ORIGINAL RESEARCH ARTICLE

Prospects of silver nanoparticles (AgNPs) synthesized by *Justicia secunda* aqueous extracts on diabetes and its related complications

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Abstract
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 is rapidly generating great research interest. Herein, the *in-vitro* study of the antidiabetic, anti-inflammatory, antiglycation, and antioxidant potentials of silver nanoparticles (AgNPs) synthesized by *Justicia secunda* aqueous extracts was evaluated. The synthesized AgNPs was characterized by UV-visible spectrophotometer, Fourier transform infrared spectroscopy (FT-IR) and Scanning electron microscopy (SEM). The antioxidant activity was evaluated via reducing power, 2,2-diphenyl-1-picrylhydrazyl (DPPH), total antioxidant capacity (TAC) and nitric oxide scavenging assays; antidiabetic activity was evaluated with α-glucosidase and α-amylase inhibitory assays; antiglycation activity was evaluated using fructosamine inhibitory assay, and anti-inflammatory activity was performed using trypsin inhibitory and albumin denaturation assays. The AgNPs from all the plant parts showed good biological potentials investigated compared to the plant extracts however, the synthesized AgNPs using the leaf aqueous extract (AgNPs-JsL) showed a better antioxidant, antidiabetic, antiglycation, and anti-inflammatory potentials with an essential quality absorption band in the ultraviolet region of 410 nm and average size distribution of 50.47nm. In conclusion, all the parts of *J. secunda* showed substantial antidiabetic, antioxidant, antiglycation and anti-inflammatory potentials with high yield of various bioactive metabolites, however, the AgNPs-JsL was more potent followed by the flower, stem and roots extracts, respectively.

Keywords: *Justicia secunda; Silver nanoparticles; Phytochemistry; Diabetes and its complications; Antioxidant; Advanced glycated end-products (AGEs); Hyperglycemia*.

Introduction

High blood sugar, commonly referred to as diabetes, is a well-known public health disease worldwide. Its complications have been associated with several deleterious effects on humans' health. It is the leading concern of the twenty-first century because of its prevalence and wide range of related complications [1]. The global prevalence of this

disorder has reached an alarming proportion of 463 million and has been projected to increase to 578 million and 700 million in the year 2030 and 2045, respectively [2]. International Diabetes Federation (IDF) in 2019 estimated diabetics in Africa and Nigeria to be 19 million and over 2 million, respectively [3]. Diabetes causes yearly detrimental effect on both individuals, societies, countries and the world at large, and it accounts for over 4 million deaths yearly [2]. The danger of developing complications associated with diabetes has been allied to a fundamental marker called advanced glycation end products (AGEs), which are usually accelerated during hyperglycemia (high blood sugar) [4]. AGEs in diabetics result in oxidative stress [5] and inflammatory reactions [6] that are inculpated in the growth and advancement of other related diabetes complications [7]. Oxidative stress occupies the foremost role in the pathophysiology of diabetes which usually results from an imbalance between free radicals such as reactive oxygen species (ROS), and free radical scavengers (antioxidants). These ROS stimulate several pathways such as, glucosamine pathway, polyol pathway, electron transport system, protein kinase-C (PKC) which plays important roles in the growth and advancement of diabetes, and may also lead to lipid peroxidation, protein glycation, and pancreatic β-cell dysfunction, etc [8]. The possibility of treating diabetes using oral hypoglycemic agents with no side effects remain an unanswered question by medical practitioners, since various adverse effects have been associated with synthetic drugs. On the other hand, medicinal plants and herbal formulations are used as better alternative to synthetic drugs majorly for their special attributes such as, less or no side effects, cheap, ecofriendly, safe and permanent cure. The world ethnobotanical has reported 800 different medicinal plants used for diabetes prevention, 450 of which have been clinically proven to possess antidiabetic potential, and 109 with a complete mode of action [9]. Several antidiabetic plants such as, *Azadirachta indica, Momordica charantia, Allium cepa, Carica papaya, Ocimum sanctum, Magnfera indica, Alium sativum, Musa paradisiaca, Terminalia catappa, Zingiber officinalis*, etc have been reported worldwide [10]. However, in this present study, *Justicia secunda* (flower, leaf, root and stem) aqueous extracts are investigated for its antidiabetic and associated biological activities.

Justicia secunda (blood root), a member of the family of *Acanthaceae*, is a tropical herbaceous plant from South America, but nowadays grown in other tropical and sub-tropical African countries such as, Côte d'Ivoire, Nigeria, and Congo [11]. The plant extracts have demonstrated various biological properties including hematinic, antihypertensive, antisickling, antinociceptive, antioxidant, anti-inflammatory and antimicrobial activities [11]. However, none of these studies covered the characterization, anti-inflammatory, antidiabetic, antiglycation, antioxidant potentials of silver-nanoparticles (AgNPs) synthesized by *Justicia secunda*, aqueous extracts.

In recent years, nanotechnology has been considered a beneficial device in the treatment of many diseases including diabetes, because of their application in medical field and drug delivery systems [12]. Nanotechnology involves nano-sized particles (ranging between 1 - 100nm) synthesis either via physical, chemical, or biological methods. The chemical and physical methods have recently faced various limitations including their harmful effect on living organisms, expensive method of synthesis, tedious nanoparticle synthesis process and hazardous toxic chemical generation [13]. Due to these problems

associated with the chemical and physical methods on human and the environment, bio-nanotechnology, which combines the principles of biotechnology to nanotechnology, to synthesize nanoparticles using biological organisms such as, medicinal plants, bacteria, yeasts, algae and fungi, have gained much attention most recently [14]. The various parts of medicinal plants including the leaves, stems, roots, flowers, etc are known to be rich in primary and various secondary metabolites. The presence of secondary metabolites acts as stabilizing, capping and reducing agents of the synthesized nanoparticles [15], which may justify the reason for silver nanoparticles synthesis by *Justicia secunda* aqueous extract. This field is rapidly generating research interest worldwide because of the use of eco-friendly, simple, easy accessibility and cost-effective noble metals. Another important advantage of plant-mediated nanoparticle synthesis is the complete synthesis within a few minutes and the reaction can take place at room temperature [16]. The use of medicinal plant is the oldest and first form of healing [17] but, the recent emergence of green nanotechnology (medicinal plant-mediated nanotechnology) has provided extensive research by taking advantage of the unique shape, size, electronic, magnetic and optical properties of metal nanoparticles, that allows these nanoparticles serve as a bridge between macromolecules and atomic structures [13]. Inflammation, diabetes and related complications are some of the diseases usually treated with medicinal plants [11, 17]. Silver nanoparticles (AgNPs) amongst other synthesized nanoparticles have received the widest use and exploration due to its various biological activities such as antibacterial, anti-inflammatory activities etc [18, 19].

Materials and Methods

Fresh matured plant parts of *Justicia secunda* (leaf, flower, stem and root) were harvested in January 2022 from Erunwen, Ikorodu, Lagos State, South-western Nigeria (Latitude: 6° 37´ 0.7140″N and 3° 30´ 29.0592″E). The plant was identified and authenticated in the Herbarium of the Department of Botany, Faculty of Science, Lagos state University, and issued voucher number LSH001049. All chemicals and reagents used were of analytical grade.

Preparation of plant extract

Justicia secunda parts (leaf, stem, root and flower) were washed severally with clean water and later with deionized water and were allowed to dry for few minutes on the laboratory table. The cleaned fresh plant parts were blended separately into possible smaller forms with a Scanfrost blender (model No: SFKAB409) and a 10% (w/v) aqueous extract each of the plant parts were prepared and filtered using Whatman filter paper 1 twice. The resulting extracts were then stored in the laboratory refrigerator for further use.

Synthesis of silver nanoparticles

Silver nanoparticles was synthesized by mixing in a 9:1 of 90 mL of silver nitrate solution (1mM $AgNO_3$ in 90 mL of deionized water) and 10mL of each extract. 2mL aliquots of each extract were taken after one hour and two (2) weeks of AgNPs synthesis, and the absorbance analyzed (200-700 nm) with UV-Visible spectrophotometer (model No: Beckman DU640).

Characterization of silver nanoparticles

Synthesized AgNPs were characterized by UV-Visible Spectroscopy to the determine if the AgNPs are within the range of silver nanoparticles synthesis; Scanning Electron Microscopy (SEM) for the determination of the shape and size of AgNPs, and FTIR (Fourier Transform Infra-Red spectroscopy (FTIR) (model: JASCO 4600) for the determination of phytochemical functional constituents present.

Phytochemical Screening

Phytochemical constituents of the extracts of *Justicia secunda* with their respective amounts were identified and measured by the methods described by Sofowora [20] and Ojewunmi *et al*. [21].

Free radical scavenging activities

DPPH scavenging activity

0.2 mL of the extract was mixed with 2 mL of 0.5 mM DPPH solution and left to stand for 30 mins at laboratory temperature before reading absorbance at 517 nm. The percentage inhibition of DPPH radical was then calculated with **% Inhibition of DPPH radical** = ($[A_{br}\ A_{ar}]/A_{br}$) X 100 and the IC_{50} calculated graphically. A_{br} = absorbance before reaction and A_{ar} = absorbance after reaction has taken place.

Nitric oxide scavenging activity

2 mL of sodium nitroprusside (10 mM) was mixed with 0.5 mL phosphate buffer saline (pH 7.4) and 0.5 mL of extract. The resulting solution was incubated at 25˚C for 150 mins. 0.5 mL aliquot of the solution was mixed with 0.5 mL of Griess reagent and re-incubated at 25˚C for another 30 mins and absorbance read at 546 nm. The percentage inhibition was then calculated as **% Inhibition** = ($[A_{br}\ A_{ar}]/A_{br}$) X 100 and the IC_{50} calculated graphically. A_{br} = absorbance before reaction and A_{ar} = absorbance after reaction has taken place.

Reducing power assay

1 mL of each extract was mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of $K_3Fe\ (CN)_6$ (1% w/v). The combined was incubated at 50˚C for 20 mins and 2.5 mL of trichloroacetic acid (10% w/v) added before centrifuging at 3000 rpm for 10 mins. 2.5 mL of supernatant is then mixed with 2.5 mL distilled water and 0.5 mL $FeCl_3$ (0.1%, w/v) before reading absorbance at 700 nm.

Total Antioxidant Capacity (TAC)

The total antioxidant capacity was determined according to the method described by Sulaimon et al. [30]. 25uL aliquot of the extract was mixed with 300ul of TAC reagent solution and incubated for 90 mins before reading absorbance at 630 nm. The total antioxidant capacity was then expressed as mg/g of ascorbic acid.

Antiglycation activity of *Justicia secunda* extracts and synthesized AgNPs

Albumin glycation and Estimation of Fructosamine production

Albumin glycation and measurement of fructosamine produced was performed as described by Safari *et al*. [23]. Albumin glycation was achieved by adding 3.5 mL 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 PBS, pH 7.4). The above mixture was then filtered and incubated at 37 °C for 72 hours.

To evaluate fructosamine production, 1 mL Trichloroacetic acid (20%) was mixed with 1 mL glycated samples. The resulting precipitate was washed thrice at 6000g for 10 mins, solubilized in 1mL PBS before adding 0.5 mL 40% TCA and centrifugation at 6000g for 10 mins. 0.5 mL of the supernatant was then mixed with 0.05 M Thiobarbituric acid (0.5mL) and incubated in water bath at 100°C for 20 mins, cooled at 25°C and absorbance read at 443 nm. Inhibition of fructosamine was calculated by **Inhibitory activity (%)** = $[(A_0 - A_1)/A_0] \times 100$. A_0: absorbance of positive control group, A_1: absorbance in presence of the extract.

Antidiabetic activity

In-vitro a-amylase inhibition

Inhibition of α-amylase assay was achieved by incorporating the method of Awote *et al*. [17]. In brief, 250 µL of each extract was mixed with 250 µL of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase solution before pre-incubating at Laboratory temperature for 10 mins, after which 250 µL of starch solution (1%) in sodium phosphate buffer (0.02 M) pH 6.9 was added at 2 mins intervals and then further incubated at 25°C for 10 mins. 500 µL of dinitrosalicylic acid (DNS) reagent was later added to terminate the reaction followed by incubation in water bath at 100°C for 5 mins and cooling at room temperature. 5 mL distilled water was then added to the above and absorbance read at 540 nm. The same procedure was followed for the control; however, distilled water substituted the extract. Inhibition of α-amylase was calculated by **% Inhibition** = $([A_c - A_s]/A_c) \times 100$ and the IC_{50} determined graphically. Ac = absorbance of control, As = absorbance of sample.

***In-vitro* α-glucosidase inhibition**

Inhibition of α-amylase assay was achieved by incorporating the method of Sulaimon *et al*. [22]. In brief, 20 µL of 10 mM pNPG (substrate) was dissolved in 20 mM PBS (pH 6.9) and incubated at 37 °C for 15 mins before stopping the reaction with 80µL of 0.2M of Na_2CO_3. The same procedure was followed for the control only that phosphate buffer substituted the extract. Acarbose served as the positive control and α-glucosidase activity (**% Inhibition** = $([A_c - A_s]/A_c) \times 100$) was calculated by reading absorbance at 405 nm. Ac = absorbance of control and As = absorbance of sample. IC_{50} value was determined graphically.

Anti-inflammatory activity of *Justicia secunda* extracts and synthesized AgNPs

Inhibition of albumin denaturation

Inhibition of albumin denaturation was achieved by incorporating the method of Govindappa *et al*. [24]. In brief, 0.5mL of BSA (1%) was mixed with 50 µL of extract and incubated at 37°C for 15 minutes, followed by heating at 60°C for 15 mins, and then cooled before reading absorbance at 660 nm. Protein denaturation was calculated as **%Inhibition** = $([A_c - A_s]/A_c) \times 100$. Ac = absorbance of control and As = absorbance of sample.

Protein inhibitory action

Protein inhibitory action was achieved by incorporating the method of Govindappa *et al*. [24]. In brief, 50 µL extract was mixed with 1 mg trypsin and 1 mL Tris HCl buffer (20 mM) pH 7.4 and then incubated at 37°C for 5 mins before adding 1mL casein (0.8%) and subsequently 2 ml perchloric acid (70%) to terminate the reaction. The reaction was centrifuges and the absorbance of the supernatant read at 210 nm against a buffer blank. Protein inhibitory action was calculated as: **% Inhibition** = $([A_c - A_s]/A_c) \times 100$. Ac = absorbance of control and As = absorbance of sample.

Statistical Analysis

Results are presented as mean ± SEM and the data evaluated with GraphPad Prism (version 5) and Microsoft Excel (version 2010). Comparison was done using analysis of variance (ANOVA), a one-way procedure, followed by Tukey Post-hoc and significance value set at $p < 0.05$.

Results

Characterization of Synthesized AgNPs:

UV-Visible spectroscopy

Figure 1 confirmed AgNPs synthesis with a color change across all extracts and the reduction of toxic Ag^+ to non-toxic Ag^0 by phytoconstituents (secondary metabolites) was also observed with an essential quality absorption band in the ultraviolet region (400 – 410 nm) using UV-Visible spectrophotometer at a wavelength ranging between 200 to 700 nm (Figure 2).

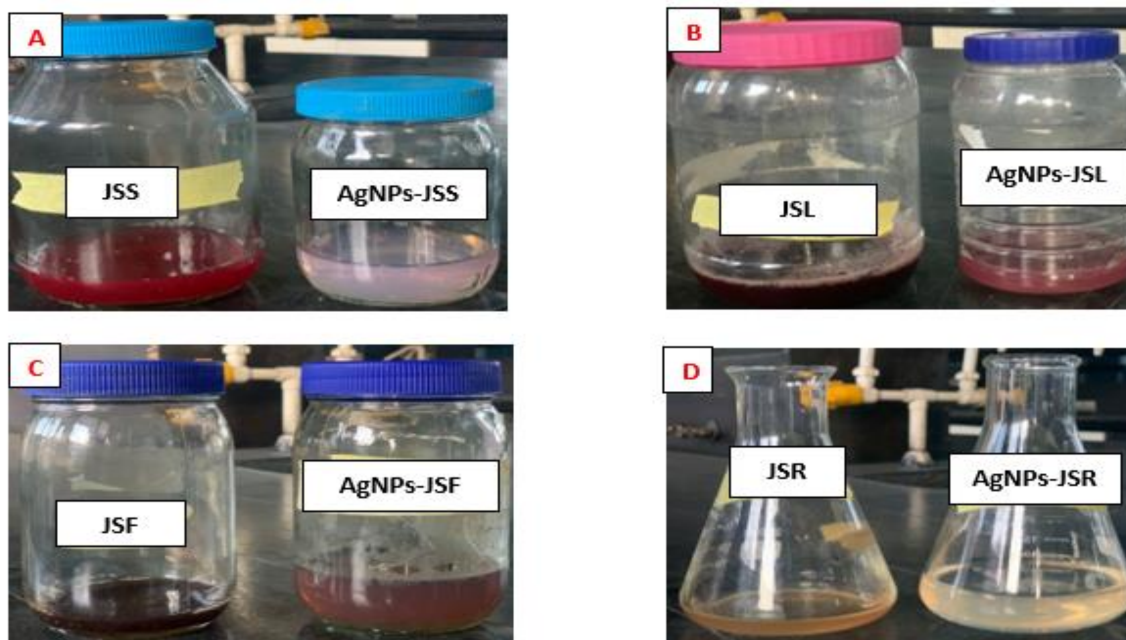


Figure 1: Color change across all the aqueous extracts of *J. secunda* (a) stem (b) leaf (c) flower (d) root.

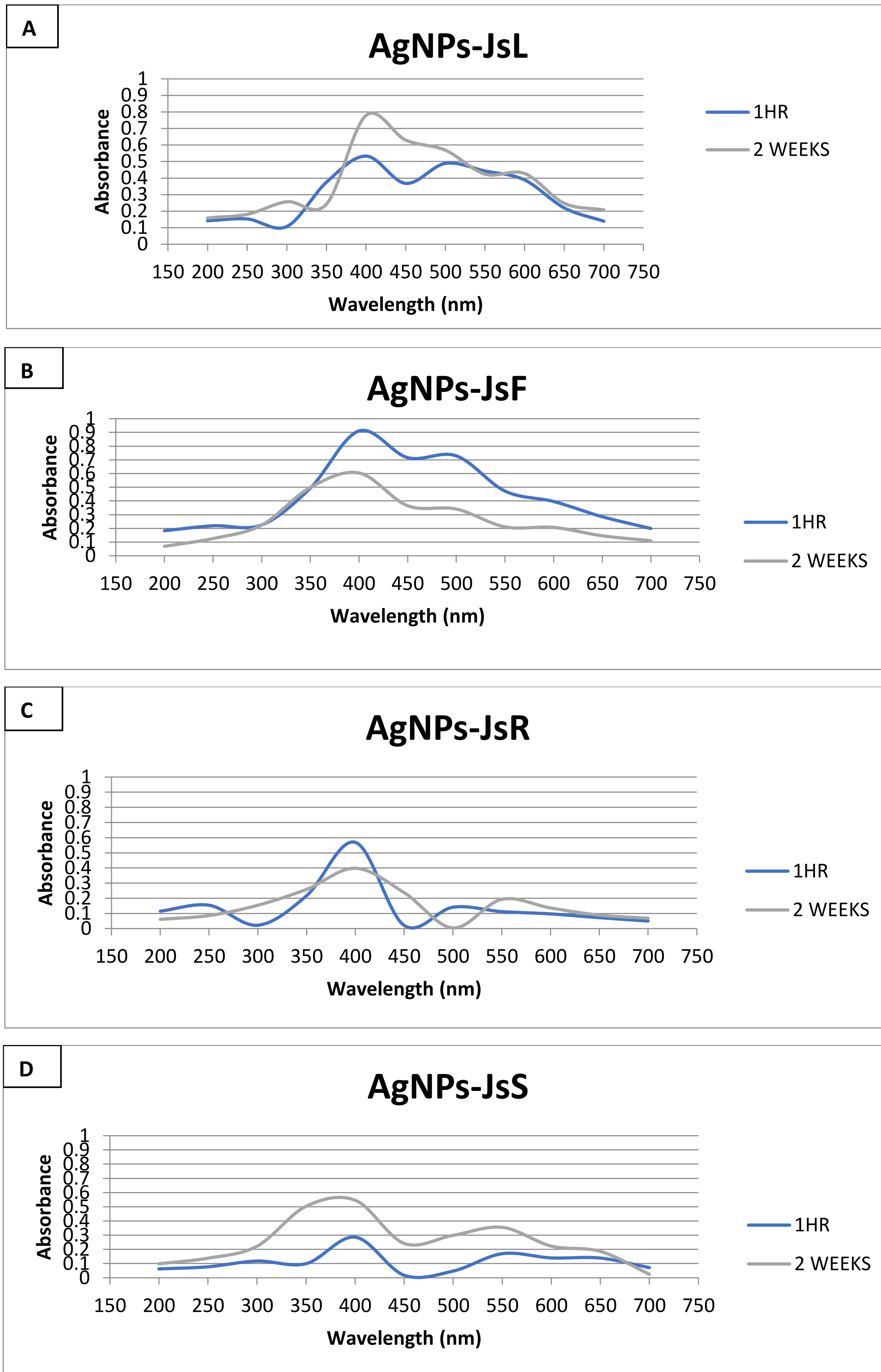
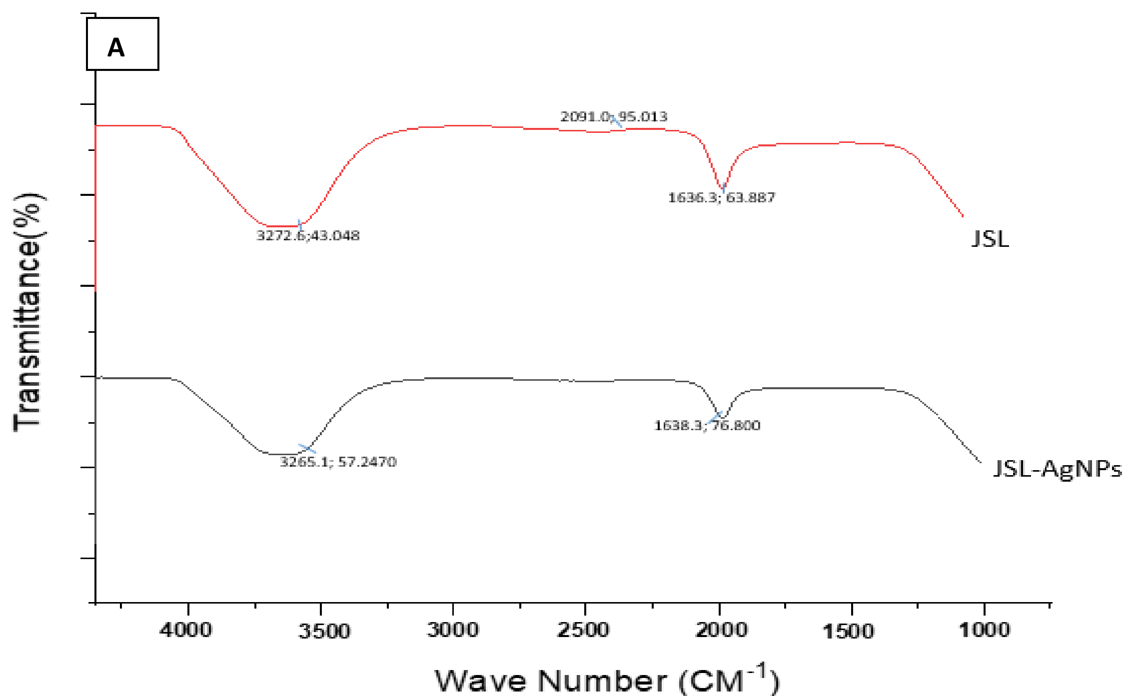
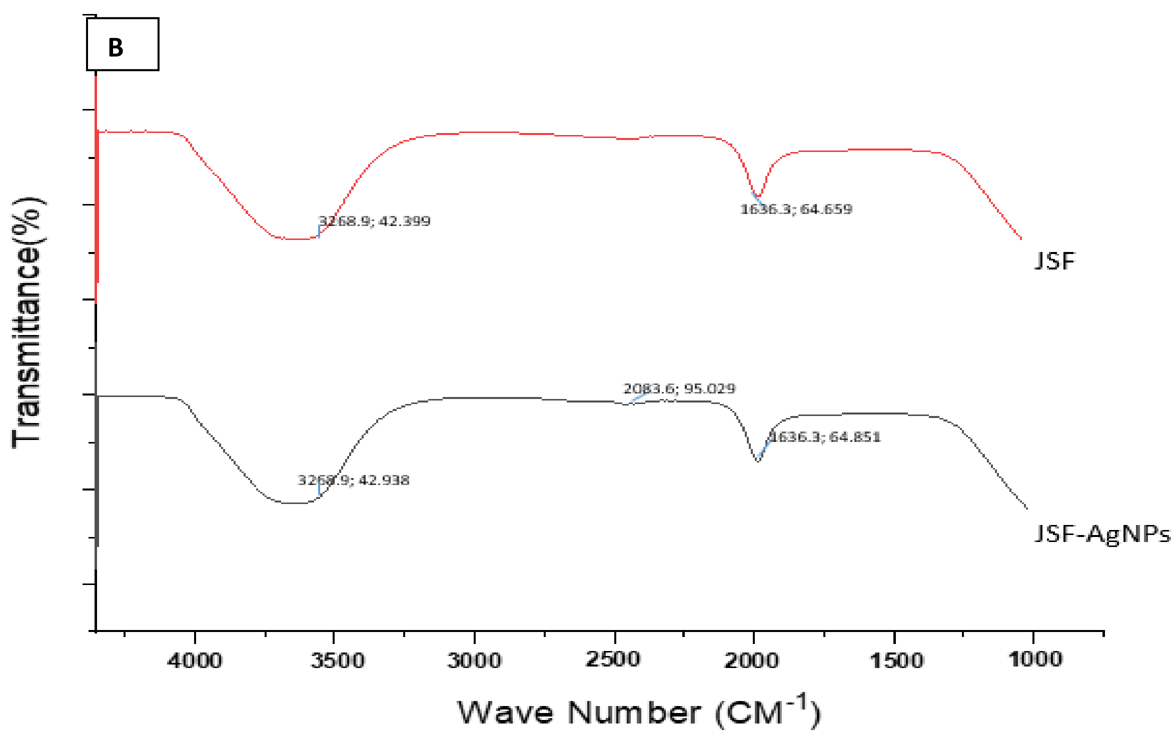


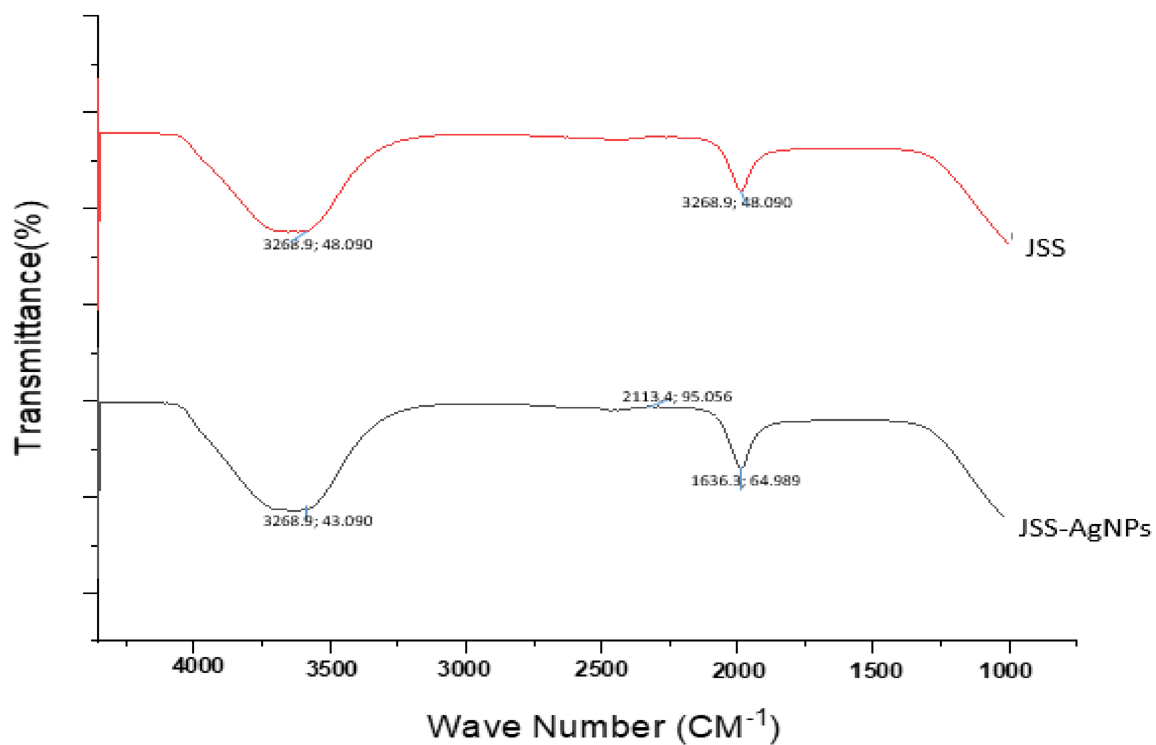
Figure 2: Absorbance readings (200 - 700 nm) for AgNPs using *J. secunda* (a) leaf (b) flower (c) root (d) stem

Fourier transform infrared spectroscopy (FTIR) analysis

Figure 3a-d shows possible functional groups present in the AgNPs. The AgNPs from the flower extract (Figure 3a) of the plant at an absorbance peak of 3268.9 cm^{-1} suggested the presence of alkynes (C-H stretch), phenol (O-H stretch), carboxylic acids and alcohols (O-H stretch); 2083.16 cm^{-1} suggested isothiocyanate group, and 1636.3 cm^{-1} suggested alkenes (C=C stretch) and amines (N-H bend). As illustrated in Figure 3b, a broad absorbance peak of AgNPs from the leaf extract of the plant was observed at 3272.6 cm^{-1} which suggested the presence of carboxylic acid (O-H stretch), alcohols (O-H stretch), phenols (O-H stretch) and alkynes (C-H stretch); 2091.0 cm^{-1} suggested isothiocyanate (N=C=S stretch), and 1635.64 cm^{-1} suggested amines (N-H bend) and alkenes (C=C stretch). Figure 3c shows the absorbance peak of the AgNPs from the root extract at 3281.2 cm^{-1} which suggested the presence of carboxylic acid (O-H stretch), alcohols (O-H stretch), phenols (O-H stretch) and alkynes (C-H stretch); 1636.3 cm^{-1} as alkenes (C=C stretch) and amines (N-H bend). The absorbance peak of AgNPs from the stem extract at 3268.9 cm^{-1} suggested the presence of carboxylic acid (O-H stretch), alcohols (O-H stretch), phenols (O-H stretch) and alkynes (C-H stretch); 2113.4 cm^{-1} suggested alkynes (C-H stretch), isothiocyanate (N=C=S), and 1636.3 cm^{-1} as alkenes (C=C stretch) and amines (N-H bend) (Fig. 3d). This functional group composition may be directly or indirectly responsible for the stabilization of the AgNPs coating the extracts of *J. secunda*.




B
Transmittance(%)
3268.9; 42.399
1636.3; 64.659
JSF
2083.6; 95.029
1636.3; 64.851
3268.9; 42.938
JSF-AgNPs
4000
3500
3000
2500
2000
1500
1000
Wave Number (CM-1)


Transmittance(%)
3268.9; 48.090
3268.9; 48.090
JSS
2113.4; 95.056
1636.3; 64.989
3268.9; 43.090
JSS-AgNPs
4000
3500
3000
2500
2000
1500
1000
Wave Number (CM-1)

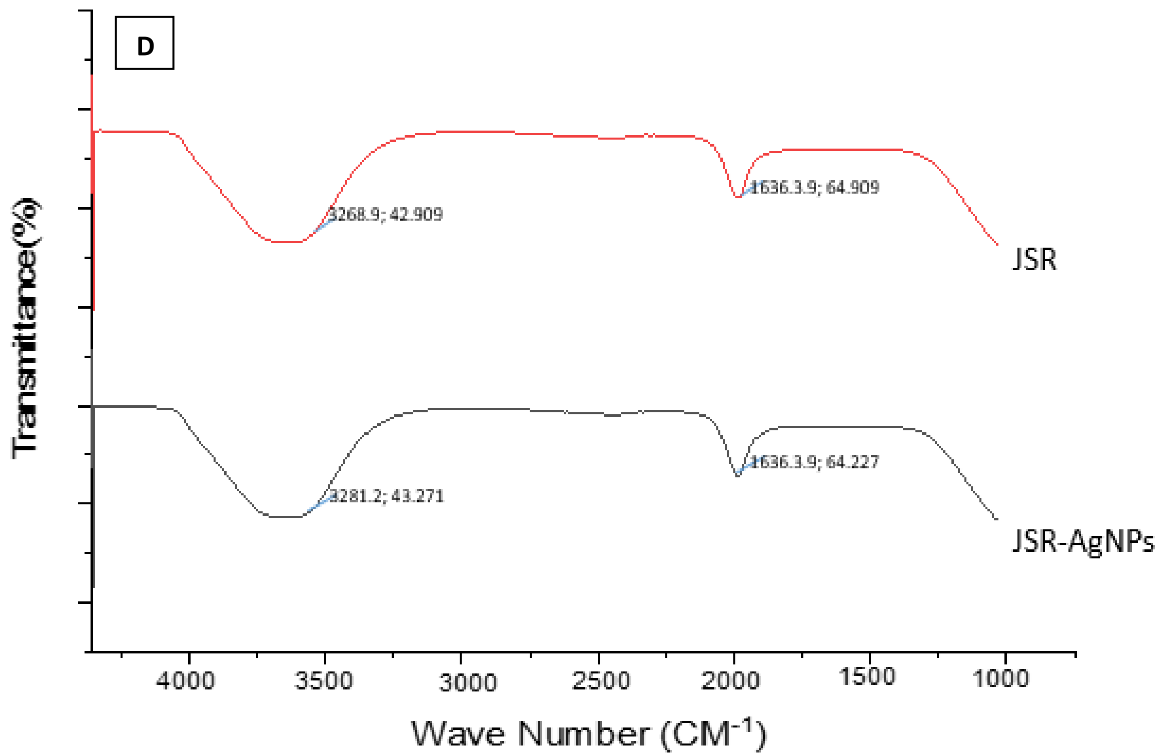


Figure 3: Functional group composition comparison using FTIR analysis (a) leaf (b) flower (c) root (d) stem.

Scanning electron microscope (SEM) analysis

Figure 4 revealed a pseudo-spherical shape for the AgNPs from all the extracts of *J. secunda* with size range of 10-70 nm (Figure 5).

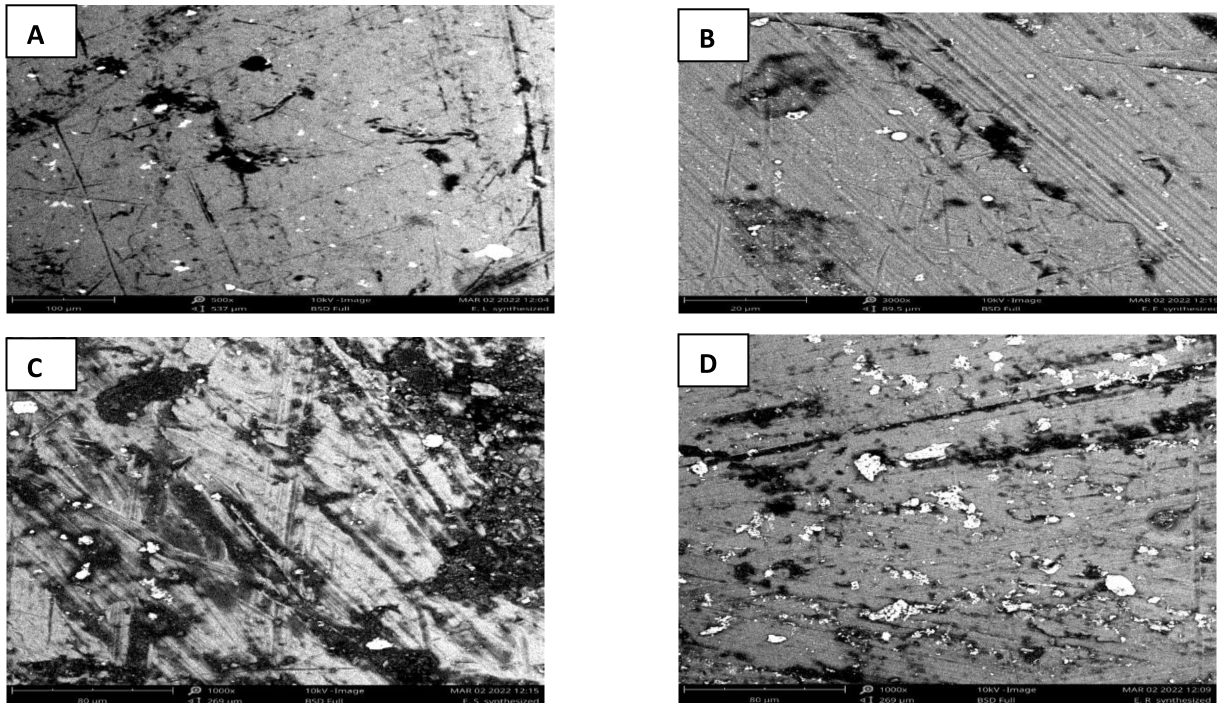


Figure 4: Morphology of AgNPs using SEM analysis (a) leaf (b) flower (c) stem (d) root

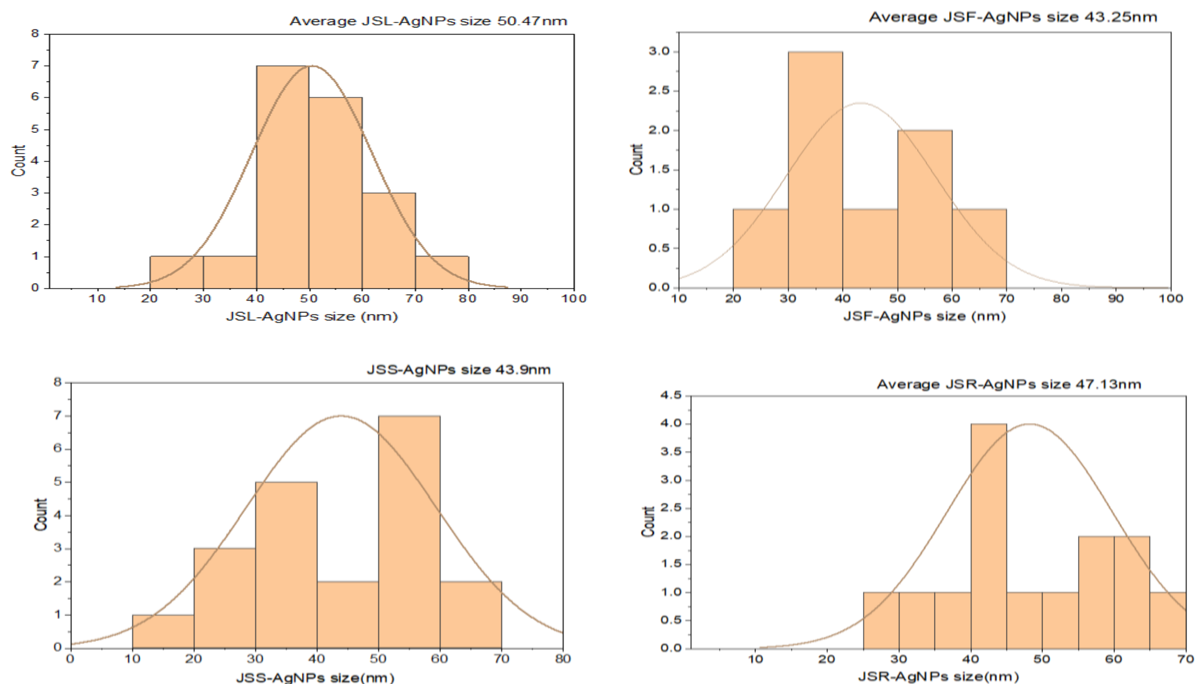


Figure 5: Size distribution of synthesized silver nanoparticles (a) leaf (b) flower (c) stem (d) root.

Table 1 revealed the qualitative phytochemical analysis of *Justicia secunda* aqueous extracts. Secondary metabolites including phenols, tannins, alkaloids, saponins, steroids, and terpenoids were present in all the extracts. Cardiac glycosides, flavonoids and reducing sugars are present in all the extracts except the root extract while, phlobatannins is absent in both leaf and root extracts.

Table 1: Qualitative Phytochemical Analysis of *Justicia secunda* aqueous extracts

PHYTOCHEMICALS	AQUEOUS EXTRACTS			
	LEAF	FLOWER	ROOT	STEM
Phenols	+	+	+	+
Flavonoids	+	+	-	+
Tannins	+	+	+	+
Alkaloids	+	+	+	+
Saponins	+	+	+	+
Reducing sugars	+	+	-	+
Steroids	+	+	+	+
Terpenoids	+	+	+	+
Cardiac glycosides	+	+	-	+
Phlobatannins	-	+	-	+

Key: + = Present and – = Absent

Table 2 shows the quantitative phytochemical analysis (amount) of each secondary metabolites present in the extracts and synthesized AgNPs. The secondary metabolite with highest quantity is tannins, followed by flavonoids, phenols, reducing sugars, alkaloids, saponins and cardiac glycosides.

Table 2: Quantitative Phytochemical Analysis (mg/100g)

Samples	Tannins	Phenols	Saponins	Alkaloids	Reducing sugars	Cardiac glycosides	Flavonoids
Fresh JsF	85.39±0.34	26.31±0.63	6.92±0.03	5.42±0.82	6.45±0.66	2.56±0.04	34.10±0.11
AgNPs-JsF	88.45±0.63	29.15±0.29	6.21±0.06	5.78±0.06	6.94±0.37	2.91±0.17	36.60±0.46
Fresh JsL	108.23±0.22	35.89±0.18	7.62±0.13	7.28±0.21	18.46±0.61	4.70±0.67	39.76±0.21
AgNPs-JsL	109.88±0.89	36.57±0.27	8.07±0.11	7.38±0.10	19.42±0.22	4.93±0.12	41.15±0.35
Fresh JsR	37.01±0.23	12.93±0.52	6.26±0.14	8.24±0.28	0.00	0.00	0.00
AgNPs-JsR	42.24±0.43	13.87±0.39	6.36±0.16	9.74±0.24	0.00	0.00	0.00
Fresh JsS	72.15±0.56	23.93±0.19	5.76±0.25	8.47±0.12	11.52±0.21	1.97±0.02	24.60±0.44
AgNPs-JsS	73.81±0.34	24.09±0.33	5.91±0.36	8.99±0.17	12.76±0.15	2.32±0.04	31.25±0.29

Values presented in Mean±SEM are of duplicate determinations

Mitochondrial oxidative phosphorylation occurs during oxygen metabolism to produce unstable molecules known as free radicals. These products such as reactive oxygen species (ROS) react with other molecules and become stable, thereby destroying cellular components. When these free radicals are confined by cellular components, they lead to various disease such as, cardiovascular disease, diabetes and its complications. The free radical scavenging potentials of synthesized nanoparticles depends on various phytochemical properties on its surface. DPPH free radical scavenging method is one of the most adequate *in vitro* antioxidant assays used because of its fast and simple method of calculation of antioxidant capacity. Table 3 revealed the antioxidant potentials of the synthesized silver nanoparticles with the leaf aqueous extract showing the best antioxidant property (for DPPH, NO, TAC and reducing power).

Table 3: Free radical scavenging potentials

SAMPLES	DPPH-IC_{50} values (μg/mL)	NO-IC_{50} values (μg/mL)	TAC (mg/100g)	Reducing power (μg/mL)
Fresh JsF	34.55±0.71	20.28±0.59	10.59±0.13	0.062±0.003
AgNPs-JsF	20.29±0.19*	18.48±0.94*	11.05±0.00	0.059±0.002
Fresh JsL	21.67±0.75	15.35±0.89	11.93±0.00	0.076±0.003
AgNPs-JsL	14.92±0.63*	12.81±0.89*	12.45±0.20*	0.094±0.003*
Fresh JsR	59.47±0.76	26.10±0.10	9.04±0.87	0.047±0.003
AgNPs-JsR	44.51±0.36*	23.07±0.51*	9.87±0.01*	0.049±0.001
Fresh JsS	60.60±0.63	22.36±0.11	9.69±0.46	0.044±0.004
AgNPs-JsS	54.68±0.56*	21.07±0.28*	10.05±0.02	0.047±0.003

Values presented in Mean±SEM are of duplicate determinations. Values carrying asterisk (*) are significantly different to the respective fresh extracts ($p < 0.05$).

Inhibitory potentials of the synthesized AgNPs and their respective extract source were evaluated on the activities of α- amylase and α- glucosidase (Table 4). The synthesized AgNPs using the leaf extract showed the best inhibitory potential against α- amylase (IC_{50} value of 7.91±0.68) and α- glucosidase (IC_{50} value of 9.24±0.65) activities.

Table 4: Antidiabetic potentials

SAMPLES	α-Amylase IC_{50} values (μg/mL)	α-Glucosidase IC_{50} values (μg/mL)
Fresh JsF	11.37±0.05	23.77±0.56
AgNPs-JsF	10.97±0.28	21.22±0.19*
Fresh JsL	9.84±0.21	11.98±0.64
AgNPs-JsL	7.91±0.68*	9.24±0.65*
Fresh JsR	26.34±0.37	37.78±0.20
AgNPs-JsR	23.59±0.66*	29.43±0.37*
Fresh JsS	17.44±0.84	26.30±0.09
AgNPs-JsS	14.36±0.56*	24.83±0.46*

Values presented in Mean±SEM are of duplicate determinations. Values carrying asterisk (*) are significantly different to the respective fresh extracts ($p < 0.05$).

Table 5 illustrates the anti-inflammatory potentials of the synthesized AgNPs. The result below showed that the synthesized AgNPs using the leaf extract have a better inhibitory potential against inflammatory reactions.

Table 5: Trypsin and Albumin denaturation inhibition

SAMPLES	Trypsin inhibition (%)	Albumin denaturation inhibition (%)
Fresh JsF	36.78±1.46	35.05±1.55
AgNPs-JsF	42.56±1.09*	44.73±1.43*
Fresh JsL	48.16±1.68	38.95±0.96
AgNPs-JsL	56.55±2.05*	58.79±1.09*
Fresh JsR	12.83±0.96	16.67±0.97
AgNPs-JsR	22.88±0.98*	18.56±0.14
Fresh JsS	24.32±0.44	17.15±1.05
AgNPs-JsS	37.61±1.23*	42.15±1.05*

Values presented in Mean±SEM are of duplicate determinations. Values carrying asterisk (*) are significantly different to the respective fresh extracts ($p < 0.05$).

Table 6 below showed that the synthesized AgNPs using *Justicia secunda* leaf extract have the best inhibitory potential against advanced glycated end products.

Table 6: Antiglycation activity

SAMPLES	Fructosamine inhibition (%)
Fresh JsF	15.17±2.05
AgNPs-JsF	52.30±2.05*
Fresh JsL	33.35±1.35
AgNPs-JsL	63.30±1.66*
Fresh JsR	18.11±1.01
AgNPs-JsR	34.18±1.48*
Fresh JsS	12.17±1.07
AgNPs-JsS	43.80±1.60*

Values presented in Mean±SEM are of duplicate determinations. Values carrying asterisk (*) are significantly different to the respective fresh extracts ($p < 0.05$).

Discussion

Several physicochemical conditions such as, size, shape and morphology of synthesized nanoparticles are important for their characterization [25]. While silver as a metal, have a resonance of strong surface plasmon that make it very important in nanoparticles synthesis [26], the emergence of bio-nanotechnology has however increased the synthesis and use of AgNPs in medical field and related sciences. In the present study, AgNPs was synthesized via green synthesis, and a color change was observed within few minutes after mixing the plant extract parts (leaf, flower, root and stem) each with $AgNO_3$ solution which signals silver nanoparticles formation (Figure 1). AgNPs are characteristically synthesized at a wavelength interval of 400 – 450 nm. The synthesized AgNPs from the leaf, flower, root and stem aqueous extracts of *J. secunda* showed maximum absorption at 400 - 410 nm from the UV-visible analysis. The AgNPs were synthesized from the solution of silver nitrate ($AgNO_3$) by the reduction of Ag^+ to Ag^0 by the phytoconstituents (secondary metabolites) such as the polyphenol content of *J. secunda*, and further led to the formation of AgNPs aggregates. This may possibly be due to the capping mechanism at the reduced silver ion that is surrounded by phytoconstituents of the plant extracts [27]. The FTIR analysis was performed on both the synthesized AgNPs and plant extracts, to check the capped phytoconstituents (functional groups) that participated in Ag^+ reduction to Ag^0; SEM which reveals the shapes and sizes of nanoparticles synthesized showed a pseudo-spherical shape and average sizes of 43.25nm, 43.9nm, 47.13nm and 50.47nm for the synthesized AgNPs from the flower, stem, root and leaf extracts, respectively.

The bioactive compounds in plants have been associated with prophylactic or therapeutic actions against various metabolic and non-metabolic diseases [28]. Phytochemical analysis of *J. secunda* aqueous extracts revealed the presence of various secondary metabolites such as tannins, alkaloids saponins, terpenoids, steroids, phlobatannins, cardiac glycosides, phenols and flavonoids. The color change observed in the extracts with silver nitrate addition may be ascribed to its embedded phytoconstituents especially the polyphenols with several reported biological activities. Tannins have been reported to improve hematological characteristics. Saponins are reported to have anti-inflammatory, anticancer, hypo-cholesterolemic, hematologic effects [29]. Alkaloids are also reported to possess anti-inflammatory and anticancer activities [30, 31]. The descending order of the number of secondary metabolites in the synthesized AgNPs using *J. secunda* aqueous extracts is tannin, followed by flavonoid, phenol, reducing sugar, alkaloid, saponin and cardiac glycoside. Herein, the synthesis of AgNPs increased the amount of each secondary metabolite detected (Table 2).

The antioxidant assays showed that the synthesized AgNPs with *J. secunda* aqueous extracts can be implored therapeutically against ROS associated diseases including diabetes mellitus and its complications, however, the AgNPs using the leaf extract showed the best antioxidant capacity. Similar result has been reported by Menon et al. [15]. At least three *in-vitro* antioxidant methods have been suggested for the confirmation of antioxidant potential because of likely variation in mechanistic reaction conditions of different protocols [32]. Hence DPPH, TAC, reducing power and NO methods were preferred in this study to determine the AgNPs and extract with the best antioxidant

potentials. The results of the antioxidant potential of the extracts and synthesized AgNPs are reported in Table 3. DPPH and NO are present as IC_{50} values and the lowest IC_{50} value represents a better antioxidant potential and *vice versa* [33]. Similar to the results from DPPH and NO assays, the AgNPs from the leaf aqueous extract exhibited a better antioxidant capacity with TAC and reducing power assays. Hence, the antioxidant activity of AgNPs is varied as Leaf > flower > stem > root in all the four assays.

Considering the traditional use of *Justicia secunda* aqueous leaf extract in the management of blood sugar level and corroborated with scientific evidence of the presence of isolated α-glucosidase inhibiting compounds using HPTLC bioautography, the inhibitory effect of the synthesized AgNPs against α- amylase and α- glucosidase activities may be linked to various bioactive compounds such as the polyphenols (phenol, tannin and flavonoid) sufficiently present in the leaf extract. Both tannins and flavonoids have hydroxyl groups. The hydroxyl group in flavonoid confers free radical scavenging ability and play a vital role in preventing lipid peroxidation while, the hydroxyl group of tannin suppresses the formation of hydroxyl radicals. Also, these secondary metabolites may protect against the advancement of resistance or dysfunction of insulin in diabetes mellitus.

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 [4]. Decreases in cationic charges in AGEs during glycation processes have been associated with the condensation of basic amino acid residues and carbohydrates [34]. Due to the formation of AGEs, there's 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. In this study, similar to our antioxidant results, the synthesized AgNPs using *J. secunda* leaf, flower, stem and root aqueous extracts, showed a good antiglycation activity, however, the synthesized AgNPs-JsL showed a better fructosamine inhibiton percentage. Hence, the antiglycation activity of AgNPs varied as Leaf > flower > stem > root. This result clearly revealed that the synthesized AgNPs using *J. secunda* can reduce protein glycation. Hence, the possibility of the synthesized AgNPs especially with the leaf of this plant, acting both against ROS-mediated oxidative stress and the formation of AGEs could make the plant effective in preventing diabetes complications onsets [7].

In addition to oxidative stress associated AGEs formation in diabetic patients, inflammatory reactions are another related condition connected to the development of various diabetes related complications [8]. Herein, the AgNPs showed a potent anti-inflammatory activity with the AgNPs-JsL showing the highest trypsin inhibition and albumin denaturation inhibition percentage, followed by AgNPs-JsF, AgNPs-JsS and AgNPs-JsR. Hence, the anti-inflammatory activity of the synthesized AgNPs is varied as Leaf > flower > stem > root. The low trypsin and albumin denaturation inhibition percentage in AgNPs-JsR may be due to the presence of small amount of phenol and lack of flavonoid contents evaluated from the quantitative analysis of the plant root extract because, phenol and flavonoid compounds from medicinal plants have been reported to have anti-inflammatory and antioxidant properties [35].

Conclusion

In conclusion, the aqueous extracts of *Justicia secunda* leaf, flower, stem and root are capable of producing AgNPs and are quite stable in solution. The synthesized AgNPs using *Justicia secunda* aqueous extracts revealed substantial antidiabetic, antioxidant, antiglycation and anti-inflammatory potentials with high yield of various bioactive metabolites which explains the plant's use as one of the traditionally important plant species in the management of diabetes mellitus. Moreso, it is noteworthy that the AgNPs-JsL showed a better potential across all the biological activities investigated in this study.

Conflict of Interest

The authors of this research hereby declare no conflict of interest in this work.

References

1. AlAmri OD, Albeltagy RS, Akabawy AM, Mahgoub S, Abdel-Mohsen DM, Moneim AEA, & Amin HK (2020). Investigation of antioxidant and anti-inflammatory activities as well as the renal protective potential of green coffee extract in high fat-diet/streptozotocin-induced diabetes in male albino rats. *Journal of functional foods*. 71:103996.

2. Ifedibalu Chukwu EI, Aparoop D, & Kamaruz Z (2020). Antidiabetic, anthelmintic and antioxidation properties of novel and new phytocompounds isolated from the methanolic stem-bark of *Vernonia amygdalina* Delile (Asteraceae). Scientific African. 10:e00578.

3. Oguntibeju OO (2019). Hypoglycaemic and anti-diabetic activity of selected African medicinal plants. *International journal of physiology, pathophysiology and pharmacology*. 11(6):224.

4. Singh VP, Bali A, Singh N, & Jaggi AS (2014). Advanced glycation end products and diabetic complications. *The Korean Journal Physiology and Pharmacology*. 18(1):1-14.

5. Sifuentes-Franco S, Padilla-Tejeda DE, Carrillo-Ibarra S, & Miranda-Díaz AG (2018). Oxidative stress, apoptosis, and mitochondrial function in diabetic nephropathy. *International Journal of endocrinology*. Article ID 1875870.

6. Ray A, Huisman M, Tamsma J, Van Asten J, Bingen B, Broeders E, Hoogeveen E, Van Hout F, Kwee V, & Laman B (2009). The role of inflammation on atherosclerosis, intermediate and clinical cardiovascular endpoints in type 2 diabetes mellitus. *European Journal of Internal Medicine*. 20(3):253-60.

7. Vasarri M, Barletta E, Ramazzotti M, & Degl'Innocenti D (2020). In vitro anti-glycation activity of the marine plant Posidonia oceanica (L.) Delile. *Journal of Ethnopharmacology*. 259:112960.

8. Sharma V, Gupta M, Chauhan DN, Chauhan NS, Goyal RK, & Shah K (2019). Novel Drug Delivery Systems for Phytoconstituents: Current and Future Prospects. *Novel Drug Delivery Systems for Phytoconstituents*. CRC Press, 3-7.

9. Prabhakar PK & Doble M (2011). Mechanism of action of natural products used in the treatment of diabetes mellitus. *Chinese Journal of Integrative Medicine*. 17(8):563-74.

10. Verma S, Gupta M, Popli H, & Aggarwal G (2018). Diabetes mellitus treatment using herbal drugs. *International Journal of Phytomedicine*. 10(1):1-10.

11. Onoja SO, Ezeja MI, Omeh YN, & Onwukwe BC (2017). Antioxidant, anti-inflammatory and antinociceptive activities of methanolic extract of *Justicia secunda* Vahl leaf. *Alexandria Journal of Medicine.* 53(3):207-13.

12. Behravan M, Panahi AH, Naghizadeh A, Ziaee M, Mahdavi R, & Mirzapour A (2019). Facile green synthesis of silver nanoparticles using *Berberis vulgaris* leaf and root aqueous extract and its antibacterial activity. *International Journal of Biological Macromolecules*. 124:148-54.

13. Abdullah JAA, Eddine LS, Abderrhmane B, Alonso-González M, Guerrero A, & Romero A (2020). Green synthesis and characterization of iron oxide nanoparticles by *Pheonix dactylifera* leaf extract and evaluation of their antioxidant activity. *Sustainable Chemistry and Pharmarcy*. 17:100280.

14. Thakur S, Sharma S, Thakur S, & Rai R (2018). Green synthesis of copper nano-particles using Asparagus adscendens roxb. Root and leaf extract and their antimicrobial activities. *International Journal of Current Microbiology and Applied Science*. 7(4):683-94.

15. Menon S, Agarwal H, Kumar SR, & Kumar SV (2017). Green synthesis of silver nanoparticles using medicinal plant Acalypha indica leaf extracts and its application as an antioxidant and antimicrobial agent against foodborne pathogens. *International Journal of Applied Pharmaceutics.* 9(5):42-50.

16. Li X, Xu H, Chen Z-S, & Chen G (2011). Biosynthesis of nanoparticles by microorganisms and their applications. *Journal of Nanomaterials*. 8: 1-16.

17. Awote OK, Adeyemo AG, Igbalaye JO, Awosemo RB, Ibrahim AB, Omolaja BE, Fidausi A, & Fajobi T (2021). In Vitro Alpha-Amylase Inhibitory Activity, Antioxidant Activity and HPLC Analysis of *Eichhornia crassipes* (water hyacinth) Methanol Extracts. *Tropical Journal of Natural Products Research*. 5(12):2174-81.

18. Christensen L, Vivekanandhan S, Misra M, & Kumar Mohanty A. Biosynthesis of silver nanoparticles using *Murraya koenigii* (curry leaf): an investigation on the effect of broth concentration in reduction mechanism and particle size. *Advanced Materials Letters*. 2(6):429-34.

19. Ali ZA, Yahya R, Sekaran SD, & Puteh R (2016). Green synthesis of silver nanoparticles using apple extract and its antibacterial properties. *Advances in Materials Science and Engineering*. Article ID 4102196.

20. Sofowora A (1996). Research on medicinal plants and traditional medicine in Africa. *Journal of Alternative and Complementary Medicines*. 2(3): .365-372.

21. Ojewunmi O, Oshodi T, Ogundele O, Chijioke M, & Adenekan S (2013). Toxicity screening and in vitro antioxidant activities of aqueous extracts of *Morinda lucida* and *Saccharum officinarum* leaves. *Biokemistri.* 25 (2): 72-78.

22. Sulaimon L, Anise E, Obuotor E, Samuel T, Moshood A, Olajide M, & Fatoke T (2020). In vitro antidiabetic potentials, antioxidant activities and phytochemical profile of african black pepper (*Piper guineense*). *Clinical Phytoscience*. 2020;6(1):1-13.

23. Safari MR, Azizi O, Heidary SS, Kheiripour N, & Ravan AP (2018). Antiglycation and antioxidant activity of four Iranian medical plant extracts. *Journal of Pharmacopuncture*. 21(2):82.

24. Govindappa M, Hemashekhar B, Arthikala M-K, Rai VR, & Ramachandra Y (2018). Characterization, antibacterial, antioxidant, antidiabetic, anti-inflammatory and antityrosinase activity of green synthesized silver nanoparticles using *Calophyllum tomentosum* leaves extract. *Results in Physics.* 9:400-408.

25. Jain S & Mehata MS (2017). Medicinal plant leaf extract and pure flavonoid mediated green synthesis of silver nanoparticles and their enhanced antibacterial property. *Scientific Reports*. 7(1):1-13.

26. Vasireddy R, Paul R, & Mitra AK (2012). Green synthesis of silver nanoparticles and the study of optical properties. *Nanomaterials and Nanotechnology*.. 2:2-8.

27. Chokshi K, Pancha I, Ghosh T, Paliwal C, Maurya R, Ghosh A, & Mishra S (2016). Green synthesis, characterization and antioxidant potential of silver nanoparticles biosynthesized from de-oiled biomass of thermotolerant oleaginous microalgae Acutodesmus dimorphus. *RSC Advances*. 6(76):72269-74.

28. Newman DJ & Cragg GM (2020). Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *Journal of Natural Products*. 83(3):770-803.

29. Anyasor GN, Moses N, & Kale O (2020). Hepatoprotective and hematological effects of *Justicia secunda* Vahl leaves on carbon tetrachloride induced toxicity in rats. *Biotechnic and Histochemistry*. 95(5):349-59.

30. Souto AL, Tavares JF, Da Silva MS, Diniz MdFFM, Athayde-Filho D, Filgueiras P, & Barbosa Filho JM (2011). Anti-inflammatory activity of alkaloids: an update from 2000 to 2010. *Molecules*. 16(10):8515-34.

31. Lu JJ, Bao JL, Chen XP, Huang M, & Wang YT (2012). Alkaloids isolated from natural herbs as the anticancer agents. *Evidence-based complementary and alternative medicine*. Article ID 485042.

32. Bolignano D, Cernaro V, Gembillo G, Baggetta R, Buemi M, & D'Arrigo G (2017). Antioxidant agents for delaying diabetic kidney disease progression: A systematic review and meta-analysis. *PloS one*. 12(6):e0178699.

33. Chavan C, Mulik S, Chavan M, Adnaik R, Patil P (2011). Screening of antioxidant activity and phenolic content of whole plant of *Barleria prionitis* Linn. *International Journal of Research in Ayurveda & Pharmarcy*. 2(4): 1313-1319.

34. Rondeau P & Bourdon E (2011). The glycation of albumin: structural and functional impacts. *Biochimie*. 93(4):645-58.

35. Zhang L, Ravipati AS, Koyyalamudi SR, Jeong SC, Reddy N, Smith PT, Bartlett J, Shanmugam K, Mu̎nch G, & Wu MJ (2011). Antioxidant and anti-inflammatory activities of selected medicinal plants containing phenolic and flavonoid compounds. J. Agric Food Chem. 59(23):12361-7.