processed in accordance with the analysis's requirements.

2.4.10 Animal model-based in vivo investigation of anti-diabetic efficacy

Wistar rats were kept in noise-controlled rooms at 25°C with a 12–12 light–dark cycle. The animals were fed a regular pellet diet and were allowed unlimited access to water. Test samples were weighed accurately and administered intraperitoneally (I.P.) in accordance with body weight. Animal urine samples were taken at the conclusion, and blood samples were obtained by heart puncture and processed in accordance with the analysis's requirements.

2.4.10.1 SGOT

SGOT catalyzes the formation of oxaloacetate and glutamate by transferring an amino group from L aspartate to keto glutamate. When malate dehydrogenase is present, the oxaloacetate that is produced combines with NADH to produce NAD. The drop in absorbance, which is correlated with the sample's SGOT activity, is used to calculate the rate of oxidation of NADH to NAD.

To make the working reagent, 1 ml of enzyme and 5 ml of start reagent were combined. After mixing 100 µl of working reagent with 20 µl of serum sample, the OD was measured at 340 nm at 25–30°C for two and three minutes. The OD difference was computed and multiplied by 952 to arrive at the final result. The measurements were expressed in IU/L. Reagent size was reduced as needed.

2.4.10.2 SGPT:

Pyruvate and glutamate are produced by SGPT from L-alanine and alpha ketoglutarate. When 2,4, Dinitrophenyl Hydrazine and Pyruvate are combined, a brown complex is produced whose intensity can be evaluated. To sum up, we combined 0.3 ml of substrate reagent, 0.2 ml of pyruvate standard, 0.1 ml of DW, and 0.05 ml of DNPH reagent, then we let it stand for 20 minutes at room temperature. The OD was examined and calibrated.

For three minutes, 0.50 ml of substrate reagent was incubated at 37°C. Add 0.1 ml of the serum sample. Stir thoroughly and let stand at 37°C for three minutes. Add 0.1 ml of the serum sample. Mix thoroughly, then incubate for 30 minutes at 37°C. Add 0.5 ml of the DNPH reagent. After thoroughly mixing, incubate for 20 minutes at RT. Add 5ml of the NaOH reagent. Mix thoroughly and leave at room temperature for ten minutes. Find the OD at 505 nm. Reagent size was reduced as necessary.

2.4.10.3 WBC:

Fill a Neubauer chamber with a 20 µl blood sample and 380 µl of WBC dilution solution. Count the cells in the chamber's WBC section. A sizable region measures 1 × 1 mm, with a depth of 0.1 mm. Four huge squares times the total area, or 4 × 1 × 0.1, equals 0.4 µL (4/10). The total WBC in a single µL is equal to Y × 10/4. Dilution is now 1:20.

Total WBC count = $Y \times 10 \times 20/4 = Y \times 50$ = Number of cells in 1µL. WBC/cu mm = total WBC - counted cells Yx 50.

2.4.10.4 RBC:

Fill a Neubauer chamber with 20 µl of blood with 3980 µl of RBC dilution solution. Count the cells in the chamber's WBC section. In the chamber's RBC section, count the cells. Factor to be multiplied = $10 \times 200 / 0.2 = 10,000$ Multiply the number of RBCs by 10,000 to get RBCs X106/mm.

2.4.10.5 Hemoglobin:

After making sure the tube and pipette for hemoglobin are dry. Pour in N/10 HCl until the 2g% mark is reached. Gently stir the EDTA sample, then transfer 0.02 ml of blood into the pipette. Ensure that the pipette is free of air bubbles. If it comes in, remove it and pipette once more. To get rid of any extra blood, wipe the pipette's exterior. Fill the tube with HCl after adding the blood. To completely mix the blood with the acid, pull in and blow out the acid several times to wash away the contents of the hemoglobin pipette. Let stand for ten minutes without moving.

(This is because the first ten minutes are when hemoglobin converts to acid hematin at its greatest rate.) After inserting the hemoglobinometer tube into the comparator, gradually add distilled water to the mixture. Work the glass rod until the color of it and the comparator glass are the same. The glass rod needs to be taken out of the solution and held upright in the tube while maintaining the same color. (Take note that the stirrer needs to be in the solution above the level rather than out of the tube.) The outcome should be noted as the reading of the solution's lower meniscus. Use g/dl to express the hemoglobin content.

2.4.10.6 HCT: Blood is centrifuged in an HCT tube. RBCs predominately will settle, and clear plasma will rise to the surface. This is the percentage-based ratio of settling cells to upper clear plasma.

2.4.10.7 Clotting Time: Blood obtained through orbital puncture and placed in a capillary tube. Every 30 seconds, a portion of the capillary tube was broken until a clot thread was recovered. Clotting time was defined as the duration during which the thread was obtained.

2.4.10.8 Platelet:

Place 3.98 milliliters of Rees-Ecker Fluid, or platelet diluting fluid, in a test tube that is dry, clean, and grease-free. Using a micropipette, add 0.02 ml or 20 μ l of Blood Specimen (1:200 Dilution) to the tube holding the diluting fluid. After thoroughly mixing for a few minutes, prepare your Neubauer's Chamber and hemocytometer.

$N \times \text{Dilution} / \text{Area} \times \text{Depth}$ $N \times 20 / (1/5 \times 0.1)$ equals the total platelet count.

Platelet count total = $N \times 1000 / \text{mm}^2$

2.4.10.9 Urine Analysis: Two independent observers examined the urine sample after it was collected in a test tube and 10ul was applied to the urine analysis strip (Acon).

3. Results

3.1 Enhancement of Silver Nanoparticle Biosynthesis UV-Vis Spectroscopy Application

The reaction mixture's color changed from green to a colorless silver nitrate solution when the green leaf extract was added and allowed to sit in the light at room temperature. As seen in Fig. 1a, the reaction mixture's color changed to brown. The reaction mixture's highest absorbance was measured at 460 nm (Fig. 1b). This is the silver surface plasmon resonance (SPR) peak. It verified that silver nanoparticles are biosynthesised. Several parameters, including the extract content (0.1–2%), silver nitrate (0.5–5 mM), and the length of light exposure (5–30 min), were assessed in order to synthesize high-quality nanoparticles.

3.2 SEM Evaluation

Scanning electron microscopy revealed a well-defined spherical silver nanoparticle (Fig. 2) free of any aggregation. The size and morphology of the nanoparticles were investigated using a scanning electron microscope (Nova Nano SEM 450, USA).

3.3 TEM Evaluation

The produced silver nanoparticles' TEM pictures were taken at various magnifications. The results indicate that the green nanoparticles produced by biosynthesis were almost spherical and ranged in size from 10 to 50 nm (Fig. 3).

3.4 XRD Schematic

The XRD pattern analysis (Fig. 6) verified that AgNPs are polycrystalline. Four significant diffraction peaks were visible in the XRD spectrum at 2θ values of 38.18° , 44.25° , 64.25° , and 77.40° . These values corresponded to lattice plane values indexed at the (111), (200), (220), and (311) planes. Bragg's reflections of metallic silver's face-centered cubic (FCC) structure. The resulting particles' identity as silver nanoparticles was verified by the XRD analysis. The crystalline structure and small particle size of silver nanoparticles are shown by their broad and prominent spectra peaks.

3.5 FTIR Analysis

Fig. 5 displayed the FTIR spectra of the biosynthesised silver nanoparticles and leaf extract. Peaks at 3897.7, 3744.7, 3681.94, 3266.94, 3030.97, 2528.86, 2307.33, 1694.52, 1513.07, 1412.33, 871.51, 784.03, 714.36, 672.50, and 613.93 cm^{-1} were visible in the leaf extract FTIR spectrum (Fig. 5). The leaf extract's absorption peaks (3897.7, 3744.7, 3681.94, 3266.94, and 3030.97 cm^{-1}) in the 4000–3000 cm^{-1} range indicated the presence of an O–H stretching hydroxyl functional group, either in strong hydrogen bonding or in the free state. The

presence of phytoconstituents such as tannins, flavonoids, and polyphenols is indicated by these peaks. Peaks (2528.86, 2307.33 cm^{-1}) in the 3000–2800 cm^{-1} wavelength range provide information regarding the presence of C–H stretching alkanes. Within the 2000–1000 cm^{-1} wave number range, the peaks were 1694.52, 1513.07, and 1412.33 cm^{-1} . These values indicate the existence of C=O, C=N, C=C, O–H, and C–H stretching, indicating the possibility of tannin, triterpene, and glycoside presence. The peaks at 871.51, 784.03, 714.36, 672.50, and 613.93 cm^{-1} in the 1000–500 cm^{-1} range suggest the presence of O–C and alkyl halide (C–X) stretching. It shows how the presence of meta-aromatic hydrocarbons and acidic components is possible. The absence of peaks in the FTIR of the nanoparticles indicates that the polyphenolic components found in the extract, which correspond to the H-bond, OH stretch, and C-H stretch, may play a significant role in the reduction and capping of silver ions.

4 Discussion

The size and shape of nanoparticles have led to a gradual increase in interest in their synthesis. Physicochemical techniques are widely employed in the production of several metal-based nanoparticles [27]. Applications of nanoparticles in medicine have steadily grown [28]. Deep within the afflicted tissues and organs, drug-loaded nanoparticles were easily absorbed [29]. Because green nanoparticle synthesis has less of an influence on the environment, demand for it has surged. It was produced quickly in large quantities with a consistent size. Plant extracts were effectively employed in the environmentally friendly manufacturing of nanoparticles [30]. These extracts are involved in capping and aid in the reduction of metal ions. Secondary metabolites with distinct functional groups included in the extract have a role in stabilizing and reducing Ag^+ to Ag^0 in the green route-based biosynthesis of silver nanoparticles. UV-Vis spectroscopy is a useful tool for monitoring the synthesis of nanoparticles. It is frequently employed to verify the biogenesis of silver nanoparticles. When *N. arbor-tristis* leaf extract was added to a silver nitrate solution, the solution's color clearly changed from green to brown. At 460 nm, the reaction mixture's absorbance peaked. The surface plasmon response phenomenon, which verified the conversion of silver nitrate to silver ions (Ag^+), provided the basis for this color shift [5]. It provides information on how silver nanoparticles are made biologically. The maximum absorbance in the 400–460 nm region provides information regarding the production of silver nanoparticles. At this wavelength, silver nanoparticle absorbance has been reported in several papers [1]. Various parameters, such as extract concentration, silver nitrate content, and light exposure length, play a significant influence in the efficient, fast, and high-quality synthesis of silver nanoparticles. These elements were assessed in other research for the environmentally friendly production of silver nanoparticles [31]. It was evident from the UV-Vis spectra that a sharp SPR band development of spherical silver nanoparticles occurred [32]. The concentration of silver nitrate determined the size of the produced silver nanoparticle. As the concentration of silver nitrate grew, so did the particle size [33]. The size of the generated silver nanoparticle is controlled by optimizing the various leaf extract concentrations and the reaction duration. It was discovered from the UV-Vis spectrum that a larger concentration of spherical silver nanoparticles formed when the reaction time length was increased, resulting in a higher-intensity, sharper SPR band [32]. The concentration of silver nitrate determines the size of the produced nanoparticle. As the concentration of silver nitrate grew, so did the particle size [33]. The AgNPs were described using a number of methods, such as FTIR analysis, XRD, TEM, and SEM. TEM examination was used to determine the size and shape of the nanoparticles [1]. The biosynthesized silver nanoparticles used in this experiment were round in shape. In a similar vein, *Cocos nucifera* was shown to synthesize spherical nanoparticles [34]. The SEM image provided insight into the pattern of nanoparticle production, including whether they were created singly or in aggregates. AgNPs' size and bioactivity were directly correlated [35]. According to the XRD data, biosynthesized materials appear to be polycrystalline. The current investigation confirmed that AgNPs are well-crystalline. FTIR research confirmed the potential groups in charge of both the capping of silver nanoparticles and the reduction of silver ions. Based on the surface chemistry of the nanoparticle, the FTIR spectrum demonstrates the straightforward fact that the material exhibits noticeable, selective absorptions in the infrared spectrum. Chemical molecules vibrate after IR light is absorbed, producing an absorption band with a broad wavelength range. It displays the transmittance peak versus characteristic absorption with the possibility of various functional groups and indicates the presence of numerous phytoconstituents, including polyphenols, glycosides, and tannins, which both serve as capping agents and verify the identity and quality of the herbs [36]. Many different ailments are treated with medicinal plants [37]. Their purpose is to regulate the activity of antidiabetic agents [38]. The existence of different secondary metabolites determines a medicinal plant's efficacy for inhibiting antidiabetic action [39]. Several flavonoids, including rutin, hesperidine, quercitrin, and kaempferol-3-oglucoside, were found in the extract according to phytochemical research. These flavonoids may have antioxidant and antidiabetic effects. According to reports, the flavonoid has antidiabetic and antioxidant properties [40, 41]. *N. arbor-tristis* has demonstrated good antidiabetic activity, suggesting that it could be a potential source of medicinal agents. In comparison to extract, nanoparticles are much more effective at preventing the growth of all clinical isolates. The antioxidant activity of leaf extract and nanoparticles was assessed using the DPPH assay, with ascorbic acid serving as the reference control. The quantity of leaf extract and silver nanoparticles increased antioxidant activity. Silver nanoparticles demonstrated strong antioxidant activity, as shown by the DPPH experiment.

Several biochemical markers are examined in an in vivo antidiabetic and antioxidant study to evaluate the impact of a certain medication or intervention on the body's metabolism and physiological functions. Below is a succinct description of the parameters discussed and how

they might be relevant in these kinds of studies:[42]

A yellow pigment called bilirubin is created as red blood cells degrade. Increased levels could be a sign of liver disease. Monitoring bilirubin levels within the framework of an antidiabetic research can aid in determining how the therapy affects liver function. When the body uses fat as fuel, ketones are created. Increased levels could indicate a change in metabolism toward fat, which happens in diseases like diabetes. Ketone level monitoring aids in assessing the effects of antidiabetic therapies as well as the metabolic alterations linked to diabetes. Blood in the urine may be a sign of renal damage or other underlying problems [41]. In the context of an antidiabetic study, blood monitoring aids in evaluating the effect of medication on renal function. Elevated protein levels in the urine could be a sign of renal impairment. In order to assess how the medication will affect kidney function—which is frequently impaired in diabetes—it is imperative to monitor protein levels. Urine nitrite levels may be a sign of bacterial growth [38]. Monitoring nitrite levels is helpful in evaluating overall health in an antidiabetic study because urinary tract infections (UTIs) can be common in patients with diabetes. Increased leukocyte counts in the urine could be a sign of infection or inflammation. This measure is crucial for evaluating how the medication affects the inflammatory response, which is frequently elevated in diabetics. In research aimed at treating diabetes, blood glucose monitoring is essential. Diabetes is characterized by elevated glucose levels, and the ability of antidiabetic therapies to control blood sugar levels is a key determinant of their effectiveness. The acidity or alkalinity of urine is reflected in its pH. Monitoring pH levels can shed light on the body's acid-base equilibrium, which is impacted by the effects of antidiabetic medications as well as metabolic changes linked to diabetes. In conclusion, these metrics aid in the comprehension of the effects of antioxidant and antidiabetic treatments on a range of physiological processes, such as inflammation, metabolic alterations, liver and kidney function, and urinary health. Keeping an eye on these metrics enables a thorough assessment of the safety and effectiveness of the treatment.[43]

Conclusion

The antioxidant and antidiabetic properties of the leaf extract are caused by the presence of flavonoids and polyphenols. Aqueous leaf extract was used to effectively create silver nanoparticles in the presence of sunshine. The produced nanoparticles were polycrystalline, spherical, and had an absorption spectra at 460 nm that was determined by a variety of methods. Their size ranged from 10 to 50 nm. Silver nanoparticles' antidiabetic effect on tested diabetics shows how effective they are as antidiabetic agents and creates new opportunities for the development of straightforward, reasonably priced medications. Different parameters like Bilirubin $\mu\text{mol/l}$, Ketone mmol/l , Protein g/l , Nitrite, Leukocytes, Glucose, Specific Gravity, pH, Blood In Urine, Body wt, WBC $103/\text{mm}^3$, RBC ($106/\mu\text{l}$), Hb (gm/dl) clotting platelet, SGOT IU/L , SGPT Urobilinogen $\mu\text{mol/l}$ also justify the antidiabetic as well as antioxidant potential of *Nyctanthes arbor tristis*. The Silver nanoparticles of *Nyctanthes arbor tristis* significantly acts better than the simple extract. The current work may make a significant contribution to the development of environmentally friendly synthetic nanoparticles as antidiabetic agents.

Declarations

Compliance with Ethical Standards

Conflict of interest: All authors declare that they have no conflict of interest.

References

1. G. Lakshmanan, A. Sathiyaseelan, P.T. Kalaichelvan, K. Murugesan, *Karbala Int. J. Mod. Sci.* 4, 61–68 (2018)
2. A. Tripathi, S. Kumari, A. Kumar, *Appl. Nanosci.* 6, 61–69 (2016)
3. M. Sathishkumar, K. Sneha, I.S. Kwak, J. Mao, S.J. Tripathy, Y.S. Yun, *J. Hazard. Mater.* 171, 400–404 (2009)
4. H.J. Lee, G. Lee, N.R. Jang, J.H. Yun, J.Y. Song, B.S. Kim, *Nanotechnology* 1, 371–374 (2011)
5. P. Velmurugan, M. Cho, S.S. Lim, S.K. Seo, H. Myung, K.S. Bang et al., *Mater. Lett.* 138, 272–275 (2015)
6. B. Kumar, K. Smita, L. Cumbal, A. Debut, *Saudi. J. Biol. Sci.* 24, 45–50 (2017)
7. V.K. Sharma, R.A. Yngard, Y. Lin, *Adv. Colloid Interface Sci.* 145, 83–96 (2009)
8. K. Yokohama, D.R. Welchons, *Nanotechnology* 18, 105101–105107 (2007)
9. M. Jeyaraj, G. Sathishkumar, G. Sivanandhan, A.D. Mubarak, M. Rajesh, R. Arun et al., *Colloids Surf. B* 106, 86–92 (2013)
10. N. Hanumanta Rao, N. Lakshmidhevi, S.V.N. Pammi, P. Kollu, S. Ganapaty, P. Lakshmi, *Mater. Sci. Eng. C* 62, 553–557 (2016)
11. K.L. Niraimathi, V. Sudha, R. Lavanya, P. Brindha, *Colloids Surf. B* 102, 288–291 (2013)
12. M.M.H. Khalil, E.H. Ismail, K.Z. El-Baghdady, D. Mohamed, *Arab. J. Chem.* 7, 1131–1139 (2014)
13. K.S. Kavitha, B. Syed, D. Rakshith, H.U. Kavitha, H.C. Yashwantha Rao, B.P. Harini et al., *Int. Res. J. Biol. Sci.* 2, 66–76 (2013)

14. S. Schultz, D.R. Smith, J.J. Mock, D.A. Schultz, Proc. Natl. Acad. Sci. USA 97, 996–1001 (2000)
15. R. Abbai, R. Mathiyalagan, J. Markus, Y.J. Kim, C. Wang, P. Singh et al., Int. J. Nanomed. 11, 3131–3143 (2016)
16. O.V. Kharissova, H.V. Rasika Dias, B.I. Kharisov, B.O. Perez, V.M.J. Perez, Trend. Biotechnol. 31, 240–248 (2013)
17. P.S. Ramesh, T. Kokila, D. Geetha, Spectrochim. Acta A 142, 339–343 (2015)
18. K. Li, C. Ma, T. Jian, H. Sun, L. Wang, H. Xu et al., J. Food Sci. Technol. 54, 3569–3576 (2017)
19. J.S. Valli, B. Vaseeharan, Mater. Lett. 82, 171–173 (2012)
20. F. Benakashani, A.R. Allafchian, S.A.H. Jalali, Karbala Int. J. Mod. Sci. 2, 251–258 (2016)
21. K. Venugopal, H.A. Rather, K. Rajagopal, M.P. Shanthi, K. Sherif, M. Illiyas et al., J. Photochem. Photobiol., B 167, 282–289 (2017)
22. S. Raja, V. Ramesh, V. Thivaharan, Arab. J. Chem. 10, 253–261 (2017)
23. J.S. Rathee, S.A. Hassarajani, S. Chattopadhyay, Food Chem. 103, 1350–1357 (2007)
24. A. Vyas, R. Sarin, Int. J. Pharm. Bio Sci. 4, 201–206 (2013)
25. A.W. Bauer, W.M.M. Kirby, J.C. Serris, M. Turck, Am. J. Clin. Pathol. 45, 493–496 (1966)
26. W. Williams Brand, M.E. Cuvelier, C. Berset, LWT Food Sci. Technol. 28, 25–30 (1995)
27. S. Iravani, H. Korbekandi, S.V. Mirmohammadi, B. Zolfaghari, Res. Pharm. Sci. 9, 385–406 (2014)
28. W.H. DeJong, P.J.A. Borm, Int. J. Nanomed. 3, 133–149 (2008)
29. C. Saraiva, C. Praça, R. Ferreira, T. Santos, L. Ferreira, L. Bernardino, J. Control. Release 235, 34–47 (2016)
30. S. Ahmed, M. Ahmad, B.L. Swami, S. Ikram, J. Adv. Res. 7, 17–28 (2016)
31. V. Manikandan, P. Velmurugan, J.H. Park, W.S. Chang, Y.J. Park, P. Jayanthi et al., 3 Biotech 7, 72 (2017)
32. H. Bar, D.K. Bhui, G.P. Sahoo, P. Sarkar, S.P. De, A. Misra, Colloids Surf. A 339, 134–139 (2009)
33. C. Dong, X. Zhang, H. Cai, C. Cao, Optik 127, 10378–10388 (2016)
34. S.M. Roopan, G. Madhumitha, A. Abdul Rahuman, C. Kamaraj, A. Bharathi, T.V. Surendra, Ind. Crop. Prod. 43, 631–635 (2013)
35. A. Ronavari, D. Kovacs, N. Igaz, C. Vagvolgyi, I.M. Boros, Z. Konya et al., Int. J. Nanomed. 12, 871–883 (2017)
36. G. Bagherzade, M.M. Tavakoli, M.H. Namaei, Asian Pac. J. Trop. Biomed. 7, 227–233 (2017)
37. N.H. Rakotoarivelo, F. Rakotoarivony, A.V. Ramarosandratana, V.H. Jeannoda, A.R. Kuhlman, A. Randrianasolo et al., J. Ethnobiol. Ethnomed. 11, 68 (2015). <https://doi.org/10.1186/s13002-015-0050-2>
38. Ivorra, M.D., Pay´aa, M., Villarb, A., A review of natural products and plants as potential hypoglycemic drugs. Journal of Ethnopharmacology, 27: 243–275, (1989).
39. Rahman, A.U., Zaman, K., Medicinal plants with hypoglycemic activity. Journal of Ethnopharmacology, 26: 1–55, (1989.)
40. Grover, J.K., Yadav, S., Vats, V., Medicinal plants of India with hypoglycemic potentials. Journal of Ethnopharmacology, 81: 81–100, (2002)
41. Saxena, A., Vikram, N.K., Role of selected Indian plants in management of type 2 diabetes: a review. Journal of Alternative and Complementary Medicine 10, 369–378. (2004)
42. Mukherjee, P.K., Maiti, K., Mukherjee, K., Houghton, P.J., Leads from Indian medicinal plants with hypoglycemic potentials, Journal of Ethnopharmacology, 106 :1–28, (2006).
43. Parotta, J.A., Healing plants of peninsular India, CAB International, Wallingford, U.K., 548-549, (2001)

Tables

Table 1: In-vitro free radical scavenging activity of silver nanoparticle and leaf extract of *N. arbor-tristis* L. evaluated by DPPH method .percentage (mean±SE)

Name of sample	Results (DPPH OD450)
Uninduced Untreated	0.60 ± 0.02
Induced Untreated	1.23 ± 0.01
<i>N. arbortristis</i> Ext	1.53 ± 0.02
<i>N. arbortristis</i> AgNPs	1.31 ± 0.01
Gilbenclamide	2.47 ± 0.03

Table 2: Reduced Glutathione (GSH) Assay of silver nanoparticle and leaf extract of *N. arbor-tristis* L. percentage (mean±SE)

Name of sample	Results (GSH OD 450)
Uninduced Untreated	1.49 ± 0.02
Induced Untreated	1.162 ± 0.01
<i>N. arbortristis</i> Ext	1.262 ± 0.02
<i>N. arbortristis</i> AgNPs	1.316 ± 0.01
Gilbenclamide	1.48 ± 0.02

Table -3 Lipid Peroxidase assay TBARS (MDA) Assay of silver nanoparticle and leaf extract of *N. arbor-tristis* L. percentage (mean±SE)

Name of sample	Results (TBARS OD450)
Uninduced Untreated	0.92 ± 0.02
Induced Untreated	1.82 ± 0.02
<i>N. arbortristis</i> Ext	1.614 ± 0.01
<i>N. arbortristis</i> AgNPs	1.44 ± 0.02
Gilbenclamide	0.87 ± 0.04

Table-4 In-vivo antidiabetic activity of silver nanoparticle and leaf extract of *N. arbor-tristis* L. percentage (mean±SE)

Name of sample	Results (Blood Glucose mg/dl)
Uninduced Untreated	77.404 ± 2.89
Induced Untreated	333.14 ± 8.50
<i>N. arbortristis</i> Ext	285.378 ± 2.79
<i>N. arbortristis</i> AgNPs	254.83 ± 2.18
Gilbenclamide	141.71 ± 7.05

Table-5 Comparison of Body Measurement between Uninduced Untreated and *N.arbortristis* AgNps ,percentage (mean±SE)

Sample	Body wt.	WBC 103/mm ³	RBC (106/ul)	Hb (gm/dl)	CLOTTING	PLATELET	SGOT IU/L	SGPT U	Urobilinogen umol/l
Uninduced Untreated	143.68 ± 3.66	7.71 ± 0.23	6.336± 0.05	10.07±2.43	96 ± 6.00	273.8 ± 21.14	107.76 ± 1.56	126.42 ± 1.04	11.6 ± 3.30
<i>N. arbortristis</i> AgNPs	133.9 ±0.81	87.49 ± 0.16	6.58 ± 0.17	12.51± 0.05	96.00 ±6.00	277.80 ± 12.57	109.12 ± 2.45	125.86 ± 0.74	11.6 ± 3.30

Table-6 Comparison of Blood Parameters in Urine. percentage (mean±SE)

Sample	Bilirubin umol/l	Ketone mmol/l	Protein g/l	Nitrite	Leukocytes	Glucose	Specific Gravity	pH	Blood In Urine
Uninduced Untreated	31.4 ± 3.60	0.7 ± 0.20	0.27 ± 0.03	Negative	26 ± 11.00	5 ± 0	1.007 ± 0	6 ± 0	Absent
<i>N. arbortristis</i> AgNPs	31.40 ± 3.6	0.90 ± 0.24	0.24 ± 0.03	Negative	26.00 ± 11.00	5.00 ± 0	1.01 ± 0	6 ± 0	Absent

Figures

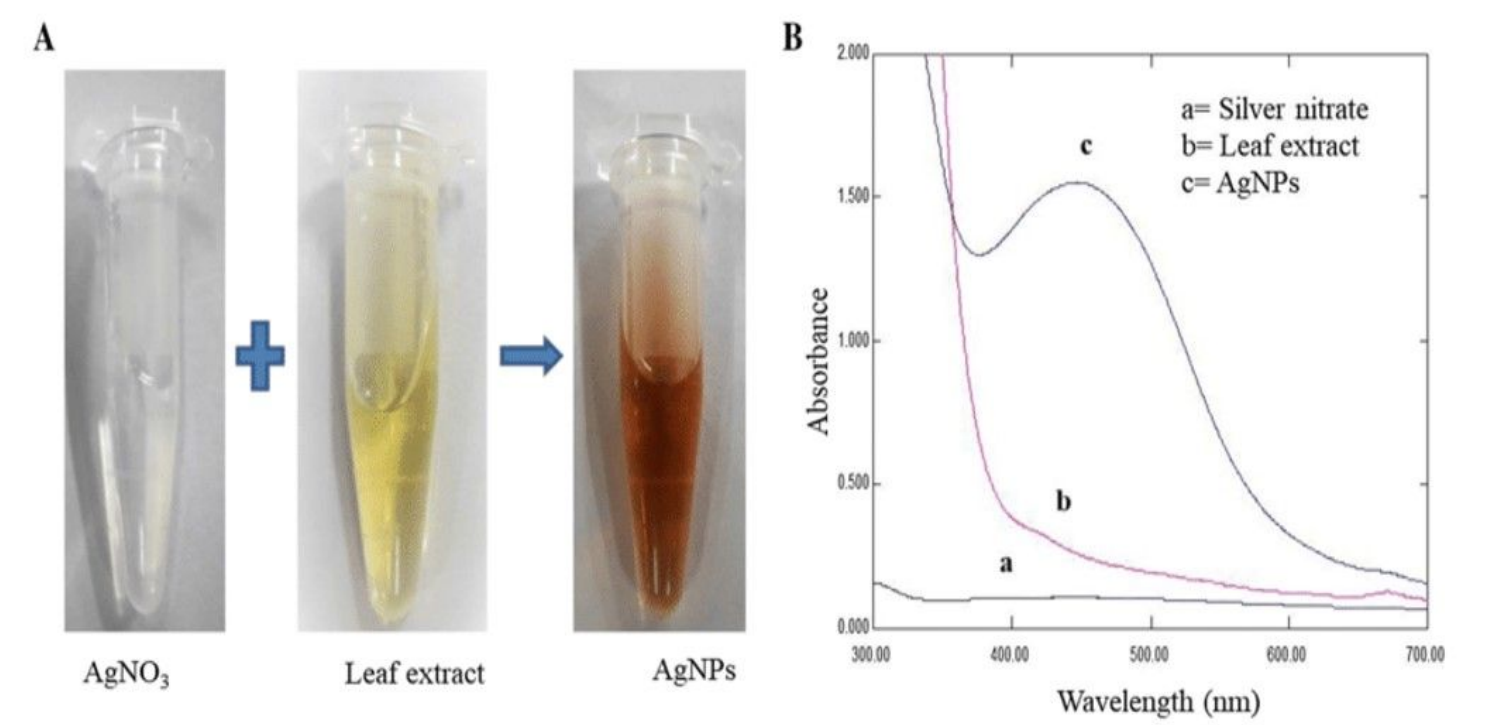


Figure 1

Green synthesis of silver nanoparticles a Change in the color of reaction mixture from yellowish green to brown after addition of leaf extract into silver nitrate solution. b UV–Vis spectrum confirmed the biosynthesis of silver nanoparticle, (λ max=460 nm) (Color figure online)

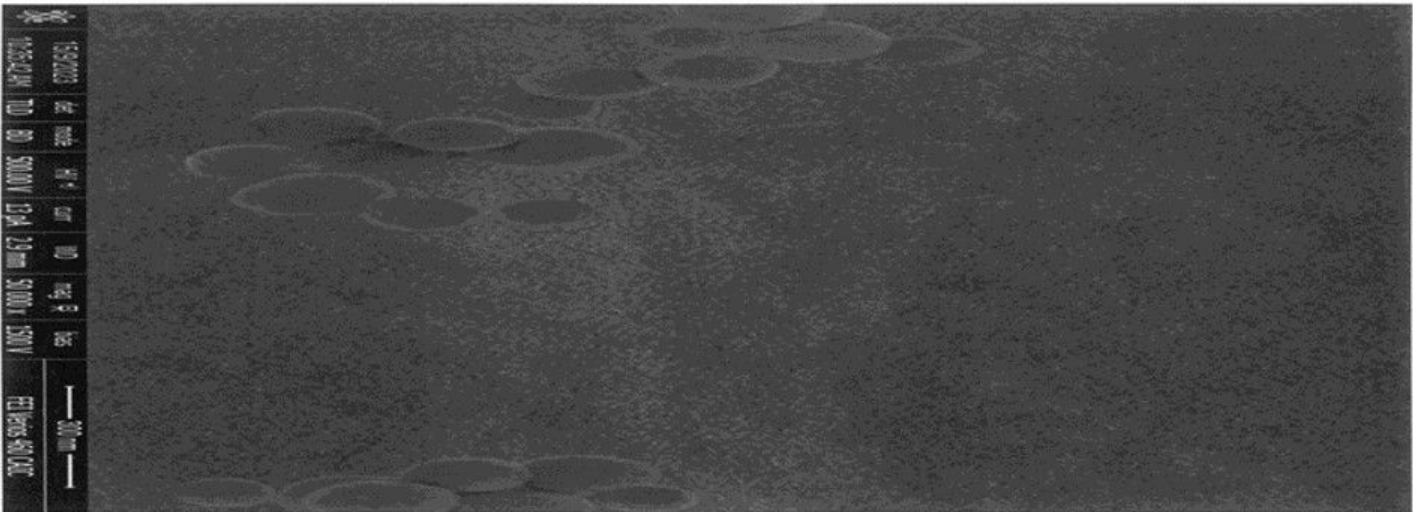


Figure 2

SEM analysis of biosynthesized silver nanoparticles a SEM image of synthesized nanoparticles *Nyctanthes arbortristis* Ag NP

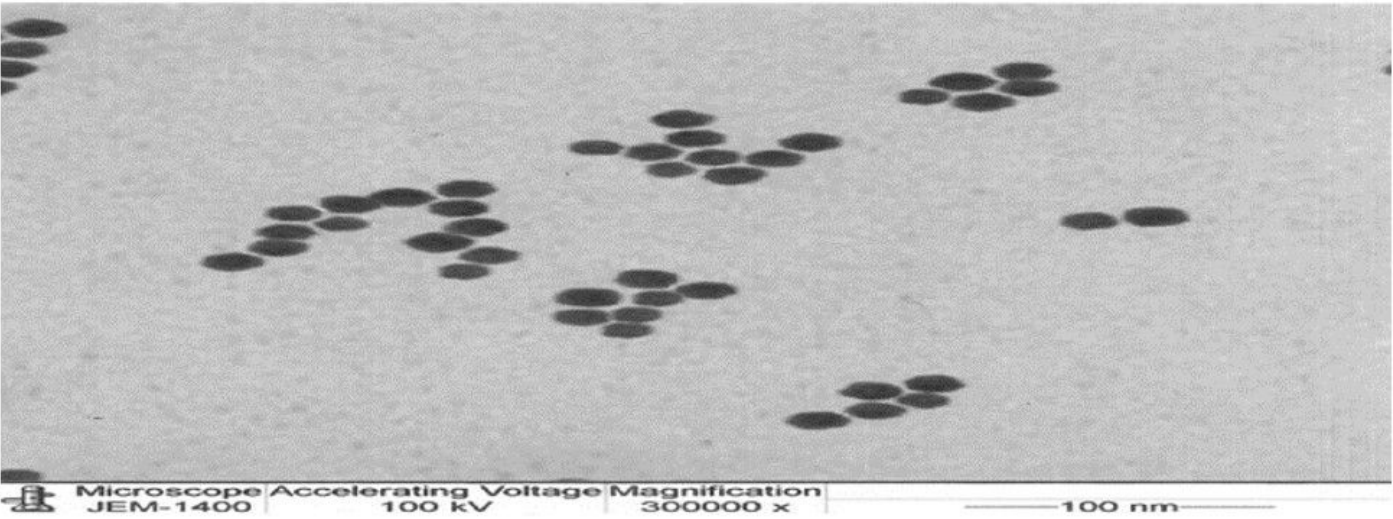


Figure 3

Topographical image of colloid silver nanoparticle from TEM analysis a–c under diferent magnification *Nyctanthes arbortristis* Ag NP

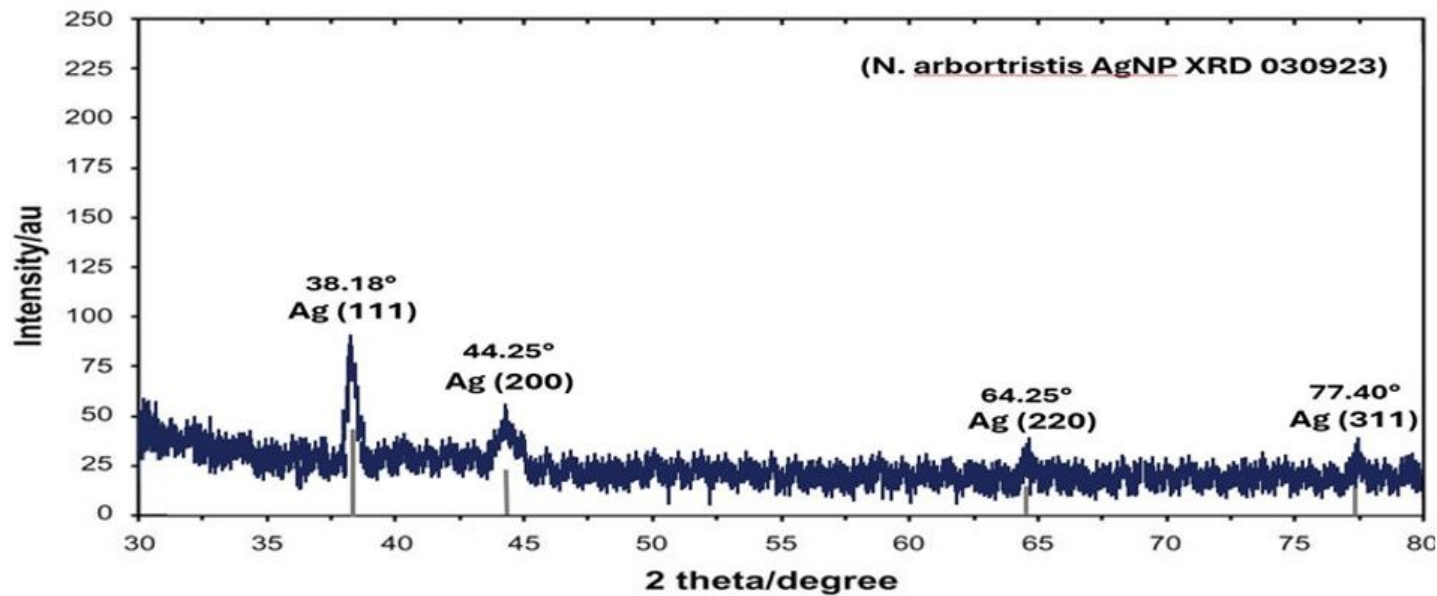


Figure 4

X-ray difraction pattern of silver nanoparticle prepared with aqueous leaf extract of *Nyctanthes arbortristis* Ag NP

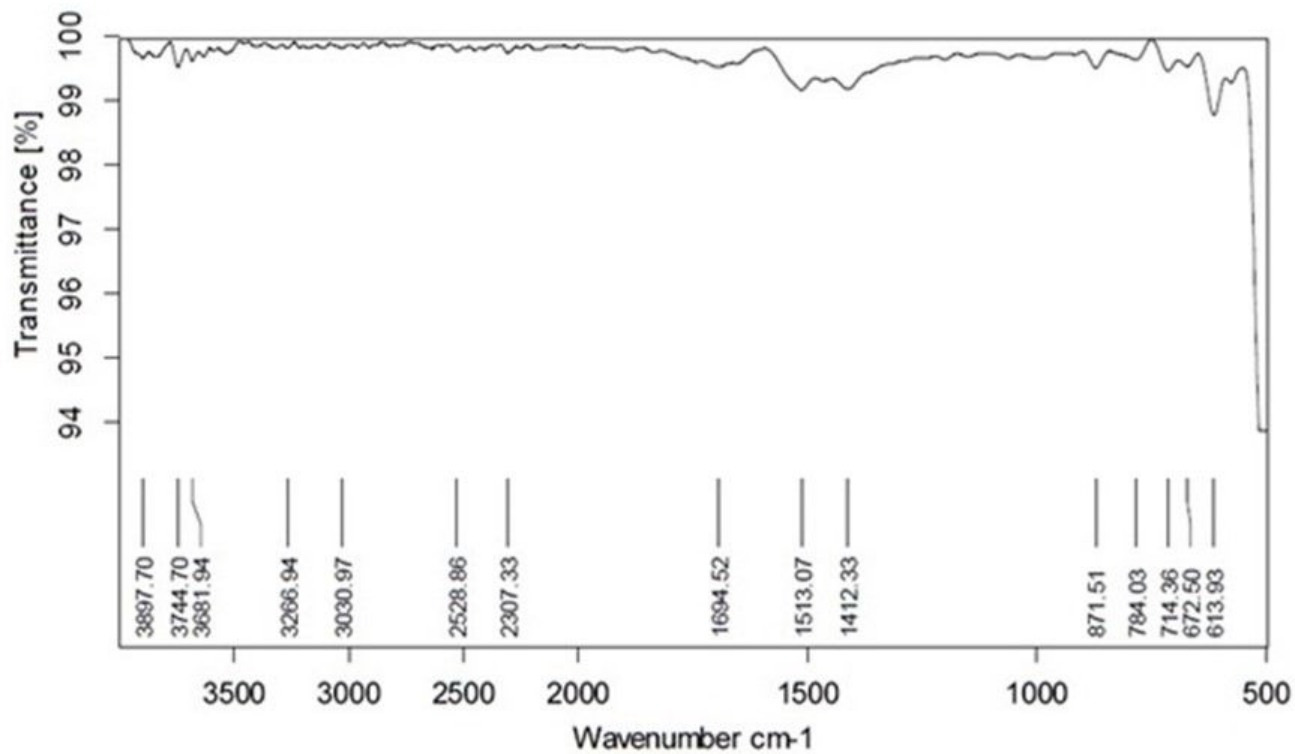


Figure 5

FTIR Spectra *Nyctanthes arbortristis* Ag NP

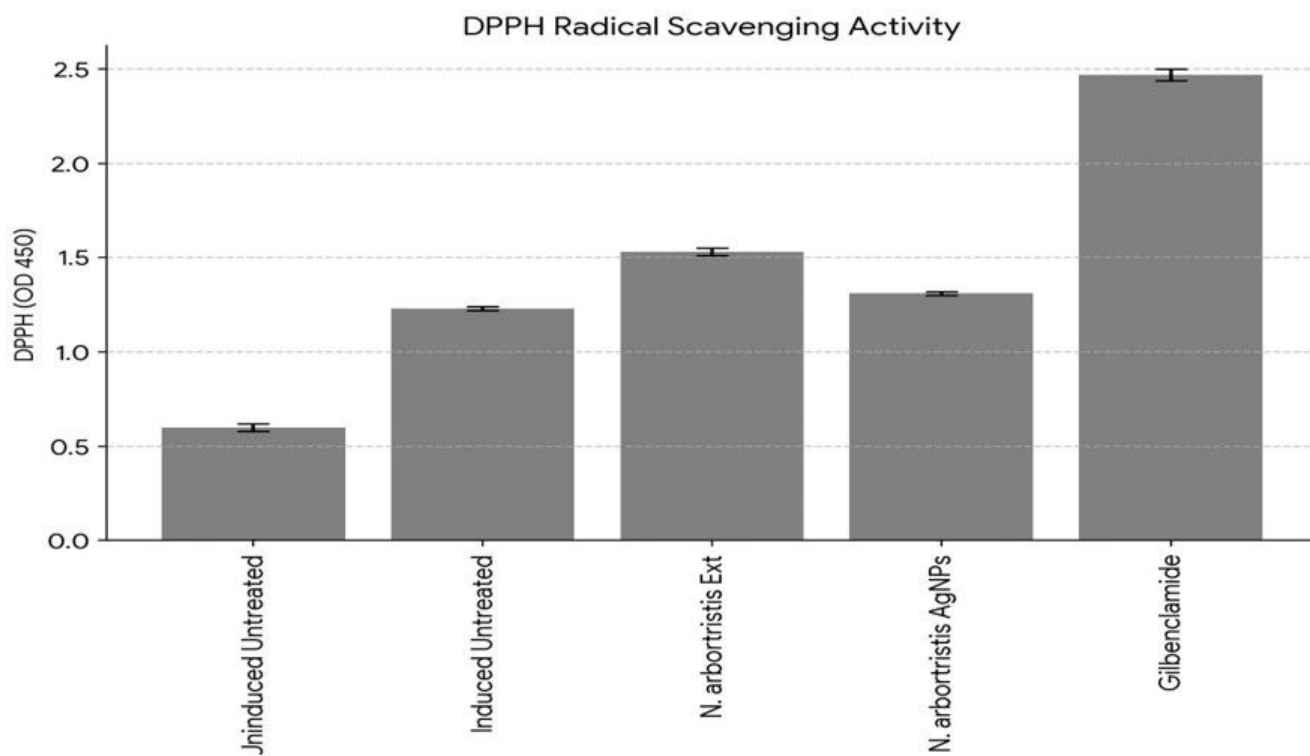


Figure 6

In-vitro free radical scavenging activity of silver nanoparticle and leaf extract of *N. arbor-tristis* L. evaluated by DPPH method

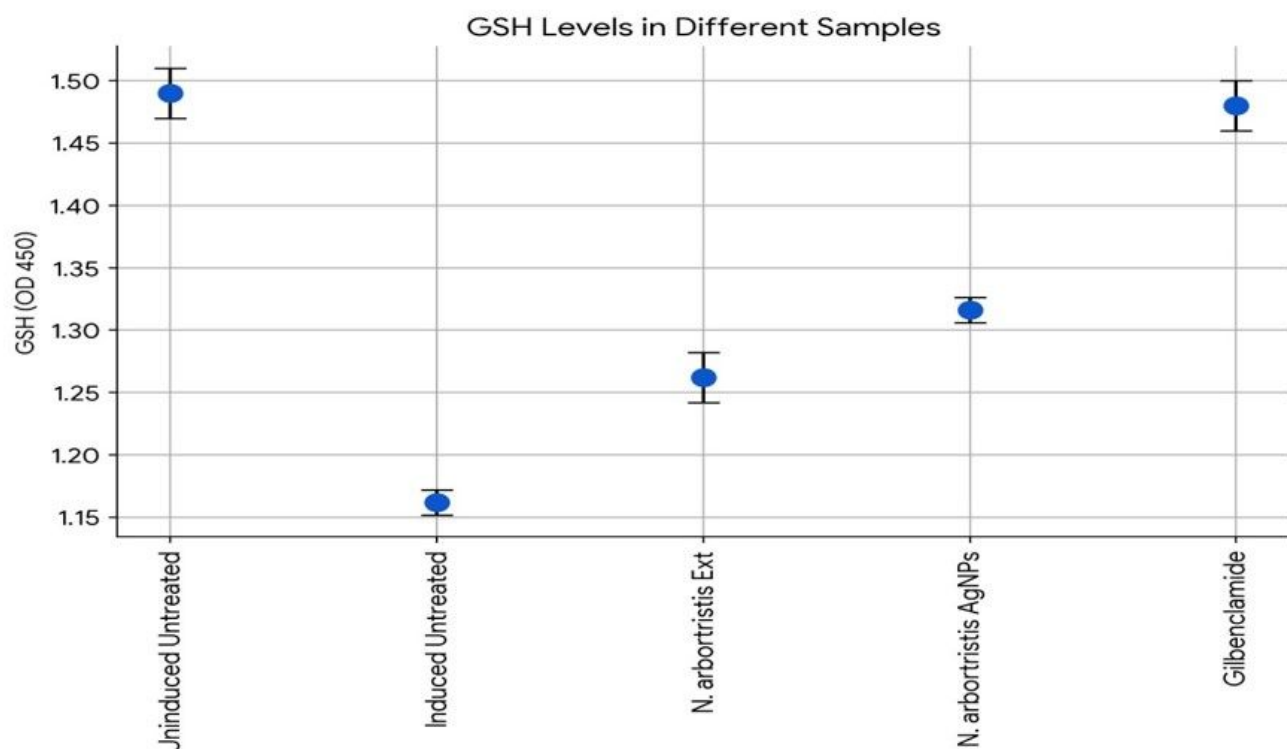


Figure 7

Reduced Glutathione (GSH) Assay of silver nanoparticle and leaf extract of *N. arbor-tristis* L.

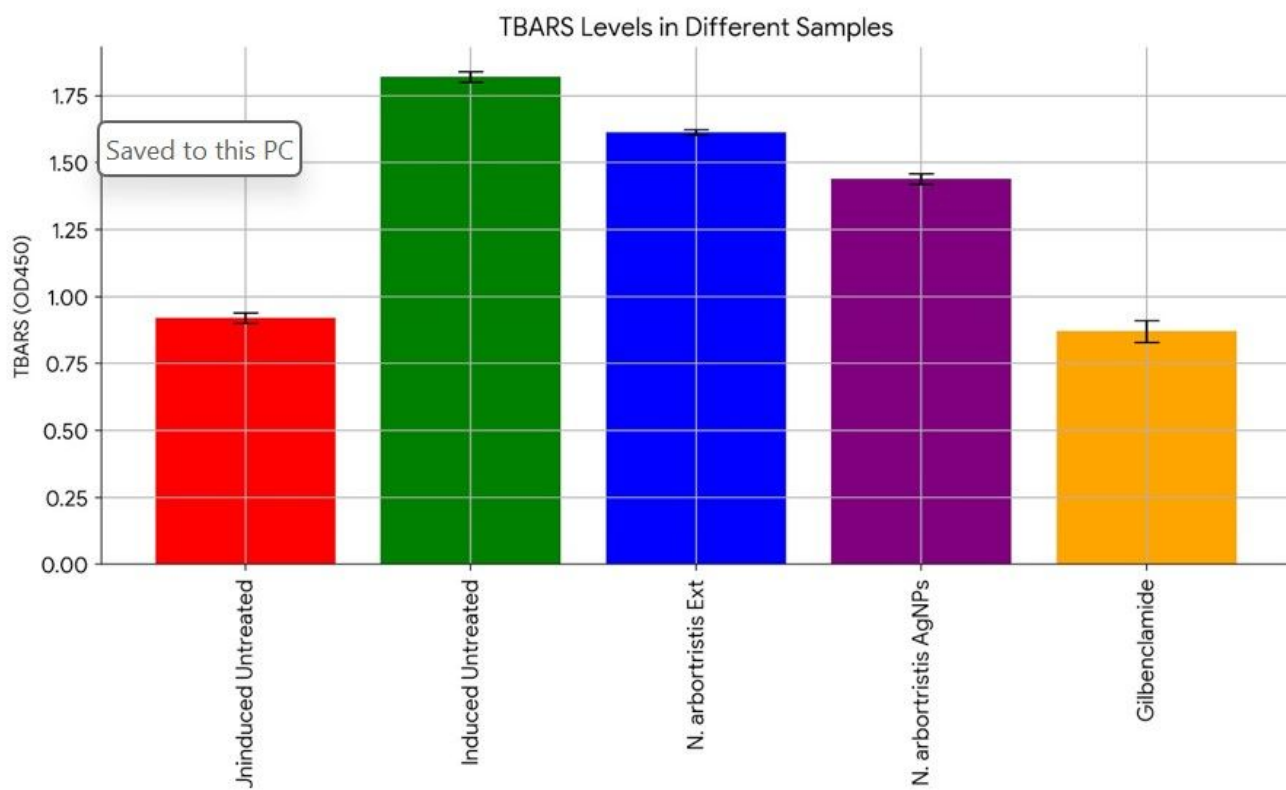


Figure 8

Lipid Peroxidase assay TBARS (MDA) Assay of silver nanoparticle and leaf extract of *N. arbor-tristis* L.

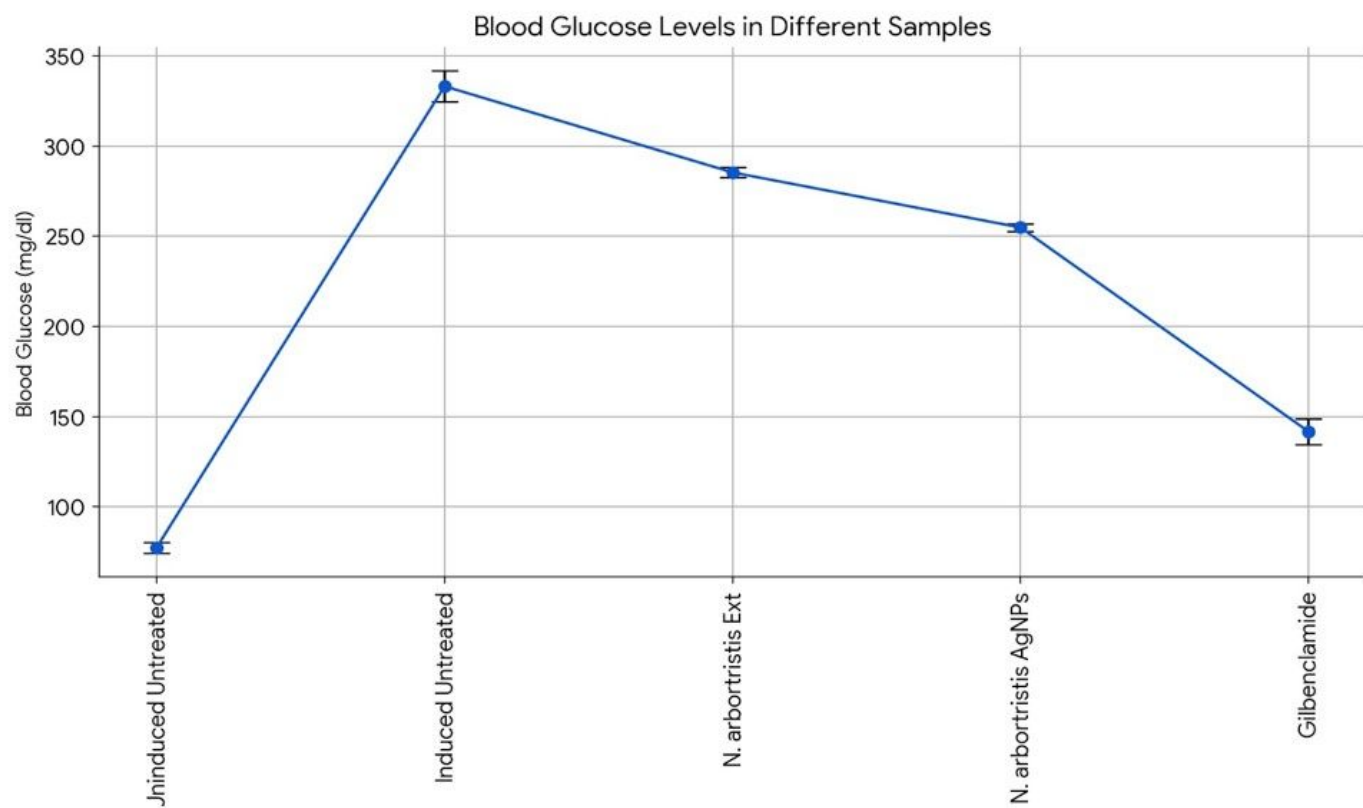


Figure 9

In-vivo antidiabetic activity of silver nanoparticle and leaf extract of *N. arbor-tristis* L.

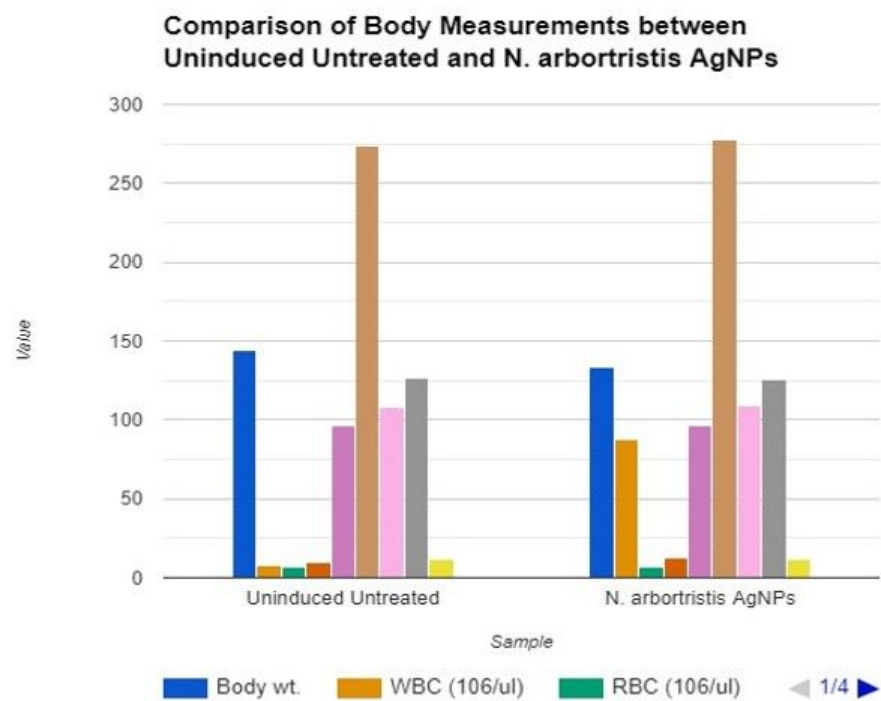


Figure 10

Comparison of Body Measurement between Uninduced Untreated and N.arbortristis AgNps

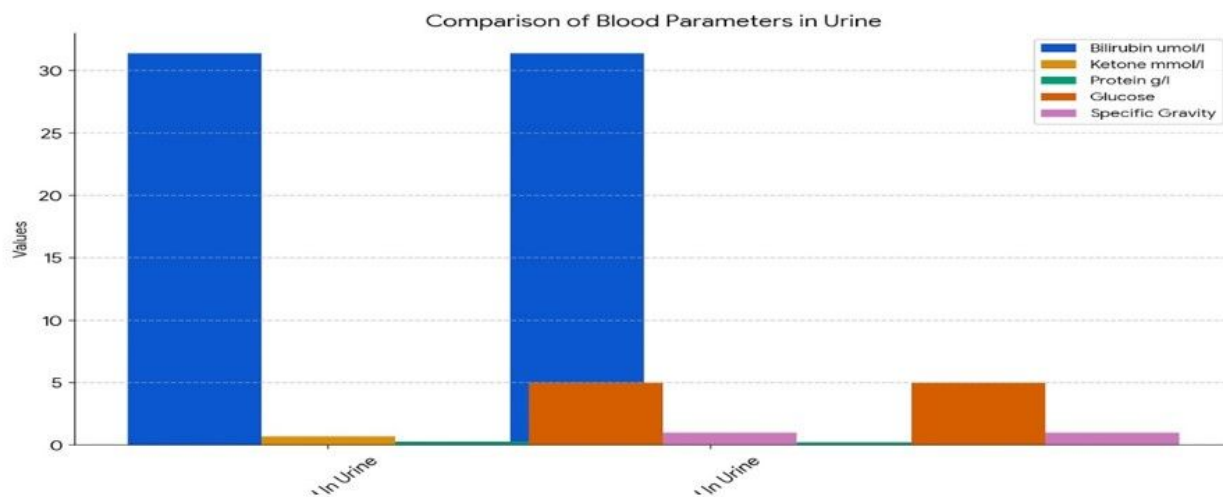


Figure 11

Comparison of Blood Parameters in Urine