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ORIGINAL RESEARCH

Jatropha tanjorensis aqueous extracts synthesized silver nanoparticles possesses antidiabetic, antiglycation, antioxidant and anti-inflammatory potentials.

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Abstract:

Introduction: The use of plant-mediated nanotechnology is gaining attention worldwide because of its low-cost and high efficiency in the synthesis of nano-sized particles that ranges between 1-100 nm.

Aims: This study investigated the antidiabetic, antiglycation, antioxidant, and anti-inflammatory potentials of synthesized silver nanoparticles (AgNPs) using *Jatropha tanjorensis* leaf and stem extracts.

Materials and Methods: AgNPs was synthesized using the aqueous extract of *J. tanjorensis* leaf and stem, respectively. The synthesized AgNPs were characterized using UV-Visible spectroscopy, Fourier Transform Infrared spectroscopy (FTIR) and Scanning Electron Microscopy (SEM). The anti-diabetic (α-amylase and α–glucosidase inhibitory assays), antiglycation (fructosamine inhibition), antioxidant (reducing power, total antioxidant capacity, DPPH and NO radical scavenging assays), and anti-inflammatory (proteinase inhibitory action and albumin denaturation inhibition) activities were evaluated using standard procedures.

Results: The synthesized AgNPs showed a maximum absorption peak at 410nm and 412nm, and an average size distribution of 42.66nm and 48.33nm using the leaf and stem extracts of the plant, respectively. The synthesized AgNPs using the leaf extract (JTL-AgNPs) showed a better antidiabetic, antiglycation, anti-inflammatory and free radical scavenging potentials which may possibly be due to the compounds adsorbed on the surface of the synthesized AgNPs as revealed by the FTIR analysis.

Conclusion: The aqueous leaf and stem extracts of *Jatropha tanjorensis* can be used to synthesize AgNPs, however, the JTL-AgNPs showed better potentials on investigated parameters, thus suggesting its exploration in the development of drugs for the treatment and management of diabetes mellitus and other associated free radical causing diseases.

To Keywords: Green synthesis, *Jatropha tanjorensis*, silver nanoparticles, Diabetes mellitus, Antioxidant, Antiglycation, Anti-inflammation.

All co-authors agreed to have their names listed as authors.

1. INTRODUCTION

Nanotechnology involves the study, imaging, measuring, modelling, or manipulation of matter at scales falling in the range of 1–100 nanometers (nm) [1]. It is a highly multidisciplinary field, drawing from fields such as applied physics, biochemistry, biology, chemistry, colloidal science, materials science, and engineering [2]. The quest for scientific understanding of the etiopathogenesis of diabetes mellitus (DM), its complications and ultimately, the development of therapeutics for its management and treatment have stimulated great scientific research interest in recent years.

An increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of many drugs and chemotherapeutics from these plants as well as from traditionally used herbal remedies [3]. Without doubts, the benefits derived from using medicine obtained from plants are that, they are relatively safer than synthetic alternatives by offering profound therapeutic benefits and more affordable treatments. More recently, plant-mediated bio-nanotechnology (green synthesis) is rapidly generating great research interest than the use of pure plant extracts because of their better drug delivery property to target cells. Silver nanoparticles (AgNPs) amongst other synthesized nanoparticles have received the widest use and exploration due to its various biological activities [4].

Jatropha tanjorensis belongs to the family Euphorbiaceae, commonly called “Hospital too far”, “Catholic vegetable”, and “Ewe Iyana-ipaja” [5] in the Yoruba language of South-western Nigeria. It is a shrub of 6 m high with spreading branches off stubby twinges and smooth grey bark which gives off whitest colored latex when cut. The leaves are delicious alternate but with apical crowded, acute to alternate basally cordate 3-5 lobe in outline, 6-40 cm broad the petioles 3-8 cm long. *Jatropha tanjorensis* is a multipurpose plant, cultivated for medicinal applications and also used as food thus, making the plant a nutraceutical. However, its medicinal and nutritional benefits depend on which part being used [6]. In Nigeria, the leaves of *J. tanjorensis* have been consumed locally as a vegetable and it is also popular as a natural remedy against diabetes especially amongst the Yoruba speakers in South-western Nigeria states. Moreso, few scientific reports on its pharmacological potentials have been documented including anti-obesity and hepatoprotective, anticancer, antimicrobial, antioxidant and antidiabetics activities [7, 8]. Despite the great potentials of *Jatropha tanjorensis* as a medicinal plant, there is dearth of scientific information on the synthesis of nanoparticles using the parts of *Jatropha tanjorensis* and their respective antidiabetic, antiglycation, antioxidant, and anti-inflammatory potentials.

2. MATERIALS AND METHODS

The fresh mature *Jatropha tanjorensis* (leaf and stem) were collected from Erunwen area of Ikorodu, Lagos State, Nigeria (Latitude: 6° 37′ 0.7140″ N and 3° 30′ 29.0592″ E) in January, 2022. The plant was authenticated and given a voucher number LSH001047 at the University herbarium, Department of Botany, Faculty of Science, Lagos State University, Ojo, Lagos, Nigeria.

2.1. Preparation of plant extract

The leaves and stems of *Jatropha tanjorensis* were washed individually under running water and later with deionized water. Thereafter, the cleaned fresh leaves and stems were chopped individually into smaller sizes and 10 g each poured into 100 mL of deionized water to obtain the aqueous extracts of the leaf and stem, respectively. The aqueous extracts were later filtered after 48 hours using a Whatman filter paper grade 1 twice, and the extracts stored separately in the refrigerator for further use.

2.2. Synthesis of silver nanoparticles

Silver nitrate solution (1 mM $AgNO_3$) was prepared in an Erlenmeyer flask in the dark at room temperature. To synthesize AgNPs, 10 mL of each extract was added to 90 mL each of the silver nitrate solution in a 250 mL beaker, and stirred periodically for 1 hour in the dark at room temperature. The change in color of the solution indicated the reduction of silver nitrate into synthesized AgNPs. Then 2 mL aliquots were taken every 1 hour until 5 hours, and the absorbance analyzed (200-700 nm) using UV visible spectrophotometer

each hour. After the completion of this process, each solution was centrifuged at 5000 rpm for 15 minutes, and the supernatant kept in the refrigerator for further analysis.

2.3. Characterization of silver nanoparticles

The synthesized AgNPs were primarily characterized by UV-visible spectroscopy as earlier described ad this was followed by Scanning Electron Microscopy (SEM) and FTIR (Fourier Transform Infra-Red spectroscopy (FTIR). SEM was used to analyze the shape and size (i.e morphology) of the synthesized AgNPs while, FTIR was used to check the capping agents (functional groups) on the surface of the AgNPs.

2.4. Phytochemical Screening

The phytochemical constituents of the aqueous leaf and stem extracts of *J. tanjorensis* with their respective quantity were identified and measured by the methods described by [4], [9], and [10].

2.5. Antioxidant activity of *Jatropha tanjorensis* 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 was diluted with 2 mL of DPPH solution (0.5 mM). After 30 minutes, the absorbance was measured at 517 nm. The percentage of the DPPH radical scavenging activity was then calculated using the equation below:

% Inhibition of DPPH radical = ($[A_{br} - A_{ar}]/A_{br}$) X 100 where, A_{br} is the absorbance before reaction and A_{ar} is the absorbance after 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 was dissolved in 0.5 mL phosphate buffer saline (pH 7.4) and mixed with 0.5 mL of the extract. The mixture was then incubated at 25˚C. After 150 minutes of incubation, 0.5 mL of the incubated solution was 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 minutes with 1 mL of naphthylethylenediamine dichloride (0.1% w/v)]. The mixture was then incubated at room temperature for 30 minutes and its absorbance measured at 546 nm. The amount of nitric oxide radical inhibition was calculated following the equation below:

% Inhibition of NO radical = ($[A_{br} - A_{ar}]/A_{br}$) X 100 where, A_{br} is the absorbance before reaction, and A_{ar} is the absorbance after reaction has taken place. Concentrations of the extracts resulting in 50% inhibition (IC_{50}) were determined graphically.

2.5.3. Reducing power Assay

Reducing power assay procedure by [12] 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 the extract. The resulting mixture was incubated at 50˚C for 20 minutes, followed by the addition of 2.5 mL of trichloroacetic acid (10% w/v). The mixture was later centrifuged at 3000 rpm for 10 minutes to collect the upper layer of the solution (2.5 mL) which was mixed with distilled water (2.5 mL) and 0.5 mL of $FeCl_3$ (0.1%, w/v). The absorbance was then measured at 700 nm against a blank. The higher the absorbance, the more potent the extract.

2.5.4. Total Antioxidant Capacity (TAC) assay

The total antioxidant capacity was determined by phosphomolybdate method using ascorbic acid as standard [12]. An aliquot of 25 µL of each extract was mixed with 300 µL of TAC reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The resulting solution was incubated in a microplate incubator for 90 minutes. The absorbance of the mixtures was then measured at 630 nm in a microplate reader. The antioxidant activity of the extract was expressed as the number of mg equivalents to ascorbic acid (AAE)/100g extract. All the experiments were carried out in triplicates.

2.6. Antiglycation activity of *Jatropha tanjorensis* extracts and synthesized AgNPs

2.6.1. Albumin glycation and Estimation of Fructosamine production

Albumin glycation and measurement of fructosamine inhibition 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 of each extract, 1mL of 5% BSA, 1 mL of 166.5 mM glucose, and 1 mL 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 inhibition, 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 minutes, and then solubilized in 1mL phosphate buffer before being added to 0.5 mL 40% TCA. Following centrifugation at 6000 g for 10 minutes, 0.5 mL of supernatant was mixed with 0.05 M Thiobarbituric acid (TBA). The mixture was incubated in a boiling water bath for 20 minutes, cooled to room temperature and the absorbance read at 443 nm. Inhibition of fructosamine was calculated as a percentage using the following formula:

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

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

2.7. Antidiabetic activity of *Jatropha tanjorensis* extracts and synthesized AgNPs

2.7.1. α-Amylase Inhibitory Assay

The α-amylase inhibitory assay was determined according to the procedure of [4] and [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 minutes, 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 minutes. The reaction was terminated by adding 500 µL of dinitrosalicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5 minutes and cooled to room temperature. The resulting mixture was diluted with 5 mL distilled water and the absorbance measured at 540 nm with the use of a spectrophotometer (Spectrum lab S23A, Globe Medical England). A control was prepared using the same procedure but replacing the extract with distilled water. The α-amylase inhibitory activity was calculated as percentage inhibition: **% Inhibition** = $([A_c - A_s]/A_c) \times 100$.

Ac = Absorbance of Control, As = Absorbance of sample (extract). Concentrations of each extract resulting in 50% inhibition of α-amylase activity (IC_{50}) were determined graphically.

2.7.2. α-Glucosidase Inhibitory Assay

This assay was conducted according to the method described by [4] and [12]. The substrate solution, p-nitrophenyl glucopyranoside (pNPG) was prepared in 20 mM phosphate buffer, pH 6.9. 20 µL of α-glucosidase (0.2 U/mL) was pre-incubated with 20 µL of the extracts for 10 minutes. 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 minutes and stopped by adding 80µL of 0.2M Na_2CO_3.The α-glucosidase activity was determined by measuring the yellow-colored paranitrophenol released from pNPG at 405 nm with microplate reader. Control contained the same reaction mixture but the same volume of phosphate replaced the extract. The α-glucosidase inhibitory activity of the extracts was calculated as percentage inhibition: **% Inhibition** = $([A_c - A_s]/A_c) \times 100$ where, Ac = Absorbance of Control, As = Absorbance of sample (extract). Concentrations of each extract resulting in 50% inhibition of α-glucosidase activity (IC_{50}) were determined graphically.

2.8. Anti-inflammatory activity of *Jatropha tanjorensis* 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 each extract and 1% stock solution of bovine serum albumin (BSA) prepared with deionized water. Briefly, 0.5 mL of BSA was mixed with 50 µL of extract. 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 triplicate. The percentage inhibition of protein denaturation was then calculated as follows: **% Inhibition** = ($[A_c - A_s]/A_c$) X 100. Ac = Absorbance of Control, As = Absorbance of sample (extract).

2.8.2. Proteinase Inhibitory Action

This method was conducted according to the modified procedure described by [15]. The reaction mixture contained 1 mg trypsin, 1 mL of 20 mM Tris HCl buffer (pH 7.4) and 50 µL of the extract. The reaction mixture was incubated at 37°C for 5 minutes and then 1 mL of 0.8% (w/v) casein was added. The mixture was incubated for an additional 20 minutes, and 2 mL of 70% perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged, and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed in triplicate and the percentage of inhibition of proteinase inhibitory activity was calculated as: **% Inhibition** = ($[A_c - A_s]/A_c$) X 100. Ac = Absorbance of Control, As = Absorbance of sample (extract).

2.9 Statistical Analysis

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

3. RESULTS

Table 1 shows the phytochemicals present and Table 2 shows the quantity of each phytochemical in the aqueous extracts of *Jatropha tanjorensis* leaf and stem extracts. Phenol, tannin, alkaloid and saponin are common to the leaf and stem extracts. Reducing sugar and cardiac glycoside are absent in the leaf and stem extracts. Flavonoid, steroid, terpenoid and phlobatannin are absent in the leaf extract but present in the stem extract. Tannin showed the highest concentration followed by phenol, flavonoid, alkaloid, saponin, respectively (Table 2). The quantitative phytochemical analysis of the plant's leaf extract showed no traces of flavonoid, cardiac glycoside and reducing sugar content, however, when the extract was used to synthesize AgNPs, it showed traces of these phytochemicals. It may be that the plants extract contains other compounds that, during the synthesis process, interact with the silver ions or nanoparticles to form complexes that mimic the properties of these phytochemicals. This interaction could lead to a false positive result for the presence of flavonoids, cardiac glycoside, and reducing sugar. Some compounds in the plant extract may also have undergone chemical transformations during the synthesis process, leading to the formation of compounds with similar characteristics of flavonoid, cardiac glycoside, and reducing sugar.

Table 1. Qualitative phytochemical analysis of *Jatropha tanjorensis* aqueous leaf and stem extracts

PHYTOCHEMICALS	AQUEOUS EXTRACTS	
	LEAF	STEM
Phenol	+	+
Flavonoid	-	+
Tannin	+	+
Alkaloid	+	+
Saponin	+	+
Reducing sugar	-	-
Steroid	-	+
Terpenoid	-	+
Cardiac glycoside	-	-

Phlobatannin	-	+

KEY: + = Present, - = Absent

Table 2. Quantitative Phytochemical screening of *Jatropha tanjorensis* leaf and stem aqueous extracts

Samples	Tannin	Phenol	Saponin	Alkaloid	Reducing sugar	Cardiac glycoside	Flavonoid
Fresh JTLE	40.55±0.03	13.45±0.12	2.56±0.01	3.42±0.33	0.00	0.00	0.00
JTL-AgNPs	41.14±0.32	13.32±0.23	2.91±0.13	3.01±0.18	0.65±0.00	2.65±0.002	4.12±0.24
Fresh JTSE	37.83±0.44	12.55±0.14	2.26±0.01	1.51±0.01	0.00	0.00	7.75±0.17
JTS-AgNPs	37.05±0.21	12.90±0.43	2.05±0.04	1.00±0.00	0.00	0.00	8.67±0.31

Values are Mean±SEM in mg/ 100 g of triplicate determinations.

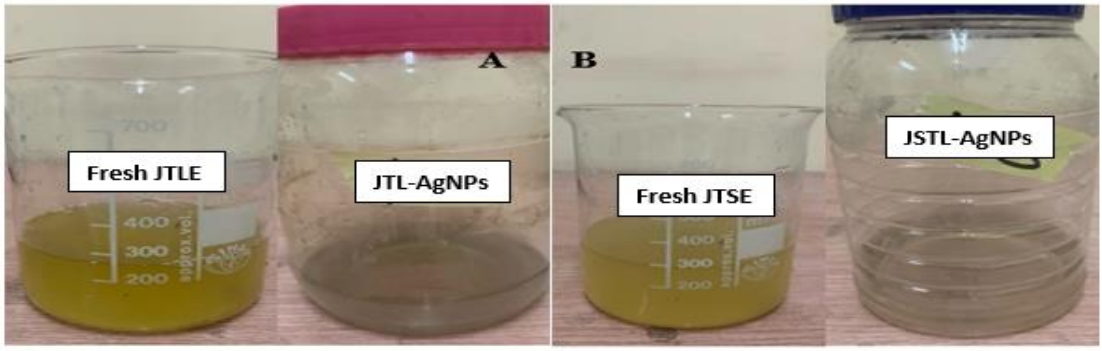
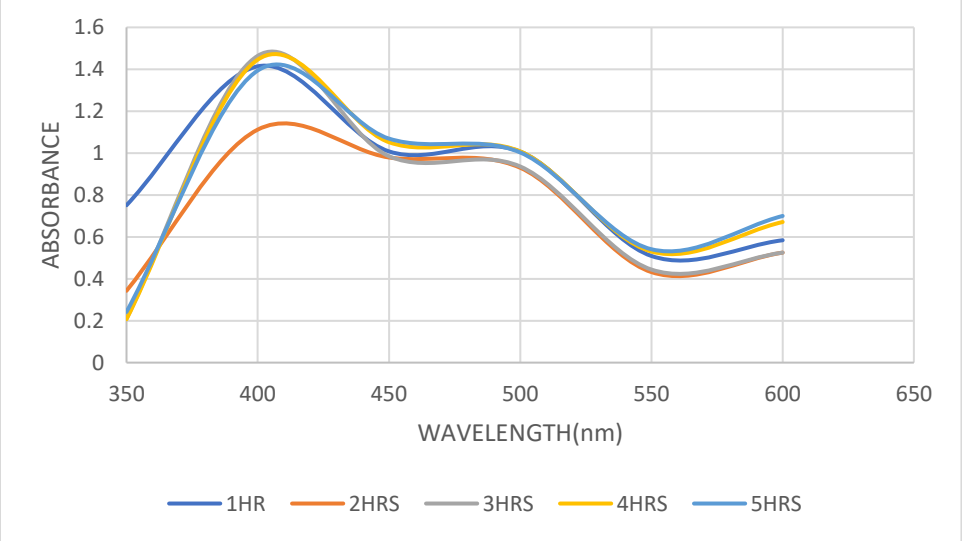


Figure 1. Visual identification of synthesized silver nanoparticles of *Jatropha tanjorensis* (a) Leaf (b) Stem. JTLE =*Jatropha tanjorensis* leaf extract, JTSE =*Jatropha tanjorensis* leaf extract, JTL-AgNPs =*Jatropha tanjorensis* leaf silver nanoparticles, JTS-AgNPs =*Jatropha tanjorensis* stem silver nanoparticles.

The bio-reduction of Ag^+ ions in $AgNO_3$ solution were observed using UV-Visible spectroscopy within the range of 350 - 650 nm. The synthesized AgNPs showed a maximum absorption peak at 410 nm and 412 nm, respectively (Figure 2).



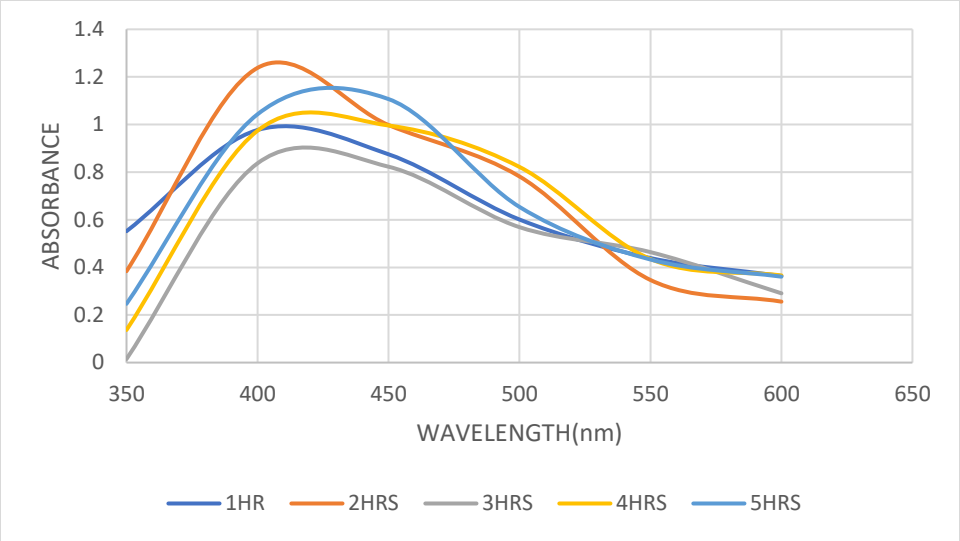
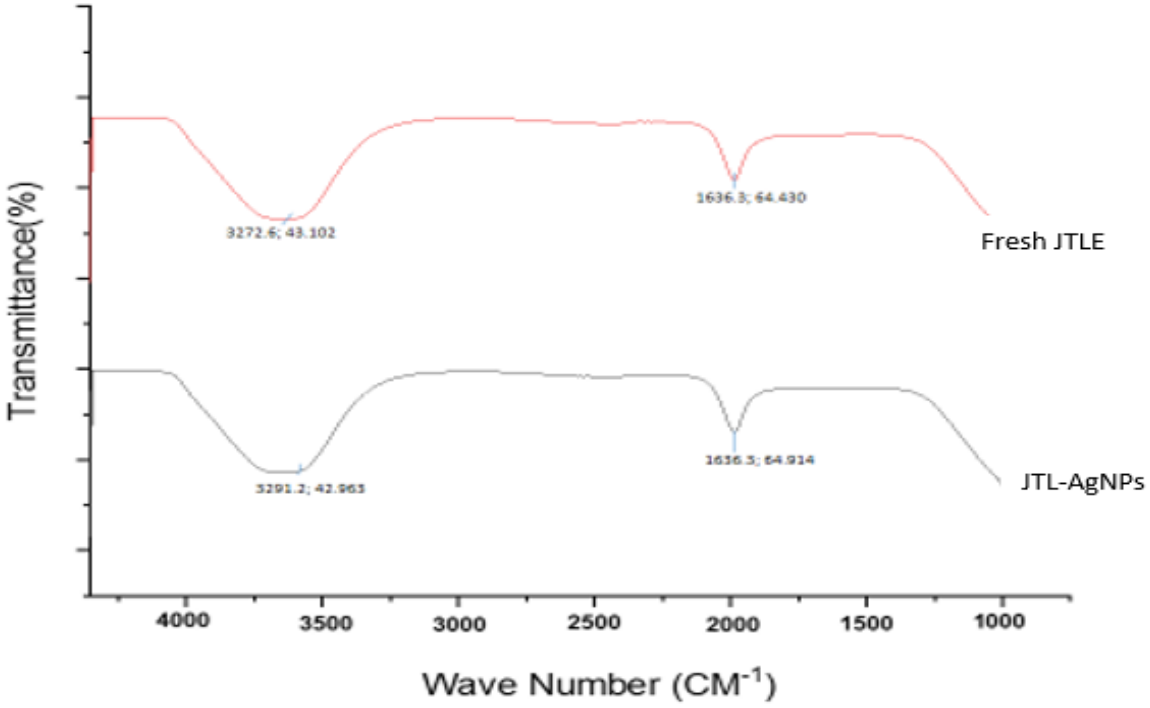


Figure 2. UV-visible readings of the synthesized AgNPs using *J. tanjorensis* (a) leaf (b) stem

Figure 3 (a & b) shows the functional groups present in the aqueous leaf and stem extracts of *J. tanjorensis* and the synthesized AgNPs using Fourier transform infrared (FTIR). Figure 3a shows the presence of functional groups from the phytochemicals present in the leaf extract. The absorbance peak at 3272.6cm^{-1} shows the presence of carboxylic acid (O-H stretch) and alcohols (O-H stretch), phenols (O-H stretch) and alkynes (C-H stretch), 1636.3cm^{-1} as alkynes (C-H stretch), isothiocyanate (N=C=S stretch) and 2102.2cm^{-1} as alkene (C=C stretch) and amine (N-H bend). Figure 3b also showed a broad absorbance peak at 3291.2cm^{-1} indicating the presence of carboxylic acid (O-H stretch), alcohols (O-H stretch), phenols (O-H stretch) and alkynes (C-H stretch), and 1636.3cm^{-1} as alkenes (N=C=S stretch). This may mean that the extracts served as reductant of silver ions into its relative nanoparticles (AgNPs). Additionally, these functional groups maybe responsible for the plant's biological properties.



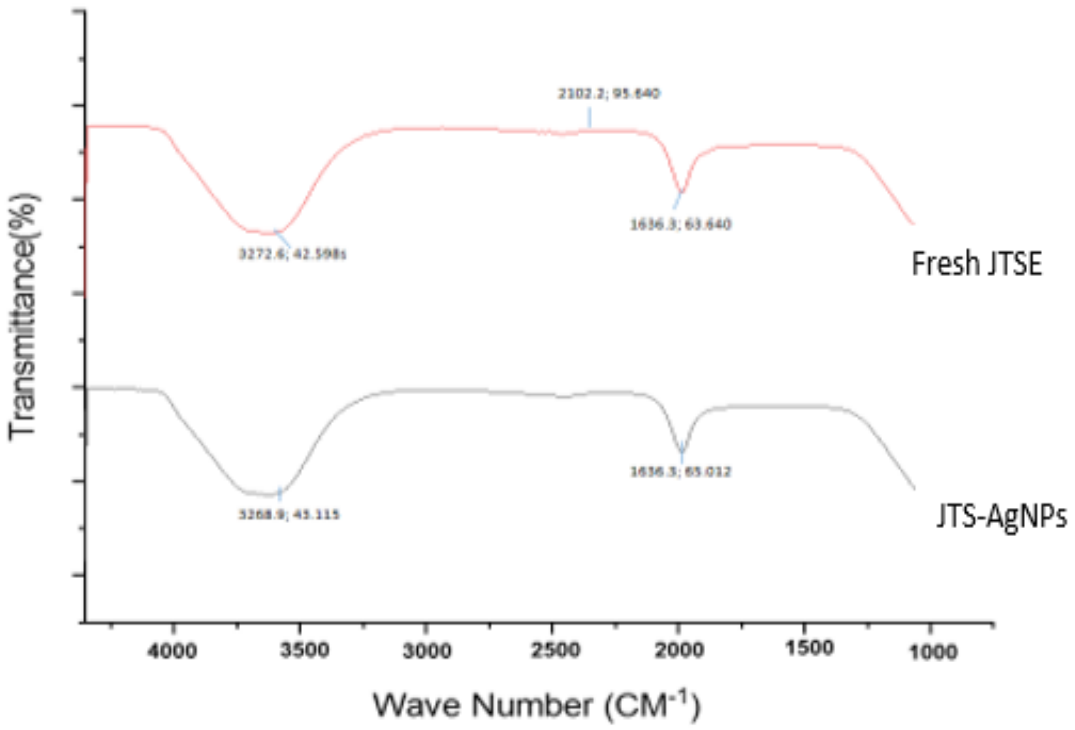


Figure 3. FTIR spectrum using *J. tanjorensis* aqueous (a) leaf extract (b) stem extract.

Figures 4 and 5 shows the morphology and average size distribution of AgNPs. It was observed that, both the JTS-AgNPs and JTL-AgNPs have a pseudo-spherical shape and are evenly dispersed with an average size distribution of 48.33nm and 42.66nm for JTS-AgNPs and JTL-AgNPs, respectively.

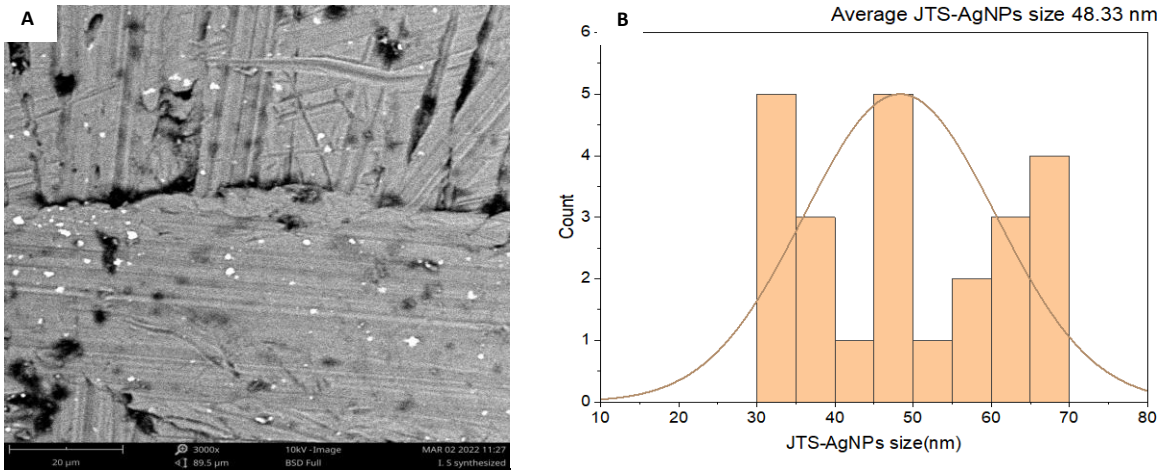


Figure 4. (a) Scanning Electron Microscopy analysis and (b) Size distribution of JTS-AgNPs.

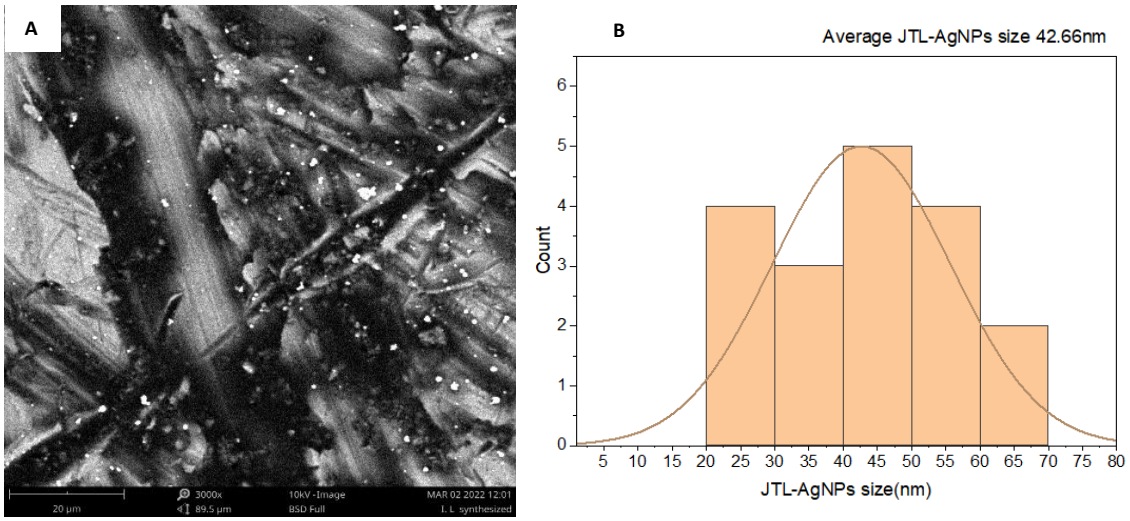


Figure 5. (a) Scanning Electron Microscopy analysis and (b) Size distribution of JTL-AgNPs.

Table 3 shows the IC_{50} values (mg/ mL) of α-amylase and α-glucosidase inhibitory activities of *J. tanjorensis* aqueous extracts (leaf and stem) and its synthesized AgNPs. The synthesized AgNPs showed a potent inhibitory activity against both α-amylase and α-glucosidase compared to the fresh extracts, however, the synthesized AgNPs using the leaf extract showed a better inhibitory potential against these carbohydrate metabolizing enzymes.

Table 3. IC_{50} values of α-amylase and α-glucosidase inhibitory activities of *J. tanjorensis* aqueous extracts and its synthesized AgNPs

SAMPLES	α- Amylase IC_{50} (mg/ mL)	α- Glucosidase IC_{50} (mg/ mL)
Fresh JTLE	76.54±1.31[a]	84.59±0.22[a]
JTL-AgNPs	29.96±0.56[b]	37.60±0.19[b]
Fresh JTSE	95.82±0.09[c]	98.50±0.55[c]
JTS-AgNPs	46.77±0.66[d]	66.59±0.12[d]

Values are Mean±SEM of triplicate determinations. Values with different alphabets in each column are statistically significant from each other at $p< 0.05$.

Figure 6 compares the antiglycation activity of the synthesized AgNPs with each aqueous extracts of *J. tanjorensis*. Here, the synthesized AgNPs showed a better antiglycation activity compared to the fresh aqueous extracts, however, the percentage inhibition of fructosamine using JTS-AgNPs was more potent than JTL-AgNPs as shown below

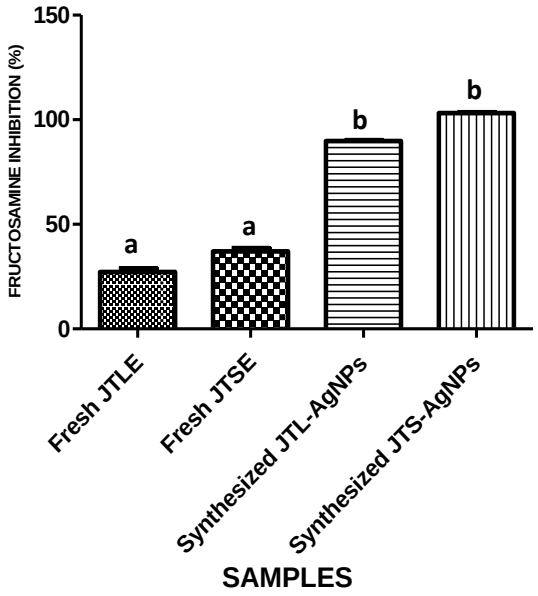


Figure 6. Antiglycation activity of the extracts of *J. tanjorensis* and their synthesized AgNPs. Bars with different alphabets are statistically significant from each other at p<0.0001.

Table 4 shows the antioxidant activities of the synthesized AgNPs and the fresh leaf and stem extracts of *Jatropha tanjorensis*. The synthesized AgNPs using the leaf extract (JTL-AgNPs) of the plant showed a better antioxidant activity in all the assayed antioxidant parameters (DPPH, Nitric oxide, Total antioxidant and Reducing power). The IC_{50} values in mg/ mL of DPPH and nitric oxide scavenging activities were calculated while, the total antioxidant capacity (mg/ 100g) and reducing power in µL/ mL were also calculated and reported as presented below.

Table 4. Antioxidant activity of *J. tanjorensis* aqueous extracts and its synthesized AgNPs

SAMPLES	DPPH. IC_{50} (mg/ mL)	NO. IC_{50} (mg/ mL)	TAC (mg/100g)	Reducing power (µL/ mL)
Fresh JTLE	28.94±1.43^{a}	34.12±0.01^{a}	4.24±0.02^{a}	0.295±0.002^{a}
JTL-AgNPs	23.26±0.20^{b}	25.98±0.68^{b}	7.01±0.00^{b}	0.687±0.012^{b}
Fresh JTSE	34.49±0.87^{c}	36.83±0.25^{a}	3.90±0.03^{a}	0.241±0.016^{a}
JTS-AgNPs	26.81±0.46^{b}	29.55±0.17^{b}	5.35±0.02^{a}	0.353±0.038^{a}

Values are Mean±SEM of triplicate determinations. Values with different alphabets in each column are statistically significant from each other at $p< 0.05$.

Figure 7 shows the anti-inflammatory activity of the synthesized AgNPs and the fresh extracts of *J. tanjorensis*. The synthesized AgNPs using *J. tanjorensis* leaf showed a better inhibitory albumin denaturation compared to JTS-AgNPs, however, the inhibitory percentage of albumin denaturation of JTL-AgNPs was lower than the standard, aspirin. On the other hand, the percentage inhibition of trypsin with JTL-AgNPs was comparable to the standard, aspirin graphically.

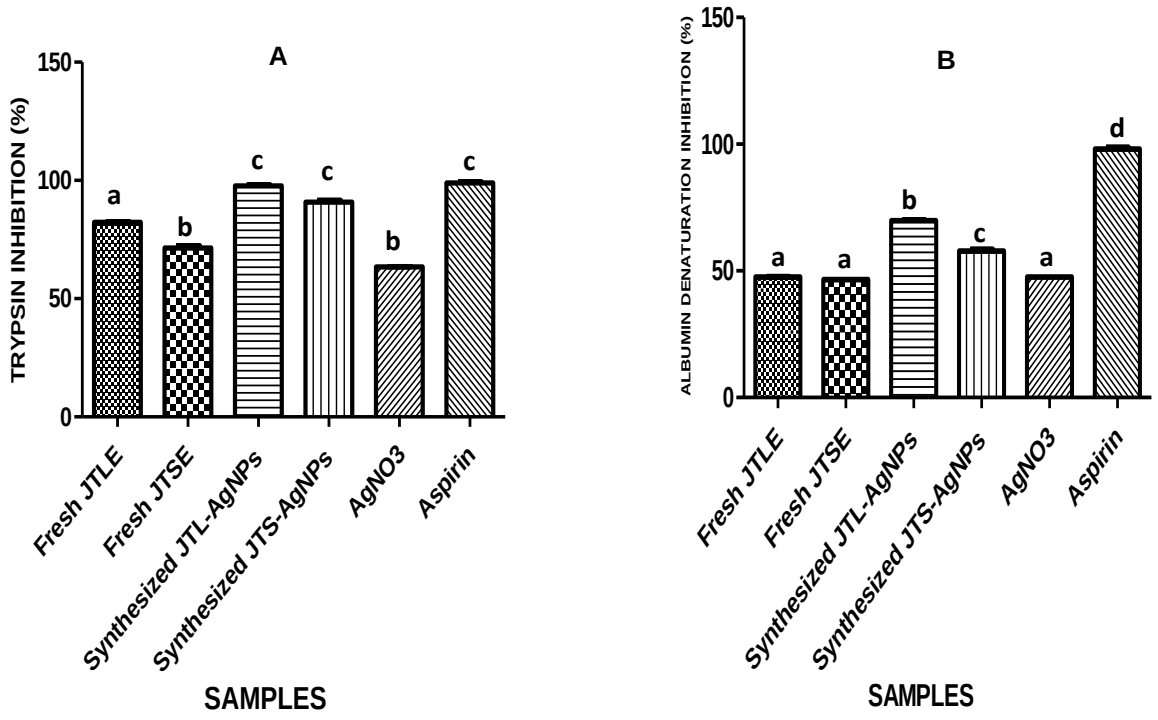


Figure 7. Anti-inflammatory activity of the extracts of *J. tanjorensis* and their synthesized AgNPs using percentage (a) trypsin inhibition (b) albumin denaturation inhibition. Bars with different alphabets are statistically significant from each other at $p<0.0001$.

4. DISCUSSION

The paucity of comprehensive information on the pharmacological properties of various indigenous vegetables in Nigeria has consequently led to the uncovering of many plants' biological potentials. Furthermore, the presence of these plants' bioactive secondary metabolites with enzyme-inhibiting activity lends some support for the traditional use of many medicinal plants in the management and treatment of diverse diseases including diabetes and its related complications [16].

The synthesis of nanoparticles using medicinal plant extracts is commonly called 'green synthesis method', and is usually designed for enhanced specificity to target cells within specific organs, thus improving the precision of drug delivery [4]. While green synthesis offers several advantages for organ-specific drug delivery, it is essential to consider the specific requirements of the application, the characteristics of the target organ, and the desired properties of the nanoparticles.

The biological method of synthesizing nanoparticles by green route is a valued alternative to chemical and physical methods which are highly expensive [17]. This method is preferred because it is non-toxic, cheap, eco-friendly and suitable for pharmaceutical and biomedical applications [4, 18]. As a result, various medicinal plants have been used for the biosynthesis of silver nanoparticles, however, there is a continuous search for better reducing natural product(s).

In this study, the addition of silver nitrate solution to *Jatropha tanjorensis* leaf and stem extracts respectively, gave a visible color change from yellow (leaf and stem) to light grey (Figure 1). The color change observed is in agreement with the findings of [19]. The color change may infer that there is a reduction of silver ions to silver nanoparticles (AgNPs). To further confirm the synthesis of AgNPs, the absorbance peak of the synthesized AgNPs were observed at 410 nm and 412 nm for the AgNPs synthesized using *J. tanjorensis*

leaf and stem extracts, respectively. These values are within the range reported by Mahiuddin *et a*l. [20] and Vishwanath and Negi, [21] for the synthesis of AgNPs.

The quantitative phytochemical analysis of the plant's leaf extract showed no traces of flavonoid, cardiac glycoside and reducing sugar content, but when the extract was used to synthesize AgNPs, it showed traces of these phytochemicals. It may be inferred that the plant extract contains other compounds that, during the synthesis process, interacted with the silver ions or nanoparticles to form complexes that mimic the properties of these phytochemicals. This interaction could lead to a false positive result for the presence of flavonoids, cardiac glycoside, and reducing sugar, respectively. Additionally, some compounds in the plant extract may also have undergone chemical transformations during the synthesis process, leading to the formation of compounds with similar characteristics of flavonoid, cardiac glycoside, and reducing sugar.

As a result of the different functional groups present in the plant leaf and stem extracts (Figure 3) from the FTIR result, it may be inferred that these organic compounds are responsible for capping and efficient stabilization of the synthesized nanoparticles coating the extracts. Moreso, the morphological analysis and the average size distribution of the synthesized silver nanoparticles (AgNPs) gave a better insight into the pseudo-spherical shape and sizes of these AgNPs (48.33nm and 42.66nm for JTS-AgNPs and JTL-AgNPs, respectively as shown in Figure 4 and 5).

The rate of diabetes mellitus prevalence is rapidly increasing globally vis-à-vis-Nigeria, and the public programs to improve diet and nutrition have largely failed to reduce the problem. Moreso, gastrointestinal adverse effects, including flatulence, diarrhea, and discomfort in the abdomen, have been reported when taking synthetic hypoglycemic medications including acarbose, miglitol, and voglibose in conjunction with other antidiabetic medications for the treatment of diabetes [22]. Conversely, medicinal plants and herbal formulations are increasingly considered as preferable alternatives to synthetic drugs due to their unique attributes, including fewer or no side effects, affordability, eco-friendliness, safety, and potential for a lasting cure [23]. The global ethnobotanical record indicates the use of 800 different medicinal plants for diabetes prevention, with 450 of them clinically proven to possess antidiabetic potential, and 109 having a well-established mode of action [24].

Many of these natural compounds have been used to synthesize nanoparticles for better drug delivery into target cells and organs for the management and treatment of various diseases including diabetes and its complications. In this study, the antidiabetic activity of the synthesized silver nanoparticles was evaluated using α-amylase and α-glucosidase inhibitory assay. The synthesized AgNPs showed a significant 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 these carbohydrate metabolizing enzymes when the respective IC_{50} values were compared in Table 3. This may infer that the leaf extract contains other phytochemical compounds that, during the synthesis process, interacted with the silver ions or nanoparticles to form complexes that improved the α-amylase and α-glucosidase inhibitory activities of the leaf extract.

Direct reactive oxygen species (ROS) attack results in the production of protein derivatives like carbonyls and charged amino acids. In hyperglycemic condition, albumin, a water-soluble globular protein that makes up 50% of the total plasma proteins is usually exposed to glycation process leading to the formation of AGEs, which is a marker for almost all diabetic complications onset. Decreases in cationic charges in AGEs during glycation processes have been associated with the condensation of basic amino acid residues and carbohydrates. Due to AGEs formation, there is a search worldwide for an ecofriendly, effective and non-toxic agents in the form of medicinal plants with an ability to inhibit glycation and treat diabetes-related conditions, hence in this study, the synthesized AgNPs using the leaf and stem extracts of *J. tanjorensis* displayed a good antiglycation potential than the fresh extracts. Moreso, the JTS-AgNPs showed a better antiglycation potential (Figure 6), and this maybe as a result of its embedded phytochemical constituents, inhibiting the formation of further advanced glycation end products (AGEs) [4, 25].

Antioxidant strategies have been thoroughly investigated, and their ability to fend off damage from free radicals have also been validated. Chronic hyperglycaemia has been demonstrated to cause oxidative

stress by the oxidation of excess glucose and the production of reactive oxygen species (ROS). Three to five antioxidant assays are necessary and a good way to ascertain the free radical scavenging ability of any sample. In this study, the antioxidant activity of both the extracts and the synthesized AgNPs were investigated using four different antioxidant assays including reducing power, total antioxidant capacity (TAC), DPPH, and NO scavenging activities. The findings of this study showed that while both the extracts and the AgNPs were capable of eliminating free radicals, JTL-AgNPs showed a superior scavenging free radical potential. Thus, it appears that the contained phytochemical concentrations were responsible for the high antioxidant property observed in JTL-AgNPs. This may infer that JTL-AgNPs may be explored therapeutically against ROS associated diseases including diabetes mellitus and its complications.

Non-steroidal anti-inflammatory drugs (NSAIDs) such as, aspirin act to prevent protein denaturation [26]. Moreso, in addition to oxidative stress associated AGEs formation in diabetic patients, inflammatory reactions are another related condition that have been implicated in the development of various diabetes related complications. Here in this study, the anti-inflammatory potentials of the synthesized silver nanoparticles and fresh extracts were investigated and our analysis showed that, JTL-AgNPs have a better and a promising potential of preventing protein denaturation (Figure 7). This may be traceable to the anti-inflammatory properties of some of the embedded phytoconstituents present in the plant extract. Additionally, this could also possibly mean that the AgNPs are able to play important anti-inflammatory role in the prevention, management, and treatment of diabetes and its complications.

5. CONCLUSION

The findings of this study validate the health benefits and traditional use of the leaf and stem aqueous extracts of *Jatropha tanjorensis* in managing and treating diabetes mellitus and its related free radical causing complications. Moreso, the synthesized AgNPs using the aqueous leaf extracts of the plant showed potent antidiabetic, antioxidant and anti-inflammatory properties, however the JTS-AgNPs showed a better antiglycation potential.

Conflicts of Interest

The authors declare no conflict of interest.

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