Biosynthesis, Characterization and Biological Applications of Silver Nanoparticles using Celosia trigyna and Solanum nigrum Extracts: Neglected Vegetables in Nigeria

ABSTRACT
Plant-mediated synthesis is gaining acceptance in many fields i.e. biology and pharmaceutical fields. This aim of this study is synthesizing Ag nanoparticles using air-dried leaves of two (2) neglected vegetables i.e. Celosia trigyna and Solanum nigrum. Ultraviolet—visible spectroscopy, fourier transform infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM) were used to characterize the formation of silver nanoparticles (AgNPs). The anti-inflammatory properties of these AgNPs were evaluated using Cell Stabilization Membrane (CSM) and lipoxidase assays, their antioxidant activity were established on DPPH and ABTS+ assays. The positive control employed are indomethacin and ascorbic acid for these activities. Nanoparticles synthesized were labelled for Celosia trigyna (CT-AgNPs) and Solanum nigrum (SN-AgNPs) were noticed through visual color change. The UV–Vis spectra of the synthesized nanoparticles displayed absorption bands at around 360-440 nm, which is a characteristic band for Ag and FTIR displayed possible functional groups responsible for Ag nanoparticles synthesized by these plants. The SEM image of the AgNPs formed displayed were spherical in morphology. CT-AgNPs exhibited the most significant inhibitory activity against HRBC (IC₅₀: 32.2 µg/ml) while SN-AgNPs displayed the most significant inhibitory activity against lipoygenases (IC₅₀: 32.8 µg/ml) when compared to the positive control used indomethacin (IC₅₀: 28.1 µg/ml). SN-AgNPs exhibited the most significant antioxidant effect against ABTS (IC₅₀: 11.4 µg/ml) while CT-AgNPs displayed the most significant antioxidant activity against DPPH (IC₅₀: 4.6 µg/ml) when compared to the positive control used ascorbic acid (IC₅₀: 4.7 µg/ml). This work showed that the synthesized AgNPs from non-cultivated vegetable can find relevance and application in health, drugs, food and environmental science.

Keywords: non-cultivated vegetables; nanoparticles; AgNPs; anti-inflammatory; antioxidant

INTRODUCTION
Nanoparticles’ synthesis comes with different dimensions and forms, coupled with the gained advantages is what Nanotechnology is. At present, an urgent pressure is evolving to acquire environmentally friendly and green techniques for the manufacture of materials made-up of nanoparticles, these will eventually replace the toxic and harmful chemicals employed in the synthesis procedures hence evade their health dangers. Silver nanoparticles (AgNPs) have been given more attention among the other nanoparticles due to their appealing physical and chemical properties. Most nanomaterial industries use silver for production of over four hundred tonnes of silver nanoparticles each year. Different methods (both chemical and physical) are employed in preparation of silver nanoparticles i.e. biological methods, chemical reduction, chemical vapor deposition, electro-irradiation, hydrothermal method, laser-mediated synthesis, microwave irradiation, microwave, photochemical reduction, reversemicelle method, sol–gel process and ultraviolet (UV) irradiation etc. Biosynthesis of nanoparticles is mostly preferred because it is cost effective, environmental friendly and human friendly. Biosynthesis of nanoparticles using plant extracts involves reduction of metal ions which has been discovered to be much faster compared to microorganisms and its stability is better. Many studies have shown the biosynthesis of AgNPs employing various plants' extracts. Celosia trigyna occurs almost throughout tropical Africa, South Africa and southern Arabia and is regarded as a weed but is used as a leafy vegetable in Benin and southern Nigeria. It is found in forest clearings and grassland, along roadsides and rivers, and as a weed in fields. It grows on a wide range of soils, but prefers fertile well-drained loamy soils. C. trigyna is employed in Africa to manage heart complaints, pustular skin eruptions and tapeworm related diseases. The leaves pulp is used to treat pains i.e. back, chest and costal, it is also used against stomach-ache and urethral disorders. The leaves and flowers are employed against diarrhea. The plant is generally believed to be among the ingredients/preparations used against various women's disorders and diseases, including ovarian troubles and excessive menstruation.
Solanum nigrum as a medicinal plant is use for food and medicine, studies have shown its anti-oxidant, anti-inflammatory and diuretic effects. In China, its chemopreventive ability is held in high esteem and is used to manage cancers related to the digestive system. It was a traditional European medicine for infirmities that needed cooling. It was considered good for cooling hot inflammations, ringworms, ulcers, testicular swellings, gout and ear pains. It was also implored in the treatment of convulsions. The Arabs used its bruised fresh leaves to ease pain and reduce inflammation.

Beside their nutritional benefits, leafy and non-cultivated vegetables have been known to possess therapeutic uses. However, many of these cheap but diseases preventing plant species are yet to be sufficiently studied and exploited. Hence, this study aims at: investigate the phytochemical screening of these non-cultivated vegetables’ leaves extract; experimentally carry out characterization and application of these medicinal plants species silver nanoparticles (AgNPs) as anti-inflammatory, antioxidant agents and acetylcholinesterase inhibitors.

MATERIAL AND METHODS

Collection of the Plants

Celosia trigyna and Solanum nigrum were collected in the month of November around Ilorin metropolis and also from Oshogbo, Osun State. The whole plants (leaf, stem and stalk) were washed with distilled water then dried in open air for two weeks to completely remove the moisture content and to effectively prepare the leaves for the next stage of preparation. After air-drying, the plants (leave stem and stalk) were crushed into powder using pestle and mortar and kept in air tight plastic containers coded.

Preparation of Extract

Extracts were prepared using solvent extraction (N-hexane and Methanol). 50 g of each of the prepared Celosia trigyna and Solanum nigrum were placed in jars and soaked in N-hexane, this lasted 4 days, then the N-hexane was concentrated using a rotary evaporator to give the crude N-hexane extract for each of the plants. The residues were exposed to air and soaked again, using methanol for 7 days after which the methanol extract was concentrated using a rotary evaporator to give crude methanol extracts for both plants.

Phytochemical Screening

Phytochemical screening was done to detect the presence of alkaloids, flavonoids, phenols, saponins, steroids and triterpenes in both plants leaf extracts. Preparation for the test was done by pouring 3 mL of the leaf extracts into separate test tubes and diluting with 2-4 ml deionized water. Standard techniques of screening and detecting secondary metabolites in plants was used.

Synthesis of Silver Nanoparticles

The synthesis of nanoparticles using Silver was carried out following the procedure reported by Bello et al. (2019). In a typical procedure, 10 mL of the methanol leaf extract was measured and poured into a clean 250 ml beaker and reacted with 90 ml of 0.01 M AgNO₃ from a burette (titration method) using AgNO₃ as the titrant and the aqueous extract as the analyte at room temperature. The synthesized mixture was left for 24 hours and then separated by centrifugation using centrifuging machine at 4000 rpm for 10 – 15 minutes the clear liquid was decanted and the settled layer (nanoparticles) was stored in a 5 ml plastic sample vial and labelled accordingly.

The following code-names were given to the synthesized nanoparticles: Celosia trigyna silver nanoparticles (CT-AgNPs) and Solanum nigrum silver nanoparticles (SN-AgNPs).

Characterization of Silver Nanoparticles

CT-AgNPs and SN-AgNPs with the plants’ extracts were characterized employing techniques such as: scanning electron microscopy (SEM), Fourier Transform Infrared (FTIR) and Ultra-violet/Visible (UV-vis).

Ultra-Violet/Visible Spectroscopy

The wavelength with the highest absorbance was determined by UV-visible spectroscopy using Biochrom Libra PCB 1500 UV-VIS spectrophotometer. The absorbance of silver nanoparticles dispersed in a quartz cuvette with a 1 cm optical path was measured by withdrawing small aliquot from the reaction mixture and wavelength scan was taken at every 60 mins interval, then 90 minutes and after 24 hours varying wavelength from 150 nm to 800 nm until a stable absorbance was obtained at maximum wavelength.

Fourier Transform Infrared Analysis (FTIR)

The FTIR analysis was done for the nanoparticles formed and the extracts of these plants were viewed employing the equipment and its software reported earlier by Bello et al., (2019).
Scanning Electron Microscopy (SEM)
The nanoparticles of these plants’ extracts were viewed employing the equipment and its software reported earlier by Bello et al., (2019).12

BIOLOGICAL ACTIVITIES

Anti-inflammation
Cell Stabilization Membrane (CSM)
The method employed in carrying out the anti-inflammatory activity of these extracts have been reported by both Bello et al., (2019)12 and Oyedapo et al., (1997; 2004).31,32 All tests and analyses were run in triplicate and averaged

Lipoxidase Assay
The inhibitory activity against lipoxigenases was studied using linoleic acid as substrate and lipoxidase as enzyme, the detail procedure for this activity was previously employed by these authors.12,33 All tests and analyses were run in triplicate and averaged

Antioxidant Activity
2, 2-diphenyl-1-picrylhydrazyl (DPPH) Activity
The DPPH activity employed has been previously been reported by some authors though with little adjustment.12,28,34 Mean ± standard error of the mean of two independent experiments run in duplicate was used to present the results.

2, 2’-azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid (ABTS) radical cation scavenging Activity (ABTS)
The ABTS’ radicals’ activity employed has been previously been reported by some authors though with little adjustment.12,28,34 All analysis was determined in duplicate.

RESULT AND DISCUSSION

Phytochemical Screening
Phytochemical constituents of the extracts of Celosia trigyna and Solanum nigrum are shown in Table 2. On the whole, polyphenol, flavonoids, triterpenes and steroids were identified in all plants’ extracts. Saponins are absent in all the plants’ extracts but alkaloids are only absent in the hexane part of all the plants’ extracts as revealed in Table 2. The hexane extract of C. trigyna gave a poor result for most groups of secondary metabolites investigated as shown in Table 2.

Characterization
UV-Visible spectroscopy study
Physical examination (visual) revealed changes in colour. The colour changes that were witnessed indicate the formation of Celosia trigyna silver nanoparticles (CT-AgNPs) and Solanum nigrum silver nanoparticles (SN-AgNPs) as shown in Table 3. Several experiments showed that AgNPs exhibited such color vicissitudes in solution made-up of water due to AgNPs surface plasmon resonance excitation (SPR), this was the first validation test that silver nanoparticles were generated 48, 49. The generated AgNPs were examined further using UV – Vis spectroscopy which is an essential and famous characterization tool. It was discovered that the aqueous extract of S. nigrum is able to reduce silver nitrate to silver nanoparticles at 420 nm being the surface plasmon absorbance peak. Among others. Looking at Figure 3, the curve in each spectrum of synthesized silver nanoparticles that have been absorbed in the wavelength range 420-440 nm of silver nanoparticle but 440 nm for of C. trigyna. This peak falls within the range of specification for nanoparticles as reported by some authors.31,32

FT-IR spectroscopy study
FTIR spectroscopy measurements were used to identify and characterize the biological reduction functional group that will give an indication of the probable group of organic metabolites contained in these hegetled vegetables responsible for the reduction of the Ag⁺ ions to elemental Ag⁰ and the subsequent capping culminating in a successful stabilization of the AgNPs.36 The FTIR spectra of the synthesized AgNPs of the two vegetables i.e. A= SN-AgNPs; B=CT-AgNPs are shown in Figure 2. The Infrared spectrum of SN–AgNPs showed the present of O-H functional group with a broadband at 3429.41 cm⁻¹, which was assigned to C=O stretch. It shows a very sharp absorption band at 3400.74 cm⁻¹ which is sp³ carbon. The IR spectrum of CT-AgNPs shows a very broad band at 3400.74 cm⁻¹ which was assigned to – OH stretch. It shows a very sharp absorption band at 1631.64 cm⁻¹ which was assigned to C=C functional group at wavenumber of 1600 cm⁻¹. Absorbance bands that are well-defined and broad were spotted at 3452.24 (-OH), 1631.41 (C=C, stretching), 1451.19-1384.70 (C-H, bending), 1141.42 (C-O) for the AgNPs synthesized (Figure 2). The OH stretching is the reason for the broad and penetrating bands spotted at around 3400 cm⁻¹ for both AgNPs, which suggests that there is like hood of flavonoids and polyphenols present in the nanoparticles formed. The –C=C– stretching give rise to the medium band seen at around 1640 cm⁻¹ in both SN-AgNPs and CT-AgNPs. The C–H stretching belonging to compounds containing aromatic rings gives peaks at 1450 cm⁻¹ (Figure 2). This give a hint about the
presence of alkaloids, flavonoids and others in this plant extract. As shown in Figure 2, most of these spectra proved distinctive functional groups of compounds i.e. Alkaloids, coumarins, flavonoids, phytosterols, tannins and phenolic acids, all these secondary metabolites may be responsible for the capping and reduction of the AgNPs that were synthesized.12

### Table 1 Phytochemical Screening Result

<table>
<thead>
<tr>
<th>Solanum nigrum</th>
<th>Celosia trigyna</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MeOH</strong></td>
<td><strong>Hexane</strong></td>
</tr>
<tr>
<td>Polyphenol</td>
<td>+++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+++</td>
</tr>
<tr>
<td>Triterpenes</td>
<td>++</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+++</td>
</tr>
<tr>
<td>Steroids</td>
<td>++</td>
</tr>
<tr>
<td>Phenols</td>
<td>++</td>
</tr>
</tbody>
</table>

+++ = Very Good, ++ = Good, + = Fair, - = Not present, MeOH = Methanol

### Table 2 AgNPs Colour Changes observed

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Colour Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Solanum nigrum</td>
<td>Black to brown</td>
</tr>
<tr>
<td>2 Celosia trigyna</td>
<td>Black to greenish to yellow</td>
</tr>
</tbody>
</table>

### Table 3 Antioxidant Activity of the Synthesized AgNPs and Extracts of the Plant Species

<table>
<thead>
<tr>
<th>Solanum nigrum</th>
<th>Celosia trigyna</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>µg/ml</strong></td>
<td><strong>ABTS</strong></td>
</tr>
<tr>
<td>SN-AgNPs</td>
<td>Me-SN</td>
</tr>
<tr>
<td>100</td>
<td>11.4±2.1</td>
</tr>
<tr>
<td>200</td>
<td>13.9±0.2</td>
</tr>
<tr>
<td>300</td>
<td>16.8±0.2</td>
</tr>
<tr>
<td>400</td>
<td>15.3±1.7</td>
</tr>
<tr>
<td>500</td>
<td>14.9±1.8</td>
</tr>
</tbody>
</table>

Me-SN = Methanol Extract of S. nigrum; Me-CS = Methanol Extract of C. trigyna; The IC₅₀ are means of three replicates (N=3 ± SD).

### Table 4 Anti-inflammatory Activity of the Synthesized AgNPs and Extracts of the Plant Species

<table>
<thead>
<tr>
<th>Solanum nigrum</th>
<th>Celosia trigyna</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>µg/ml</strong></td>
<td><strong>HRBC</strong></td>
</tr>
<tr>
<td>SN-AgNPs</td>
<td>Me-SN</td>
</tr>
<tr>
<td>100</td>
<td>32.2±0.1</td>
</tr>
<tr>
<td>200</td>
<td>32.5±0.1</td>
</tr>
<tr>
<td>300</td>
<td>33.1±0.1</td>
</tr>
<tr>
<td>400</td>
<td>34.4±0.1</td>
</tr>
<tr>
<td>500</td>
<td>35.9±0.1</td>
</tr>
</tbody>
</table>

Me-SN = Methanol Extract of S. nigrum; Me-CS = Methanol Extract of C. trigyna; The IC₅₀ are means of three replicates (N=3 ± SD).
compared employing two different antioxidant assays as presented in Table 4. The AgNPs and the methanol extract for each of these plants were evaluated for in-vitro activity employing DPPH and ABTS assays. The results are expressed in terms of IC$_{50}$ (the concentration that caused a 50 % inhibition) and presented in Table 4. These were carried out with in-vitro method at various concentrations (100, 200, 300, ...500 µg/mL) of the extracts and AgNPs formed. The synthesized AgNPs of these wild but eaten plant species and the extracts tends to display a significant antioxidant activity at the dose 100 µg/mL concentration, this was noticed with the positive control too. The higher the concentration the less the antioxidant effect that was noticed though there was a climax at 400 µg/mL as shown in Table 4. From Table 4, it is observed that there is an obvious trend, the synthesized AgNPs displayed a better activity when compared to the extracts of these plants i.e. AgNPs from Celosia trigyna and Solanum nigrum displayed a better in-vitro antioxidant activity (IC$_{50}$: 11.4 and 12.4 µg/mL) with ABTS assay and (IC$_{50}$: 15.4 and 9.4 µg/mL) using DPPH assay but the methanol extracts of these plants displayed a lower value to the former. SN-AgNPs showed the most significant antioxidant activity against ABTS (IC$_{50}$: 11.4 µg/mL) while CT-AgNPs displayed the most significant antioxidant activity against DPPH (IC$_{50}$: 9.4 µg/mL) when compared to the positive control used ascorbic acid (IC$_{50}$: 4.7 µg/mL). Most of the AgNPs formed showed the most significant result at 100 µg/mL though the positive control gave the best result at this dose also (Table 4). Higher plants always contain constituents and substances with antioxidant effect. Flavonoids and polyphenols are one of naturally occurring substances that are widely renowned to exert scavenging ability against superoxide, free and hydroxyl radicals. In this study, we assess the antioxidant of the AgNPs of these wild vegetables and their methanol extracts because of the multifaceted and complex nature of compounds in plants, the antioxidant nature of these AgNPs and their extracts cannot be studied by only a single assay (method). As a result of this, the generally accepted assays i.e. DPPH and ABTS methods were used in this study. Though, CT-AgNPs display a significant antioxidant effect in both assays employed but SN-AgNPs only showed a good antioxidant activity in DPPH assay only. The DPPH and ABTS antioxidant assays proves that these neglected vegetables with their synthesized AgNPs portend a good antioxidant activity. Bello et al., (2019A) reported the health benefits of non-cultivated plants species beyond dietary uses. Their antioxidant activity via ABTS assay is well acknowledged in this study as compared with DPPH. Some secondary metabolites have been reportedly isolated from C. trigyna, these compounds include chondrillasterol, chondrillasterol acetate, Pheophytin A and lutein. These molecules and others reportedly identified in this plant exhibit antiulcer, antioxidant, antiuretic, against heart diseases. Celosia trigyna
Biosynthesis, Characterization and Biological Activity

Anti-inflammatory Activity

The methanol extracts of the two non-cultivated vegetables with their corresponding synthesized nanoparticles were evaluated and compared using cell-based assays for their anti-inflammatory activity as shown in Table 5. The AgNPs and the methanol extract for Celosia trigyna and Solanum nigrum were evaluated for in-vitro activity employing the Human Red Blood Cell Membrane Stabilization (HRBC) method and lipoxygenase Assay. The results are expressed in terms of IC$_{50}$ (the concentration that caused a 50% inhibition) and presented in Table 5. These were carried out with in-vitro method at various concentrations (100, 200, 300...500 μg/mL) of the extract. The extract tends to display a significant anti-inflammatory activity at 100 μg/mL concentration, this was noticed with the positive control too. The higher the concentration the less the anti-inflammatory effect that was noticed though there was a climax at 400 μg/mL as shown in Table 5. From Table 5, it is observed that there is an obvious trend, the synthesized AgNPs displayed a better activity when compared to the extracts of these plants i.e. AgNPs from S. nigrum and C. trigyna displayed a better in-vitro anti-inflammatory activity (IC$_{50}$: 32.2 and 38.5 μg/mL) against Human Red Blood Cell Membrane (HRBC) and (IC$_{50}$: 57.6 and 32.8 μg/mL) against lipoxygenases but the methanol extracts of these plants generally exhibited a lower value to the former. SN-AgNPs showed the most significant inhibitory activity against HRBC (IC$_{50}$: 32.2 μg/ml) while CT-AgNPs showed the most significant inhibitory activity against lipoxygenases (IC$_{50}$: 32.8 μg/ml) when compared to the positive control used indomethacin (IC$_{50}$: 28.1 μg/mL). Most of the AgNPs formed showed the most significant result at 100 μg/ml though the positive control gave the best result at this dose too. CT-AgNPs displayed good activity against LOX assay employed, they could serve well as LOX inhibitors. It is so surprising to note that they display a moderate activity in the other assay used. Some authors have reported the anti-inflammatory activity of C. trigyna through LOX assay. Some secondary metabolites have been reported to be isolated from C. trigyna, these compounds include chondrillasterol, chondrillasterol acetate, Phaeophytin A and lutein.$^{41}$ Eboh et al., (2019) reported that ethanol extract of C. trigyna has a high content of flavonoids (4.72 ± 0.97 mgQE/g extract) and phenolic acid (2.51 ± 0.21 mgGAE/g extract).$^{44}$ Celosia trigyna extract could be a promising nutraceutical for preventing and managing some radicals causing diseases. Flavonoids, polyphenols, bioactive phytosterols and triterpenoids are widely broadly distributed in the plants species globally, these have been documented as important contributing to nutrients for human health.$^{45}$ These compounds are considered to have chelating properties, scavenging free radicals by donating hydrogen, antioxidants and agents preventing low-density lipoproteins oxidation. These are excellent Lipoxygenase (LOX) inhibitors, hence this plant could have some advantages for the treatment of psoriasis, osteoporosis arthritis, cancer, allergic rhinitis, asthma and atherosclerosis.$^{46,47}$

Future Consideration and Conclusion

The CT-AgNPs, SN-AgNPs, extracts of C. trigyna and S. nigrum exhibited some lipoxygenase activity and are therefore possess a significant inhibition towards inflammation but these conclusions should be carefully seen because in-vitro assays were only applied. In this study only HBRC and 5-LOX inhibitory activities were studied. These results were produced from in-vitro assays using artificial radicals ABTS and DPPH for the antioxidant activity while HBRC and LOX are also artificial in-vitro assays for anti-inflammatory. Therefore, the extracts from these wild vegetables and their corresponding nanoparticles need further in-vivo activity to be employed in order to fully validate their antioxidant and anti-inflammatory activities.

CONFLICT OF INTEREST

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FUNDING

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REFERENCES


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