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Antidiabetic, Antioxidant, Antiglycation and Anti-inflammatory potentials of green-synthesized silver nanoparticles using fresh leaf and stem aqueous extracts of *Telfairia occidentalis*: an *in-vitro* approach

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Abstract: Over the years, due to its remarkable functional properties, nanoparticles have been widely used in the treatment and diagnosis of diseases. This study synthesized easy and eco-friendly silver nanoparticles (AgNPs) using fresh leaf and stem aqueous extracts of *Telfairia occidentalis*, and evaluated its antidiabetic, antiglycation, antioxidant, and anti-inflammatory potentials. The biosynthesized AgNPs were characterized using UV-visible spectroscopy, FTIR, SEM, and EDXS techniques. The antidiabetic (α-amylase and α–glucosidase inhibitory assay), antioxidant (Reducing power, total antioxidant capacity, DPPH, and NO radical scavenging assay), anti-inflammatory (Protein inhibitory action, albumin denaturation inhibition), and antiglycation (fructosamine inhibition) activities were carried out using standard procedures. Biosynthesized AgNPs showed maximum absorbance at 412nm, and the SEM analysis revealed a pseudo-spherical shape with an average size distribution of 43.90 nm. The possible functional groups revealed by FTIR analysis include phenols, alkenes, alcohols, alkynes, carboxylic acids, isothiocyanates, and amines. AgNPs-TOL showed a better biological potential. In conclusion, the aqueous leaf and stem extracts of *Telfairia occidentalis* can be used to synthesize AgNPs with promising antidiabetic, antiglycation, antioxidant, and anti-inflammatory potentials. However, AgNPs-TOL showed a better biological potential that can be explored in developing drugs for treating diabetes mellitus and its complications.

Keywords: *Telfairia occidentalis*, Diabetes mellitus & its complications, silver-nanoparticles, phytochemistry, antioxidant, antiglycation, anti-inflammatory, SEM, FTIR, EDXS.

1. Introduction

Nano-biotechnology is a fast-emerging branch of science**,** and the quest for the scientific understanding of the etiopathogenesis of diabetes mellitus (DM) and the ultimate development of definite curative and/or prophylactic options in its management have stimulated great scientific research interest in recent years. Drug management of diabetes without associated adverse effect**s** has remained a challenge for orthodox medical practice, necessitating the exploration and screening of medicinal plants with acclaimed therapeutic efficacies [1]. The wide range of secondary metabolites in medicinal plant extracts are used to treat and manage various diseases worldwide**,** but, more recently**,** plant-mediated bio-nanotechnology (green synthesis) is rapidly generating great**er** research interest than using pure extracts. Silver nanoparticles (AgNPs)**,** amongst other synthesized nanoparticles such as selenium

nanoparticles, gold nanoparticles**,** etc**.**, have received the widest use and exploration due to their various potential biological activities such as antibacterial and anti-inflammatory activities etc**.** [2].

Several antidiabetic plants, such as Carica papaya, Azadirachta indica, Momordica charantia, Magnfera indica, Allium cepa, Alium sativum, Musa paradisiaca, etc., have been reported worldwide [3]. Telfairia occidentalis is a tropical green leafy vegetable known as Ugwu in South-western Nigeria and belongs to Curcurbitaceae family. It is mainly used in soups and herbal medicines [4], and widely cultivated in the West, East, and Central African countries, including Benin Republic, Cameroon, Nigeria, Sierra Leone, Angola, and Uganda. It is called "ugu" or "ugwu" by the South-western Nigerians. The various extracts of Telfairia occidentalis leaves have been reported to possess anticancer, antioxidant [4], profertility and reversible-antifertility [5], hepatoprotective [6], anti-inflammatory [7], antidiabetic [8], anti-hepatitis and aphrodisiac activities. It has also been reportedly used in the management of hyper-cholesterolemia, liver problems, and impaired immune system, remedies reproductive and fertility issues [4], treatment of malaria as well as replenishment of lost blood due to its high iron content. Here, we report the syntheses of silver nanoparticles (AgNPs) by using Telfairia occidentalis (leaf and stem) aqueous extract and silver nitrate ($AgNO_3$) as precursors. The structural properties of the synthesized AgNPs have been confirmed using UV–Vis, FT-IR (Fourier Transform Infrared Spectroscopy), SEM (Scanning Electron Microscopy), and EDX (Energy-Dispersive X-ray analysis) techniques and, thus, investigated for antidiabetic, antioxidant, antiglycation and anti-inflammatory activities.

2. Materials and Methods

The fresh mature *Telfairia occidentalis* (leaf and stem) were collected from Lagos State University farm, Lagos State, Nigeria, in January 2022. The plant was authenticated and given voucher number LSH001048 at the University Herbarium, Department of Botany, Lagos State University. The chemicals, including $AgNO_3$, ferric chloride (0.1%), phosphate buffer (0.2 M, pH 6.6), trichloroacetic acid (10%), ascorbic acid (1%), and potassium ferricyanide (1% w/v), DPPH, α-amylase, α-glucosidase were purchased from Sigma-Aldrich and were of analytical grades.

2.1. Preparation of plant extract.

The leaves and stems of *Telfairia occidentalis* were washed individually under running water and then with deionized water. The cleaned fresh leaves and stems were ground individually into slurry form to obtain fresh aqueous extracts, and 10% w/v each of the plant parts was prepared. The aqueous extracts were further boiled separately at 40˚C using a water bath for 2 hours. The extracts were later subjected to centrifugation at 3000 rpm for 5 minutes, followed by filtration using Whatman filter paper 1 twice, and the extracts were stored at 2-4˚C in the refrigerator for further use.

2.2. Synthesis of silver nanoparticles.

The synthesis of silver nanoparticles was performed in an Erlenmeyer flask that consisted of 90 ml of silver nitrate solution (1mM $AgNO_3$ in 90 ml of deionized water). The solution was prepared at room temperature. In brief, 10ml of each extract was added to 90ml silver nitrate solution in a 250ml beaker and stirred periodically for 1 hour in the dark at room

temperature. The change in color of the solution after one week indicated the reduction of silver nitrate into AgNPs. Then 2ml aliquots were taken after 1-5 hours of synthesis, and the absorbance was analyzed (200-700 nm) using a UV visible spectrophotometer. After the completion of the reaction, the solution was centrifuged at 5000 rpm for 15 minutes, and the supernatant was kept in the refrigerator for further analysis.

2.3. Characterization of silver nanoparticles.

The synthesized AgNPs were primarily characterized using UV-visible spectroscopy followed by Scanning Electron Microscopy (SEM) and FTIR (Fourier Transform Infra-Red spectroscopy (FTIR). SEM was used to analyze the shape and size of the synthesized AgNPs, while FTIR was used to check the capping agents on the surface of AgNPs.

2.4. Phytochemical screening.

Phytochemical constituents of the aqueous leaf, flower, stem and root extracts of *Telfairia occidentalis* with their respective amounts were identified and measured by the methods described by [9] and [10].

2.5. Antioxidant activity of Telfairia occidentalis extracts and synthesized AgNPs.

2.5.1. DPPH Free radical scavenging activity.

DPPH assay procedure by [10] was followed. Briefly, 0.2 mL of the extract is diluted with methanol, after which 2 mL of DPPH solution (0.5 mM) is added. After 30 mins, the absorbance is measured at 517 nm. The percentage of the DPPH radical scavenging is then calculated using the equation given below:

$$\textbf{\% Inhibition of DPPH radical} = ([A_{br}\ A_{ar}]/A_{br})\ X\ 100$$

where A_{br} is the absorbance before the reaction, and A_{ar} is the absorbance after the reaction has taken place. Concentrations of the extracts resulting in 50% inhibition (IC_{50}) were determined graphically.

2.5.2. Nitric oxide scavenging activity.

Nitric oxide assay procedure by [11] was followed. Briefly, 2 mL of 10 mM sodium nitroprusside dissolved in 0.5 mL phosphate buffer saline (pH 7.4) was mixed with 0.5 mL of sample. The mixture is then incubated at 25˚C. After 150 mins of incubation, 0.5 mL of the incubated solution is withdrawn and mixed with 0.5 mL of Griess reagent [(1.0 mL sulfanilic acid reagent (0.33% in 20% glacial acetic acid at room temperature for 5 min with 1 mL of naphthylethylenediamine dichloride (0.1% w/v)]. The mixture is then incubated at room temperature for 30 min, and its absorbance pouring into a cuvette is measured at 546 nm. The amount of nitric oxide radical inhibition is calculated following this equation:

$$\textbf{\% Inhibition of NO radical} = ([A_{br}\ A_{ar}]/A_{br})\ X\ 100$$

where A_{br} is the absorbance before the reaction, and A_{ar} is the absorbance after the reaction has taken place with Griess reagent. Concentrations of the extracts resulting in 50% inhibition (IC_{50}) were determined graphically.

2.5.3. Reducing power assay.

The reducing power assay procedure by [10] was followed. Briefly, 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of $K_3Fe(CN)_6$ (1% w/v) were added to 1.0 mL of sample dissolved in distilled water. The resulting mixture is incubated at 50˚C for 20 min, followed by the addition of 2.5 mL of trichloroacetic acid (10% w/v). The mixture is centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution (2.5 mL), mixed with distilled water (2.5 mL) and 0.5 mL of $FeCl_3$ (0.1%, w/v). The absorbance is then measured at 700 nm against a blank sample.

2.5.4. Total antioxidant capacity (TAC) assay.

The total antioxidant capacity was assessed using the phosphomolybdate technique with ascorbic acid as the standard [12]. 300ul of TAC reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) was combined with an aliquot of 25ul of each (1 mg/ml) solution. The microplate was incubated in a microplate incubator for 90 minutes. In a microplate reader, the mixtures' absorbance was evaluated at 630 nm. The amount of mg equivalents to ascorbic acid (AAE)/100g extract was used to express the extract's antioxidant activity.

2.6. Antiglycation activity of Telfairia occidentalis extracts and synthesized AgNPs.

2.6.1. Albumin glycation and Estimation of Fructosamine production.

Albumin glycation and measurement of fructosamine produced were performed according to the method described by [13]. Albumin glycation was performed with a total volume of 3.5 ml glycation reaction solution containing (0.5 ml different extracts, 1ml of 5% BSA, 1 ml of 166.5 mM glucose, and 1ml gentamicin (20mg/dl in 0.01M phosphate buffer, pH 7.4). All solutions were filtered and incubated at 37 °C for 72 hours.

To evaluate fructosamine production, 1 ml of 20% Trichloroacetic acid (TCA) was added to 1 ml of glycated samples; the precipitate was washed three times at 6000g for 10 min, solubilized in 1ml phosphate buffer and added to 0.5 ml 40% TCA. Following centrifugation at 6000g for 10 min, 0.5 ml of supernatant was mixed with 0.05 M Thiobarbituric acid (TBA). The mixtures were incubated in a boiling water bath for 20 mins, cooled to room temperature, and the absorbance was read at 443 nm. Inhibition of fructosamine was calculated as a percentage using the following formula:

$$\textbf{Inhibitory activity (\%)} = [(A_0 - A_1)/A_0] \times 100$$

Where A_0: absorbance of control, A_1: absorbance in the presence of the extract sample.

2.7. Antidiabetic activity of Telfairia occidentalis extracts and synthesized AgNPs.

2.7.1. *In vitro* α-amylase inhibitory assay.

The α-amylase inhibitory assay was determined according to the procedure of [14]. A total of 250 µL of the extract was placed in a tube, and 250 µL of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase solution was added. This solution was pre-incubated at 25°C for 10 min, after which 250 µL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added at 2 minutes timed intervals and then further incubated at 25°C for 10 min. The reaction was terminated by adding 500 µL of dinitrosalicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5 mins and cooled to room temperature. The resulting mixture was diluted with 5 mL distilled water, and the absorbance was measured at 540 nm with the use of a spectrophotometer (Spectrum lab S23A, Globe Medical England). A control was prepared using the same procedure replacing the extract with distilled water. The α-amylase inhibitory activity was calculated as percentage inhibition.

$$\textbf{\% Inhibition} = ([A_c - A_s]/A_c) \text{ X } 100$$

where Ac = Absorbance of Control, As = Absorbance of the sample. Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC_{50}) were determined graphically.

2.7.2. *In vitro* α-glucosidase inhibitory assay.

This assay was conducted according to the method described [12]. The substrate solution, p-nitrophenyl glucopyranoside (pNPG) was prepared in 20 mM phosphate buffer, and pH 6.9. 20 µL of α- glucosidase (0.2 U/mL) was pre-incubated with 20 µL of the different concentrations of the extracts for 10 min. Then 20 µL of 10.0 mM (pNPG) as a substrate dissolved in 20 mM phosphate buffer (pH 6.9) was then added to start the reaction. The reaction mixture was incubated at 37 °C for 15 mins and stopped by adding 80µL of 0.2m of Na_2CO_3.The α-glucosidase activity was determined by measuring the yellow-colored paranitrophenol released from pNPG at 405 nm with a microplate reader. The control contained the same reaction mixture and the same volume of phosphate was added instead of the sample solution. Acarbose was dissolved in 10% Dimethyl sulfoxide (DMSO) and used as a positive control. The α-glucosidase inhibitory activity was calculated as percentage inhibition.

$$\textbf{\% Inhibition} = ([A_c - A_s]/A_c) \text{ X } 100$$

Ac = Absorbance of control, As = Absorbance of the sample. Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC_{50}) were determined graphically.

2.8. Anti-inflammatory activity of Telfairia occidentalis extracts and synthesized AgNPs.

2.8.1. Inhibition of albumin denaturation.

This method was conducted according to the procedure described by [15]. The reaction mixture consisted of test extracts and a 1% stock solution of bovine serum albumin (BSA) prepared with deionized water. Briefly, 0.5ml of BSA was mixed with 50 µl of test samples. The reaction mixtures were incubated at 37°C for 15 minutes, heated to 60°C for another 15 minutes, and then cooled before the absorbance was read spectrophotometrically at 660 nm. The experiment was performed in duplicate. Percent inhibition of protein denaturation was calculated as follows:

$$\textbf{\% Inhibition} = ([A_c - A_s]/A_c) \text{ X } 100$$

where Ac = Absorbance of control, As = Absorbance of the sample.

2.8.2. Protein inhibitory action.

This method was conducted according to the modified procedure described by [15]. The reaction mixture contained 1 mg of trypsin, 1 ml of 20 mM Tris HCl buffer (pH 7.4), and 50 µl test samples. The reaction mixture was incubated at 37°C for 5 mins, and then 1ml of 0.8% (w/v) casein was added. The mixture was inhibited for an additional 20 min, and 2 ml of 70% perchloric acid was added to terminate the reaction. The cloudy suspension was centrifuged, and the absorbance of the supernatant was read at 210 nm against the buffer as blank. The experiment was performed in triplicate. The percentage of inhibition of proteinase inhibitory activity was calculated as:

$$\textbf{\% Inhibition} = ([A_c - A_s]/A_c) \text{ X } 100$$

Where Ac = Absorbance of control, As = Absorbance of the sample.

2.9 Statistical analysis.

The results are expressed as mean ± SEM, and the data analyzed with GraphPad Prism (version 5.0) and Microsoft Excel (version 2010). The comparison was made using one-way analysis of variance (ANOVA) followed by Tukey Post hoc and significance value set at $P < 0.05$.

3. Results and Discussion

The synthesis of nanoparticles using biological methods is a valuable alternative to chemical and physical methods [16]. The synthesis of silver nanoparticles using the green route became popular because it is non-toxic, cheap, eco-friendly, and suitable for pharmaceutical and biomedical applications [17].

Biosynthesized silver nanoparticles have been reported to have a wide range of applications in biomedical sciences [18]. In this context, diverse natural products have been used to synthesize silver nanoparticles. However, there is a continuous search for better reducing natural products for synthesizing stable and broadly effective silver nanoparticles [19]. In addition, natural products are an important part of traditional medicinal systems for human health maintenance and combatting diseases. Several secondary metabolites of plants, including flavonoids, tannins, and alkaloids, have been attributed to various medicinal properties [20].
Table 1 revealed the qualitative phytochemical analysis of the aqueous leaf and stem extracts of *Telfairia occidentalis*. Flavonoids, alkaloids, saponins, reducing sugars, and cardiac glycosides are common in the leaf and stem extracts. Steroids, terpenoids, and phlobatannins are absent in the leaf extracts, while only phenol and tannin are absent in the stem extracts.

Table 1. Preliminary qualitative phytochemical analysis of leaf and stem extracts of *Telfairia occidentalis.*

PHYTOCHEMICALS	**AQUEOUS EXTRACTS**	
	LEAF	**STEM**
PHENOLS	+	-
FLAVONOIDS	+	+
TANNINS	+	-
ALKALOIDS	+	+

SAPONINS	+	+
REDUCING SUGARS	+	+
STEROIDS	-	+
TERPENOIDS	-	+
CARDIAC GLYCOSIDES	+	+
PHLOBATANNINS	-	+

KEY: + = Present, - = Absent. To-LE= *T. occidentalis* leaf extract, To-SE= *T.occidentalis* stem extract

Table 2 illustrates the quantitative analysis (amount) of each secondary metabolites present in the extracts and synthesized AgNPs. Tannins showed the highest amount as a secondary metabolite, followed by flavonoids and phenols, reducing sugars, alkaloids, saponins, and cardiac glycosides.

Table 2. Quantitative phytochemical analysis (mg/100g) of *Telfairia occidentalis* extracts.

SAMPLES	Tannins	Phenols	Flavonoids	Alkaloids	Saponins	Cardiac glycosides	Reducing sugars
Fresh ToLE	52.51 ±0.12	17.42 ±0.21	17.42 ±0.13	5.27 ±0.14	4.41 ±0.06	1.61 ±0.01	5.20 ±0.13
AgNPs-ToL	53.48 ±0.09	17.36 ±0.13	18.08 ±0.04	5.83 ±0.15	4.46 ±0.03	1.70 ±0.10	5.34 ±0.07
Fresh ToSE	0.00	0.00	8.82 ±0.12	3.16 ±0.08	3.56 ±0.11	0.85 ±0.12	4.74 ±0.02
AgNPs-ToS	3.60 ±0.07	1.00 ±0.04	16.24 ±0.06	3.47 ±0.05	3.66 ±0.12	1.03 ±0.02	4.40 ±0.03

Values are Mean±SEM of duplicate determinations.

The addition of silver nitrate solution to *Telfairia occidentalis* leaf and stem extracts gave a visible color change from light yellow (stem) and dark yellow (leaves) to almost colorless (stem) and light grey (leaves) (Figure 1 a&b). The color change observed is in agreement with the findings of [21]. The figure below shows the visual observation of the synthesis of silver nanoparticles (AgNPs) using *Telfairia occidentalis* leaf and stem extracts respectively, due to the reduction of silver ions to AgNPs.

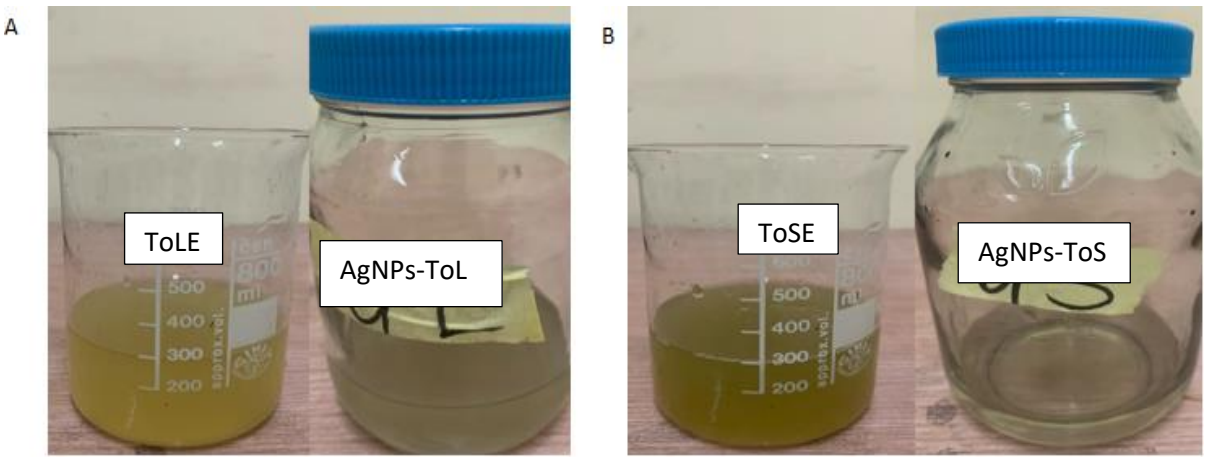


Figure 1. Visual observation of color change in *Telfairia occidentalis* leaf and stem extracts after 72 hours**. To-LE**= *T. occidentalis* leaf extract, **To-SE**= *T.occidentalis* stem extract, **AgNPs**= Silver nanoparticles.

The UV-visible spectra of the synthesized AgNPs using *Telfairia occidentalis* leaf and stem extracts read between the wavelengths range of 350-600nm gave an absorbance peak at 412nm, which is within the absorbance peak range of 400-450 nm as reported in the previous studies of [22]. This confirmed the rapid bio-reduction of silver nitrate into silver nanoparticles using *Telfairia occidentalis* leaves and stem extract as a reducing agent [23]. The silver nanoparticles exhibited a surface plasmon resonance band due to the free electron excitation,

which is similar to the results obtained in a previous report by [24]. Figure 2 (a&b) shows the UV-Vis absorption spectra of obtained synthesized silver nanoparticles using *Telfairia occidentalis* leaf and stem extracts, respectively. The absorption peak of the synthesized AgNPs was observed at 412 nm using the spectrophotometry method.

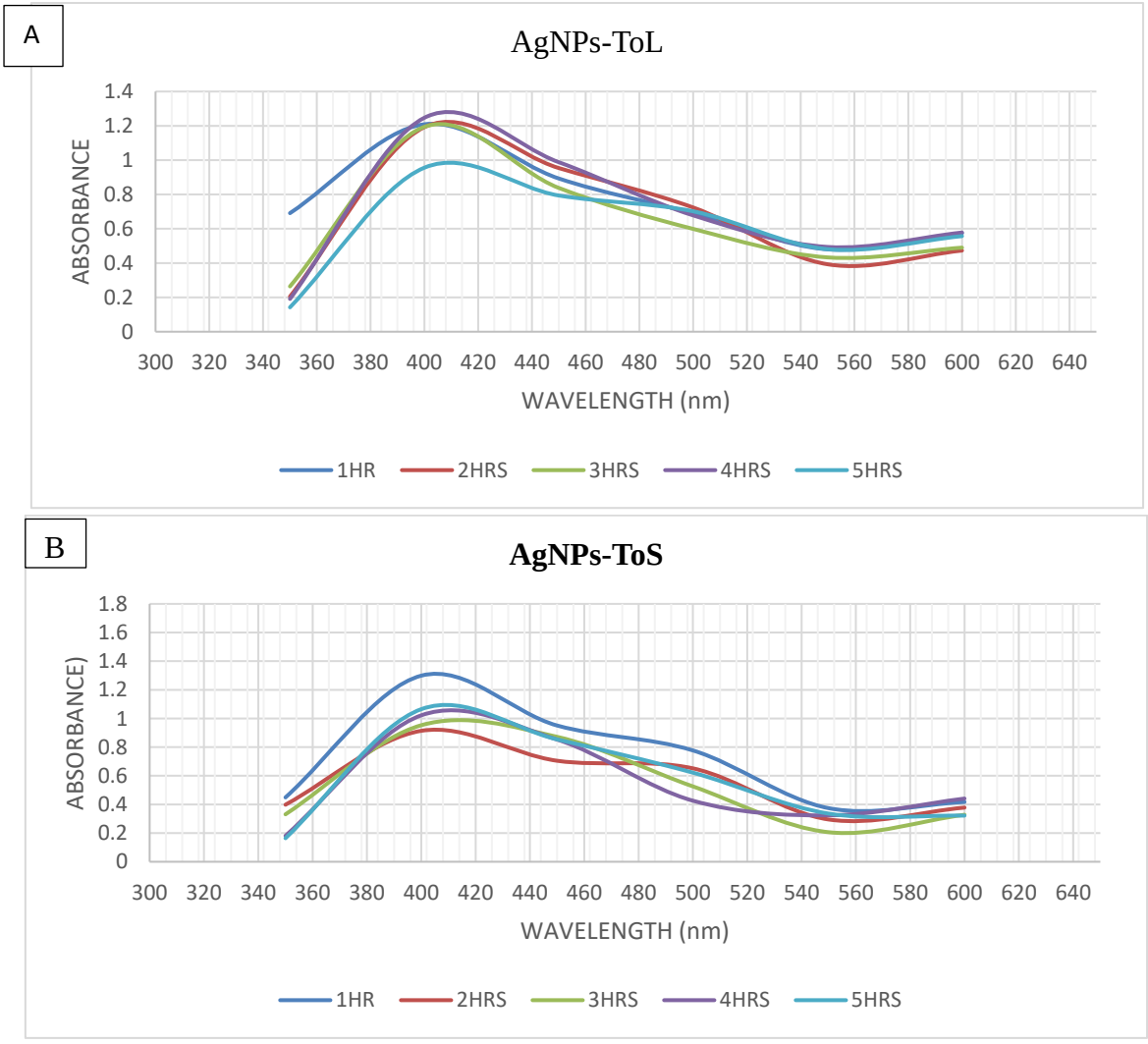
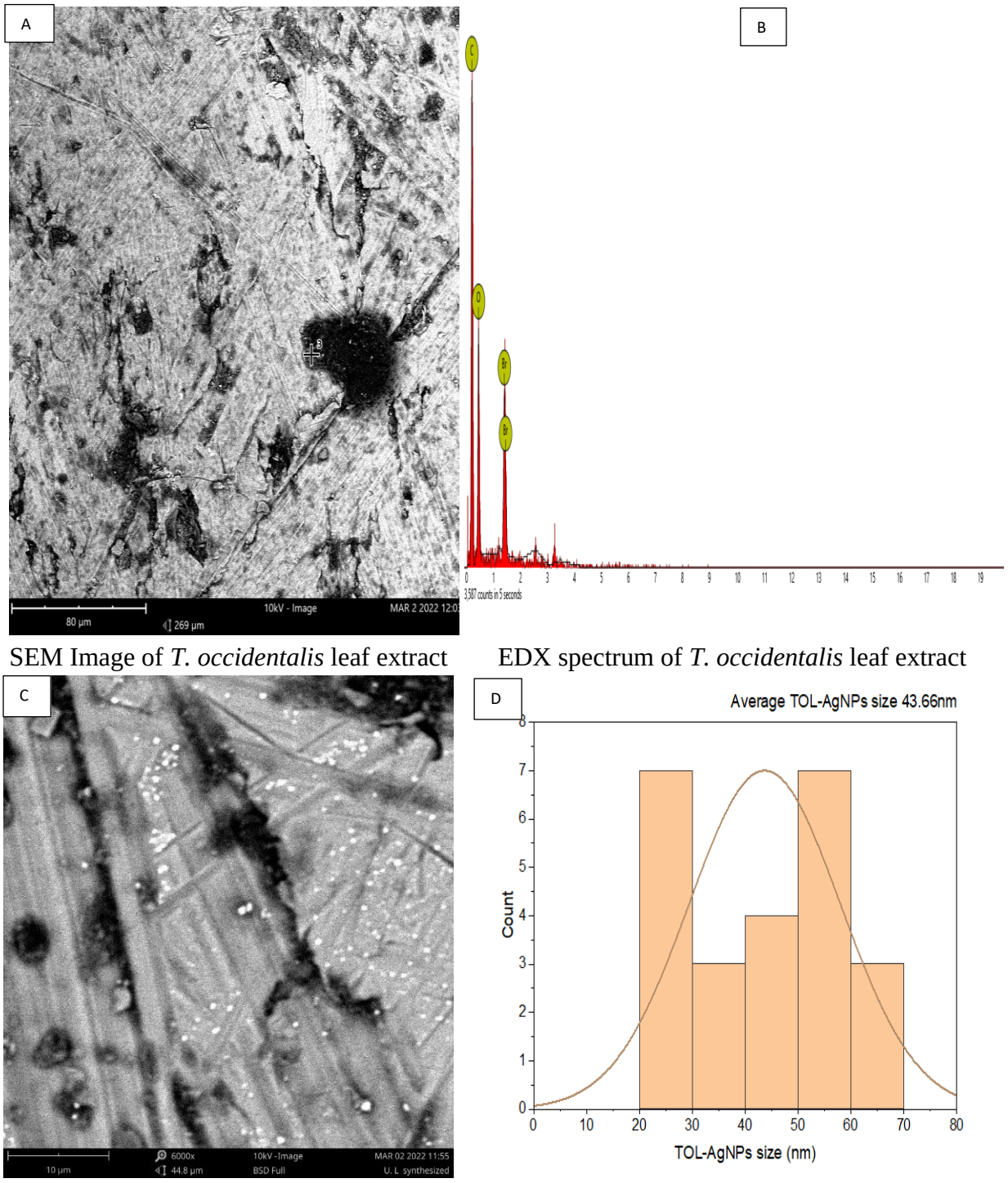


Figure 2. UV-Vis absorption spectra of synthesized silver nanoparticles at different time intervals (1-5 hours) using aqueous (a) leaf and (b) stem extracts of *Telfairia occidentalis.*

The scanning electron microscopy (SEM) analysis revealed the morphology and size of the synthesized AgNPs using *Telfairia occidentalis* leaves and stems. The SEM results confirmed the pseudo-spherical shape of the synthesized AgNPs, and the investigation of the size distribution of the synthesized AgNPs from the pure extracts varied between 10-70 nm and 20-80 nm for the synthesized AgNPs using stem and leaf extracts, respectively. Thus, the average size of the synthesized AgNPs using the leaf and stem were 50.47 nm and 43.90 nm, respectively. The figures below revealed the SEM image of the leaf extract (Figure 3a), the elemental composition of the leaf extract (using EDXS) (Figure 3b), shape and size of the synthesized silver nanoparticles (AgNPs) using Scanning electron microscopy (SEM) imaging, Image J and Origin softwares (Figures 3 c&d). The SEM result revealed that the synthesized AgNPs have a pseudo-spherical shape, and the size ranged between 22 - 68 nm with an average size of 43.66 nm. In addition, the energy dispersive analysis by X- rays (EDXS) spectrum of *T. occidentalis* leaf extract showed a number of elements, including carbon, oxygen, and bromine, in appreciable amounts.

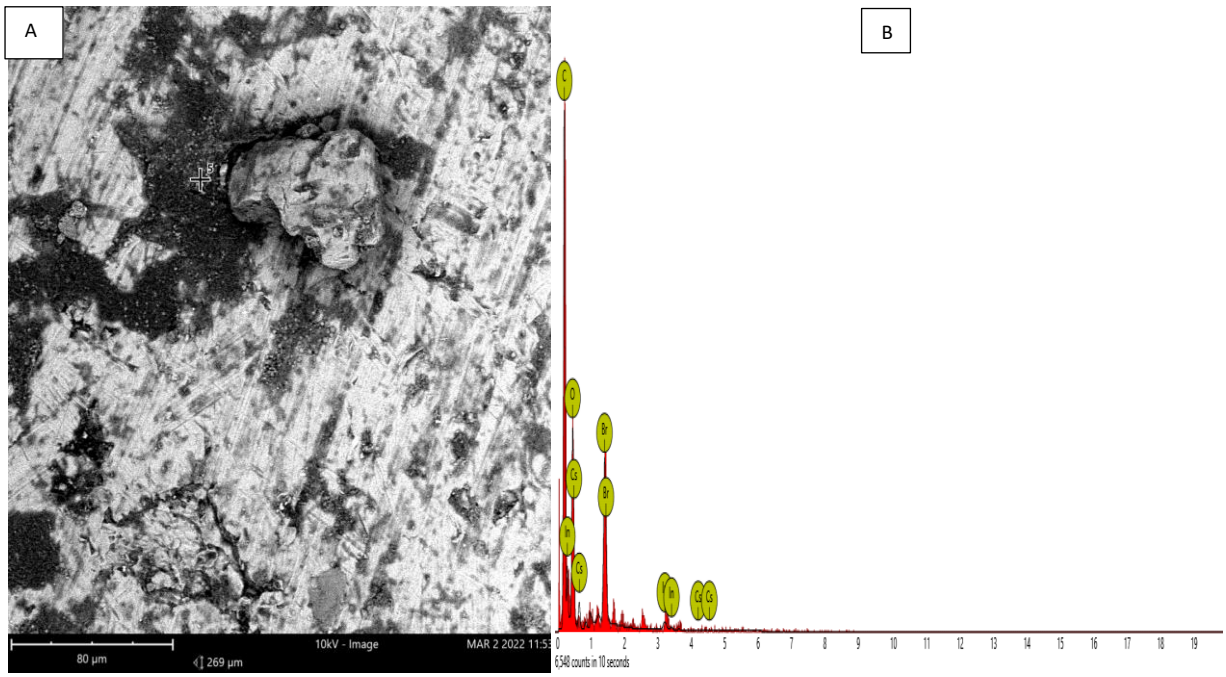


SEM Image of *T. occidentalis* leaf extract

EDX spectrum of *T. occidentalis* leaf extract

Figure 3. (a) SEM image of leaf extract; (b) EDXS of leaf extract; (c) SEM image and (d) Size distribution of synthesized AgNPs using aqueous leaf extract of *T. occidentalis*.

Figure 4 (a-d) revealed the shape (using SEM imaging), the elemental composition of the stem extract (using EDXS), and the size of the synthesized AgNPs using *Telfairia occidentalis* stem extracts. Figure 4a below revealed the SEM image of the stem extract while, the elemental composition of the stem extract is represented in the Figure 4b below. The shape and size of the synthesized silver nanoparticles (AgNPs) using Scanning electron microscopy (SEM) imaging, Image J, and Origin are represented in Figures 4c & d below. The SEM result revealed that the synthesized AgNPs using *Telfairia occidentalis* stem extracts have a pseudo-spherical shape with an average size of 48.40 nm. The energy dispersive analysis by X-rays (EDXS) spectrum of *T. occidentalis* stem extract showed a number of elements, including carbon, oxygen, bromine, indium, and cesium, in appreciable amounts.



SEM Image of *T. occidentalis* stem extract

EDX spectrum of *T. occidentalis* stem extract

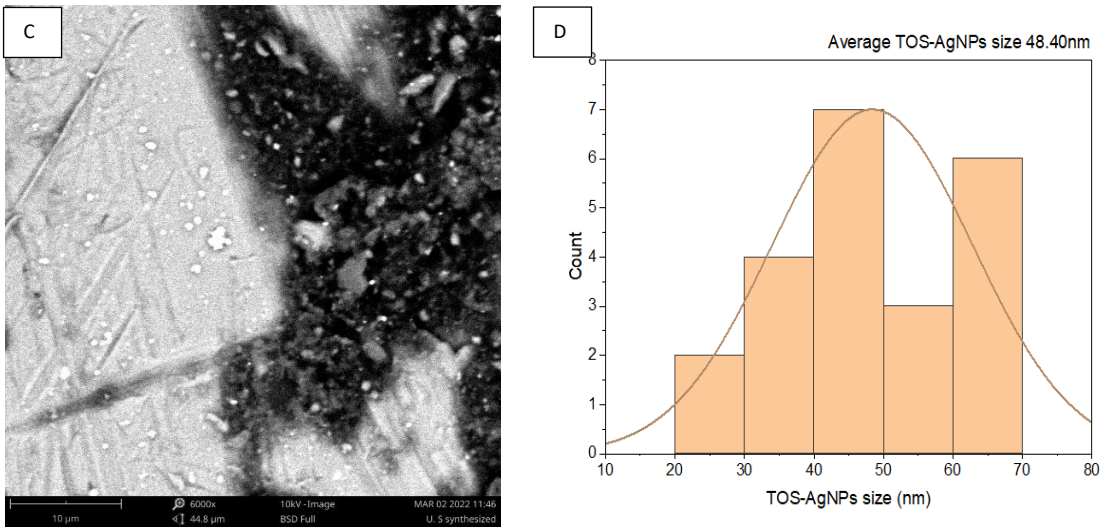


Figure 4. (a) SEM image of stem extract (b) EDXS of stem extract (c) SEM image and (d) Size distribution of synthesized AgNPs using aqueous stem extract of *T. occidentalis*

FT-IR is used to determine functional groups (capping agents) present in the phytochemicals of plants used for the synthesis of nanoparticles hence, Figure 5 a-d in the present study revealed the possible functional groups involved in the reduction of Ag^{+} in all the synthesized AgNPs using *Telfairia occidentalis* extracts (leaf and stem). Figures 5 a&c revealed the same absorbance peak observed at 3268.9 cm^{-1} from the leaf extract and AgNPs-ToL (with slight difference in their transmittance) and predicted alkynes (C≡C stretch), carboxylic acid (O–H stretch), alcohols (O–H stretch), phenols (O-H stretch), aliphatic primary amine (N-H) and secondary amine (N-H); 1636.3cm^{-1} as alkenes (C=C stretch), secondary amide (C=O stretch), tertiary amide (C=O stretch), δ-lactam (C=O stretch), conjugated alkene (C=C), cyclic alkene (C=C stretch), amine (N-H bend). Meanwhile, the absorbance peak observed at 3272.6 cm^{-1} from the stem extract predicted carboxylic acid (O–H), aliphatic primary amine (N-H), secondary amine (N-H) and alcohols (O–H), 2109.7 as alkynes (C≡C stretch), ketene (C=C=C stretch), isothiocyanate (N=C=S stretch); and 1636.3cm^{-1} as alkenes (C=C stretch), secondary

amide (C=O stretch), tertiary amide (C=O stretch), δ-lactam (C=O stretch), conjugated alkene (C=C), cyclic alkene (C=C stretch), amine (N-H bend) whereas, the synthesized AgNPs using *T. occidentalis* (AgNPs-ToL) showed absorbance peak at 3268.9cm^{-1} predicted to be alkynes (C≡C), carboxylic acid (O-H), phenols (O-H stretch), aliphatic primary amine (N-H), secondary amine (N-H) and alcohols (O-H), and 1636.3cm^{-1} as alkenes (C=C stretch), secondary amide (C=O stretch), tertiary amide (C=O stretch), δ-lactam (C=O stretch), conjugated alkene (C=C), cyclic alkene (C=C stretch), amine (N-H bend).

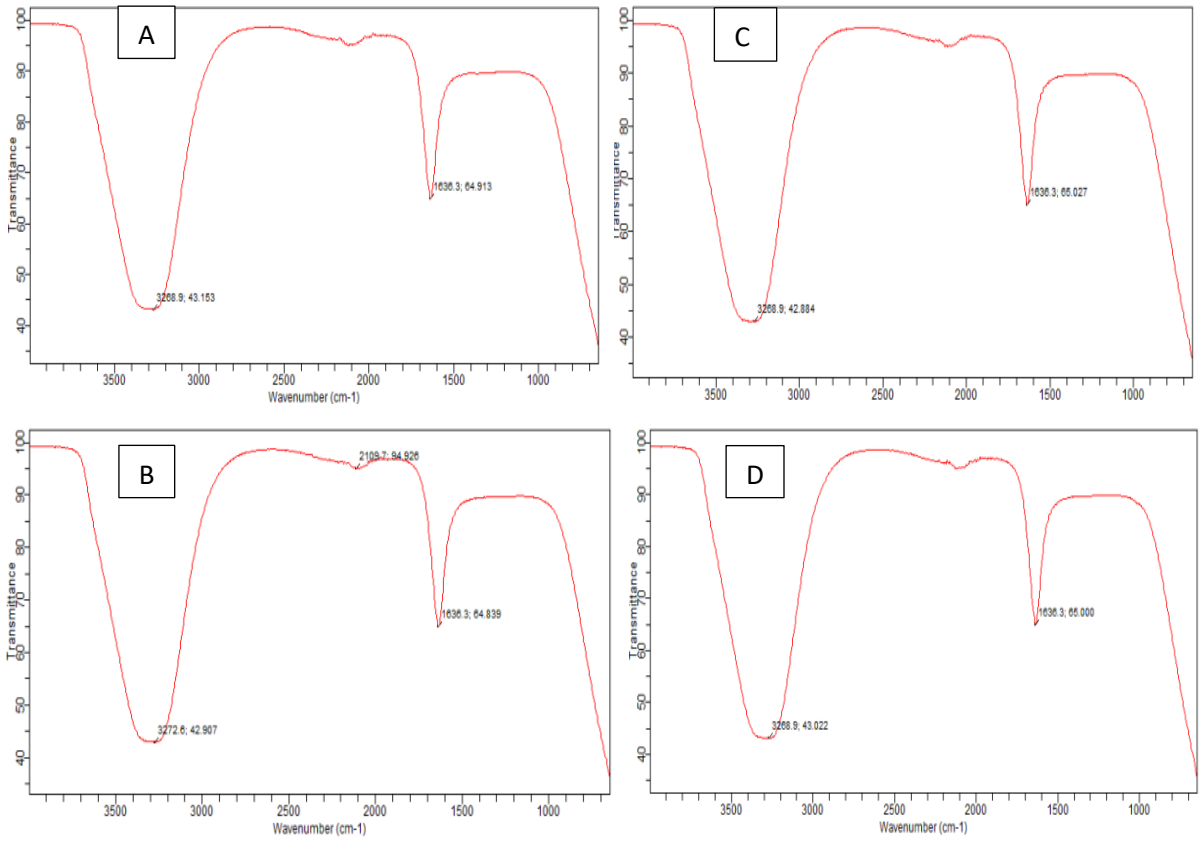


Figure 5. Fourier Transform Infra-red (FT-IR) spectrum of (a) *T. occidentalis* leaf extract (b) *T. occidentalis* stem extract (c) AgNPs using *T. occidentalis* leaf extract (AgNPs-ToL) (d) AgNPs using *T. occidentalis* stem extract (AgNPs-ToS).

It may be inferred that these organic compounds are responsible for the capping and efficient stabilization of synthesized nanoparticles by coating the extracts [25]. Moreover, these functional groups may be responsible for the various biological activities (antidiabetic, anti-inflammatory, antioxidant, and anti-glycation) observed in the synthesized nanoparticles (AgNPs) using the extracts of *Telfairia occidentalis* in this study [26].

Diabetes mellitus is a metabolic disease that elevates blood sugar levels over a long period. A therapeutic approach to decrease hyperglycemia (high blood sugar) is to inhibit the carbohydrate digestive enzyme [27]. The antidiabetic activity of the synthesized silver nanoparticles was evaluated using α-amylase and α-glucosidase inhibitory assay. α-amylase is responsible for the breakdown of carbohydrates into monosaccharides for absorption while α-glucosidase breaks down starch and disaccharides into glucose [28]. Thus, natural compounds using traditional medicinal plants that could inhibit the digestive enzyme would be effective for the treatment of non-insulin diabetes [29]. From the present study, *Telfairia occidentalis* mediated nanoparticles showed significant inhibition of α-glucosidase and α-amylase which is comparable to the control (acarbose) (Table 3). The synthesized AgNPs using *Telfairia occidentalis* leaf extract showed higher inhibitory activity against α-glucosidase and α-amylase

as compared to *Telfairia occidentalis* stem extracts. This is in agreement with the findings of Riyaphan, Pham [30]. The mechanism behind this is that the phytochemicals in *Telfairia occidentalis* adhered to the silver nanoparticle surfaces, providing superior antidiabetic potential [31]. This result depicts the potential effectiveness of synthesized silver nanoparticles using *Telfairia occidentalis* leaves and stem extract to treat and/or manage diabetes and its related complications, and this could be considered in the approach for developing diabetes drugs.

Table 3 revealed the antidiabetic activity of the synthesized AgNPs and fresh extracts. The synthesized AgNPs showed a potent antidiabetic inhibitory activity against α- amylase and α- glucosidase compared to the fresh extracts; however, the synthesized AgNPs using the leaf extract showed a better inhibitory potential against the carbohydrate metabolizing enzymes comparing their respective IC_{50} values.

Table 3. Antidiabetic activity of AgNPs using fresh aqueous extract of *Telfairia occidentalis*.

SAMPLES	α- Amylase IC_{50} VALUES	α- Glucosidase IC_{50} VALUES
Fresh ToLE	13.22±0.21[a]	19.93±0.03[a]
AgNPs-ToL	10.74±0.05[b]	16.75±0.11[b]
Fresh ToSE	14.74±0.53[a]	26.62±0.36[a]
AgNPs-ToS	12.05±0.01[b]	23.16±0.14[b]

Values are Mean±SEM of duplicate determinations, and different superscripts denote statistical significance from respective synthesized AgNPs at $p < 0.05$ in each column.

In the present study, the reducing power radical scavenging activity, nitric oxide inhibition, DPPH inhibition, and Total antioxidant capacity (Table 4) shows that the synthesized AgNPs have a reducing power similar to the control (ascorbic acid), which may be due to the presence of reductants in the synthesized AgNPs, reducing Fe^{3+} to Fe^{2+}. However, the synthesized AgNPs using *Telfairia occidentalis* leaves extract exhibited a higher reducing power compared to the synthesized AgNPs using *Telfairia occidentalis* stems extract. Additionally, the synthesized AgNPs using *Telfairia occidentalis* leaves and stem extract showed higher reducing power as compared to the pure extracts suggesting that the synthesized silver nanoparticles enhanced the antioxidant potential of the extracts. The antioxidant potential of the synthesized AgNPs was further confirmed using other antioxidant assays, including Total antioxidant capacity, DPPH, and NO % inhibition. Table 4 revealed the antioxidant activities of the fresh extracts (leaf and stem) and synthesized AgNPs using aqueous leaf and stem extracts of *Telfairia occidentalis*.

Table 4. Antioxidant activity of synthesized AgNPs using fresh aqueous extract of *Telfairia occidentalis*.

SAMPLES	DPPH IC_{50} VALUES	NO IC_{50} VALUES	TAC (mg/100g)	REDUCING POWER (Absorbance at 700nm)
Fresh ToLE	42.47±0.33[a]	30.93±0.01[a]	5.86±0.00[a]	0.046±0.01[a]
AgNPs-ToL	22.29±0.05[b]	24.17±0.04[b]	8.55±0.02[b]	0.071±0.07[b]
Fresh ToSE	64.96±0.09[a]	36.06±0.03[a]	5.49±0.05[a]	0.040±0.05[a]
AgNPs-ToS	42.99±0.03[b]	31.95±-0.01[b]	6.98±0.03[a]	0.054±0.02[b]

Values are Mean±SEM of duplicate determinations and different superscript denotes statistical significance from respective synthesized AgNPs at $p < 0.05$ in each column.

The synthesized AgNPs showed a higher antioxidant activity than the fresh extracts (leaf and stem). However, the AgNPs-ToL showed the most potent antioxidant activity, comparing their IC_{50} values for DPPH and NO activities. The synthesized AgNPs showed a high total antioxidant capacity, although AgNPs-ToL was more potent. The prevention of protein

denaturation is the main mechanism of action of non-steroidal anti-inflammatory drugs (NSAIDs). In this study, *Telfairia occidentalis* leaf and stem extracts and the synthesized AgNPs using these extracts showed a promising potential of preventing the denaturation of proteins, and this may be due to their anti-inflammatory properties. However, the synthesized AgNPs showed a more potent anti-inflammatory potential than the pure extracts.

Figures 6 a&b below revealed the anti-inflammatory activity of the synthesized AgNPs and fresh extracts. The AgNPs showed a better anti-inflammatory potential than the fresh extracts, and AgNPs-ToL gave the best anti-inflammatory activity compared to AgNPs-ToS.

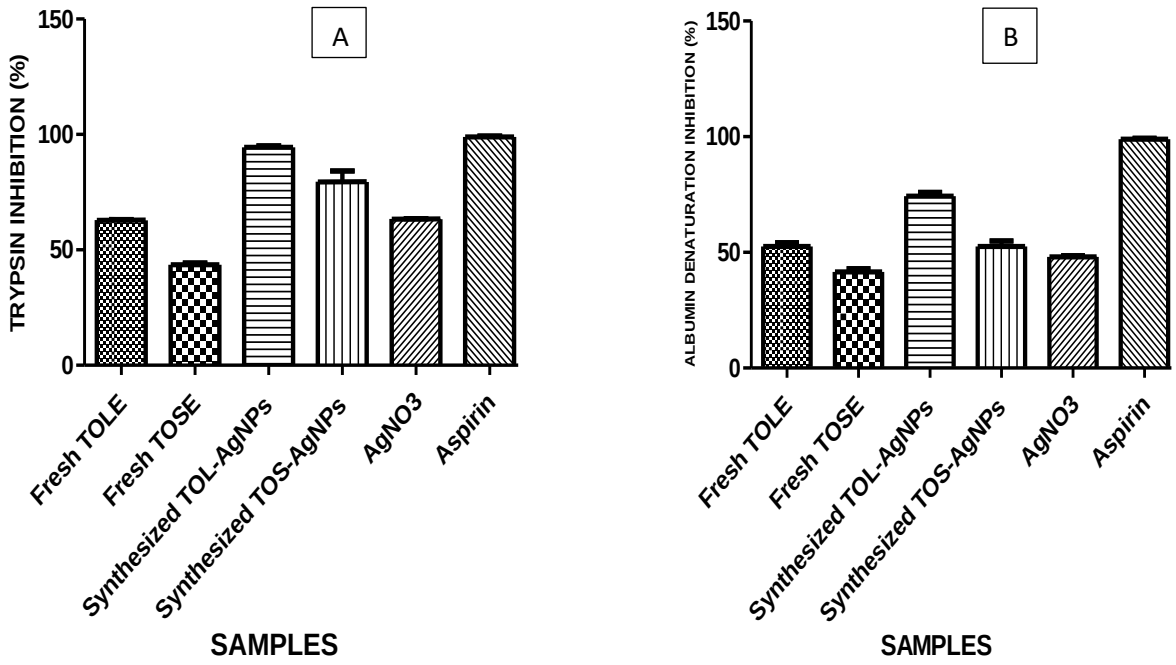


Figure 6. Anti-inflammatory activity of fresh extracts and synthesized AgNPs using *Telfairia occidentalis* leaves and stems extracts (a) Trypsin inhibition (b) Albumin denaturation inhibition.

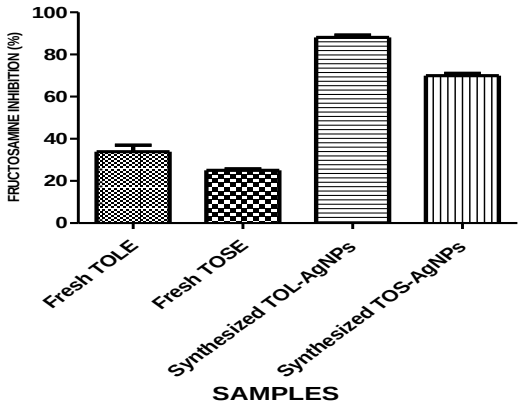


Figure 7. Anti-glycation activity of fresh extracts of *Telfairia occidentalis* leaf and stem extracts and synthesized AgNPs

The leaf and stem extracts displayed a good antiglycation potential, although the synthesized AgNPs using *Telfairia occidentalis* leaf extract showed the highest potential than the pre-extracts and the synthesized AgNPs using *Telfairia occidentalis* stem extract. The synthesized AgNPs in this study have been observed to have high antioxidant potential, and agents that possess good antioxidant activity by mopping up free radicals can simultaneously inhibit the formation of advanced glycation end products (AGEs) [32]. Figure 7 below shows

that the synthesized nanoparticles have a better antiglycation potential than the fresh samples; however, the synthesized AgNPs using the leaf extract showed a better antiglycation activity.

The leaf and stem extracts displayed a good antiglycation potential, although the synthesized AgNPs using *Telfairia occidentalis* leaf extract showed the highest potential than the pre-extracts and the synthesized AgNPs using *Telfairia occidentalis* stem extract. The synthesized AgNPs in this study have been observed to have high antioxidant potential, and agents that possess good antioxidant activity by mopping up free radicals can simultaneously inhibit the formation of advanced glycation end products (AGEs) [32].

This study's findings strongly recommend applying *Telfairia occidentalis*-mediated silver nanoparticles as useful natural antioxidants for health preservation against different oxidative stress-associated diseases. However, an *in-vivo* evaluation and confirmation of this research findings is highly recommended for further studies.

4. Conclusions

In conclusion, the aqueous leaf and stem extracts of *Telfairia occidentalis* can be used to synthesize AgNPs with promising antidiabetic, antiglycation, antioxidant, and anti-inflammatory potentials. In addition, the AgNPs-TOL showed a better biological potential that can be explored in developing drugs for treating diabetes mellitus and its complications.

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Conflicts of Interest

The authors declare no conflict of interest.

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