



# Phytobiological-facilitated Production of Silver Nanoparticles From Selected Non-cultivated Vegetables in Nigeria and Their Biological Potential

## Nijerya'da İşlenmemiş Sebzelerden Fitobiyolojikler ile Kolaylaştırılmış Gümüş Nanopartiküllerinin Üretimi ve Biyolojik Potansiyelleri

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### ABSTRACT

**Objectives:** Plant-mediated synthesis [silver (Ag) to form Ag nanoparticles (AgNPs)] is becoming progressively well accepted in many scientific and pharmaceutical fields. The aim of this study was to synthesize AgNPs using air-dried leaves of four neglected vegetables, i.e. *Ceratotheca sesamoides*, *Ceiba pentandra*, *Crassocephalum crepidioides*, and *Launaea taraxacifolia*.

**Materials and Methods:** Ultraviolet-visible (UV-Vis) spectroscopy, fourier transform infrared (FTIR) spectroscopy, and scanning electron microscopy (SEM) were used for characterization. Cell stabilization membrane and lipoxidase assays were used to determine used to assess the antiinflammatory activities while 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS+) assays were used to assess the antioxidant activities of AgNPs [*L. taraxacifolia*-AgNPs, *C. sesamoides* Ag nanoparticles (CS-AgNPs), *C. pentandra* Ag nanoparticles (CP-AgNPs), and *C. crepidioides* AgNPs (CC-AgNPs)].

**Results:** The UV-Vis spectra of the synthesized NPs displayed absorption bands at around 360-440 nm, which is a characteristic band for AgNPs. The SEM image showed that the AgNPs formed were spherical in morphology. CC-AgNPs exhibited the most significant inhibitory activity against human red blood cell membrane stabilizasyonu [median inhibitory concentration (IC<sub>50</sub>): 32.2 µg/mL] while CS-AgNPs displayed the most significant inhibitory activity against lipoxygenases (IC<sub>50</sub>: 32.8 µg/mL). CP-AgNPs exhibited the most significant antioxidant effect against both ABTS and DPPH (IC<sub>50</sub>: 5.5 and 6.4 µg/mL) when compared to ascorbic acid (IC<sub>50</sub>: 4.7 µg/mL).

**Conclusion:** The synthesized AgNPs were found to be stable and the FTIR evidence suggested that the phytochemicals in the vegetables might have played an important role in the reduction and stabilization of AgNPs. This work showed that the synthesized AgNPs from non-cultivated vegetables can find relevance and application in health, drugs, food and environmental science. The evidences herein further confirmed their ethnopharmacological applications.

**Key words:** AgNPs, antiinflammatory, antioxidant, non-cultivated vegetables, nanoparticles

### ÖZ

**Amaç:** Bitkiler aracılıklı sentez [gümüş nanopartikülleri (AgNP) oluşturmak için gümüş (Ag)] birçok bilimsel ve farmasötik alanda artan bir şekilde kabul görülmektedir. Bu çalışmanın amacı havada kurutulmuş işlem görmemiş sebze (*Ceratotheca sesamoides*, *Ceiba pentandra*, *Crassocephalum crepidioides* ve *Launaea taraxacifolia*) yapraklarını kullanarak AgNP'lerinin sentezidir.

**Gereç ve Yöntemler:** Karakterizasyon için ultraviyole-görünür bölge (UV-Vis) spektroskopisi, fourier transforme kızılötesi (FTIR) spektroskopisi ve taramalı elctron mikroskopisi (SEM) kullanılmıştır. AgNP'lerin [*L. taraxacifolia*-AgNP'ler, *C. sesamoides* Ag nanopartikülleri (CS-AgNPs), *C. pentandra* Ag nanopartikülleri (CP-AgNP) ve *C. crepidioides* Ag nanopartikülleri (CC-AgNP)] antienflamatuar aktivitesini belirlemek için hücre stabilizasyon membranı ve lipoksidaz yöntemleri kullanılırken antioksidan aktivitelerini değerlendirmek için 2,2-difenil-1-pikrilhidrazil hidrat (DPPH) ve 2,2'-azinobis(3-etilbenzotiyazolin-6-sulfonik asit (ABTS+) yöntemleri için kullanılmıştır.

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**Bulgular:** Sentez edilen NP'lerin UV-Vis spektrumları AgNP'leri için karakteristik bir bant olan 360-440 nm arasında absorpsiyon bantları göstermiştir. SEM görüntüleri AgNP'lerin küresel morfolojilerinin olduğunu göstermiştir. CC-AgNP'ler insan kırmızı kan hücresi membrane stabilizasyonu [medyan inhibitör konsantrasyon ( $IC_{50}$ ): 32,2  $\mu\text{g/mL}$ ] için en yüksek inhibitör etkiyi gösterirken, CS-AgNP'leri lipoksijenazlara karşı en belirgin inhibitör etkiyi göstermiştir ( $IC_{50}$ : 32,8  $\mu\text{g/mL}$ ). CP-AgNP'leri hem ABTS+ hem de DPPH için ( $IC_{50}$ : 5,5  $\mu\text{g/mL}$  ve 6,4  $\mu\text{g/mL}$ ) askorbik asitle karşılaştırıldığında ( $IC_{50}$ : 4,7  $\mu\text{g/mL}$ ) en belirgin antioksidan etki göstermişlerdir.

**Sonuç:** Sentez edilen AgNP'ler stabil bulunmuştur ve FTIR verileri sebzelerdeki fitokimyasalların AgNP'lerin redüksiyonunda ve stabilizasyonunda önemli rol oynadıklarını göstermiştir. Bu çalışma ilelenmemiş bitkilerden sentez edilen AgNP'lerin sağlık, ilaç, gıda ve çevresel bilimlerde ilgi ve uygulama alanı bulabileceğini göstermiştir. Buradaki bilgiler etenoparmakolojik uygulamalarını onaylamıştır.

**Anahtar kelimeler:** AgNP'ler, antiinflamatuvar, antioksidan, işlem görmemiş sebzeler, nanopartiküller

## INTRODUCTION

For centuries, cultures around the world have continuously employed and taken advantage of edible but non-cultivated plants for sufficient nutrition, food security, and wealth creation.<sup>1-3</sup> These non-cultivated plants supply necessary and essential components of the human diet, supplying the body with various minerals, protein, and certain precursors of human hormones besides helping in the build-up of energy.<sup>4-6</sup>

Some of the plants studied here are non-cultivated due to their being tagged as “poor man’s” vegetables but are eaten by the locals. *Ceratotheca sesamoides*<sup>7</sup> belongs to the family Pedaliaceae. It is mostly found in Africa and it grows as a wild and non-cultivated plant. However, in some parts of Africa, it is being cultivated, and because of its similarities with common sesame (*Sesamum indicum*), some call it false sesame.<sup>8,9</sup> Although widely regarded as a delicacy in most West African countries, literature on this plant and its consumption is scanty and not sufficient.<sup>10</sup> *C. sesamoides* is traditionally employed in the management of diarrhea in Nigeria. The plant is used as an aphrodisiac and in the treatment of jaundice, snake bites, and skin ailments. *C. sesamoides* leaf infusions are used to facilitate delivery in both humans and animals.<sup>10-13</sup> In northern Nigeria, *C. sesamoides* seeds are used to relieve circumcision pains.

*Ceiba pentandra* belongs to the family Malvaceae.<sup>7</sup> It is native to the Caribbean, Central America, northern South America, Mexico, and tropical West Africa. Besides its young leaves' nutritional benefits, in Nigeria many locals use its leaves for treating many ailments. This plant has many ethnobotanical uses (Table 1), i.e. to treat headache and diabetes and as a diuretic and aphrodisiac. Its use as one of the main ingredients in a hallucinogenic drink has also been reported.<sup>14,15</sup>

*Crassocephalum crepidioides*<sup>7</sup> is also called thickhead, fireweed, Okinawa spinach, and red flower ragleaf in English, Ebolo, or Ebire (Yoruba) in Nigeria. Its use is widespread in many tropical and subtropical regions, but is especially prominent in tropical Africa. It has also been widely cultivated in Asia due to its medicinal and nutritional properties.<sup>16,17</sup> In southern Nigeria, *C. crepidioides*' leaves have been reported to be valuable in the management of indigestion, stomach ache, and fresh wounds (in Uganda) and its leaves' decoction is employed in Nigeria against headache (Table 1). In Tanzania, a mixture of the leaf sap of *C. crepidioides* and *Cymbopogon giganteus* is taken by mouth against epilepsy. Its dried leaves are used to stop nose bleeds and aid in sleeping.<sup>18</sup>

*Launaea taraxacifolia* (synonymous to *Lactuca taraxacifolia*)<sup>7</sup> is a greenish leafy vegetable that is mainly eaten in the western part of Nigeria. This vegetable is eaten in most countries in Africa either cooked or as salad, i.e. Dahomey, Ghana, Senegal, and Sierra Leone.<sup>19</sup> Most people in West Africa call *L. taraxacifolia* by the name African lettuce or wild lettuce.<sup>20</sup> There are many ethno-medicinal applications of *L. taraxacifolia*. This leafy vegetable has been employed in managing many ailments for centuries, ailments such as diabetes, eye diseases (conjunctivitis), measles, skin diseases, and yaws (Table 1). Some cultures in Nigeria rubbed a concoction of its leaves on the limbs of toddlers to facilitate walking.<sup>21,22</sup>

Many studies have reported the green synthesis of leafy vegetable extracts employing various metals, i.e. the green synthesis of copper nanoparticles (NPs) using *Ocimum sanctum*<sup>23</sup> green synthesis of palladium NPs employing *Origanum vulgare* leaf extract<sup>24</sup> lemon fruits and turmeric powder to steady the green synthesis employing manganese NPs<sup>25</sup> and the synthesis of silver NPs (AgNPs) from *Curcuma longa*<sup>26</sup> and *Calotropis*. Beside their nutritional benefits, leafy and non-cultivated vegetables (Figure 1) are known to possess therapeutic uses.<sup>13,27,28</sup> However, many of these cheap but disease-preventing plant species are yet to be sufficiently studied and exploited. Hence, the aim of the present study was to investigate the phytochemical screening of these non-cultivated vegetables' leaves extract and experimentally carry out characterization and application of these medicinal plants species' AgNPs as anti-inflammatory and antioxidant agents and acetylcholinesterase inhibitors.

## MATERIALS AND METHODS

Fresh green plants of *Crassocephalum crepidioides* (I.U. 0345), *Ceratotheca sesamoides* (I.U. 011), *Ceiba pentandra* (UILH/001/957), and *Launaea taraxacifolia* (UILH/002/1020) were obtained in December 2016 from “Oja-Oba” market in Ilorin, in Kwara State of Nigeria located in the rain forest zone at latitude 10°00' North of the equator and longitude 8°00' East of the Greenwich meridian. The plants were identified and authenticated at the Plant Biology Department, University of Ilorin, and voucher numbers collected. The authenticated plant materials were air-dried at ambient temperature for 2 weeks to completely remove the moisture content and to effectively prepare the plants for the next stage of preparation. After drying, the dried leaves were crushed into fine powder using a ceramic pestle and mortar and the samples were kept in an air-tight plastic container.

### Equipment and reagents

The equipment used comprised a pestle and mortar, extraction jar, rotary evaporator, centrifuging machine, ultraviolet-visible (UV-Vis) spectrophotometer, and fourier transform infrared spectrophotometer (FTIR). The reagents included n-hexane, methanol, silver nitrate, ferric chloride, potassium

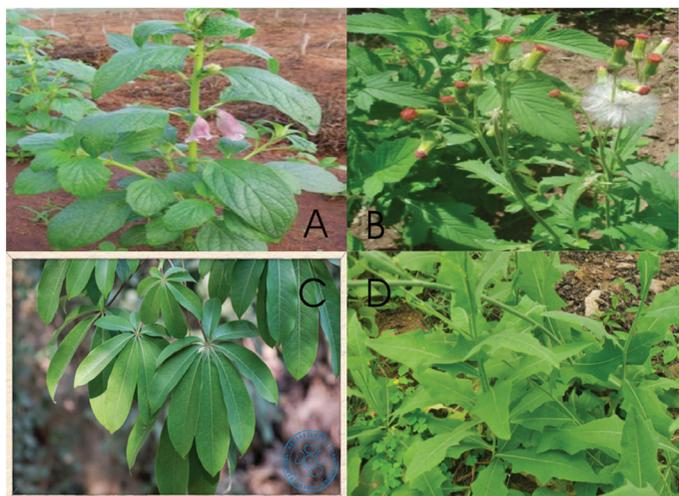
ferricyanide, chloroform, sulfuric acid, lead acetate, acetic anhydride, potassium hydroxide, and Fehling solution. They were purchased from Labtrade and Sunaf Nig. Ltd. All solvents used were of analytical grade.

### Preparation of extracts

Powdered *C. sesamoides*, *C. pentandra*, *L. taraxacifolia*, and *C.*

**Table 1. Ethnomedicinal importance of the non-cultivated<sup>13-84</sup>**

S/N	Plant name	Other names	Country found	Ethnomedicine	Biological activities	Phytochemical present	References
1	<i>Crassocephalum crepidioides</i>	Thickhead, fireweed, red flower ragleaf (English); Okinawa spinach (Igbo); Efo Ebolo or Ebire (Yoruba), Sekkoteka Ekyakiragala (Southern Uganda)	Uganda, West African countries, Bangladesh, India, and Malaya	Epilepsy, indigestion, sickness, sleeping disorder, stomach-ache, swollen lips, tumor, diabetes, dizziness, fever, headache, hypertension, leprosy, mental diseases, peptic ulcer, crop yield improvement	$\beta$ -cell protection, antidiabetic, antioxidant, anticholinesterases	Polyphenolic, pyrrolizidine alkaloid, tannin, dihydroisocoumarins, monoterpenes	52-58
2	<i>Ceratotheca sesamoides</i>	Ekú (Yoruba-Western Nigeria); Bungu (Nigeria); Tchaba-laba (Guinea Bissau); Lалу-caminho (Senegal)	Senegal, Guinea Bissau, Angola, Namibia, Tanzania, Democratic Republic of Congo, Nigeria, Botswana, Mozambique, Zimbabwe, and Zambia	Diarrhea, conjunctivitis, emollient and lubricant, stomach ache, leprosy, tumor, relieve circumcision pains, malaria, aphrodisiac, jaundice, snake bites and skin ailments	Antiviral, antidiarrheal, antiplasmodial, antioxidant, hyaluronidase, phospholipase A2, proteolytic	Flavonoids, saponins, alkaloids, tannins, phenols, phenolics	59-67
3	<i>Launaea taraxacifolia</i>	Yarin/Yamurin/Odundun-Odo (Yoruba); Nononbarya, namijin dayii (Hausa); Ugu (Igbo); Yantotoé/yantoto (Fon); Lantoto/yantotoé (Mahi); Odôdô/Odôdôlodôdô (Idaach)	Nigeria, Benin, Togo, Ghana, Cameroon	Malaria, ulcer, against high blood pressure, diabetes mellitus, pain in fresh wounds, dysentery, eye diseases (conjunctivitis), measles, skin diseases, and yaws	Antioxidant, hypolipidemic/antidiabetics, antibacterial, antimalarial, antiviral, anticancer	Flavonoids, phenols, chlorogenic acid	13,22,59,68-78
4	<i>Ceiba pentandra</i>	Kapok, the Ceiba, Java cotton, Hara kapok, Silk cotton and Samauma is also known as Rimi (Hausa), Bamtami (Fulani), Araba ogungun (Yoruba) and Akpi (Igbo)	Indonesia, Nepal, Bahamas, the Caribbean, Mexico, South America, West African countries, Cape Verde, Chad and Angola	Diuretic, aphrodisiac, headache, diabetes, to banish evil spirits. hallucinogenic drink, bowel complaints, diarrhea, hypertension, headache, dizziness, constipation, mental diseases, fever, peptic ulcer, and leprosy	Antibacterial, antiinflammatory, antiallergic, antiviral, antioxidant, antimicrobial, antidiarrheal	Naphthaquinone, flavonoids, linoleic acids, fatty acids	15,79-84



**Figure 1.** The leaves of A: *Ceratotheca sesamoides*, B: *Ceiba pentandra*, C: *Crassocephalum crepidioides*, D: *Launaea taraxacifolia*

*crepidioides* were macerated in 3 L of n-hexane in an extraction jar such that the level of the solvent was above that of the plant materials. The macerated mixtures were then left for 72 h at ambient temperature. The extracts were filtered out from the macerated mixture using Whatman 185  $\mu\text{m}$  filter paper. The n-hexane extracts were concentrated in a vacuum rotary evaporator under reduced pressure and suitable temperature, transferred to appropriately labeled 250 mL beakers, and allowed to stand at ambient temperature to permit evaporation of residual solvents. The procedure was repeated using methanol after the residue of the n-hexane extract has been air-dried.

#### Phytochemical screening

Preparation for the test was done by pouring 3 mL of the leaf extracts into separate test tubes and diluting with 2-4 mL of deionized water. The various tests were carried out following the procedures described below. Standard techniques of screening and detecting secondary metabolites in plants were used.<sup>29,30</sup> The metabolites tested for were alkaloids, anthraquinones, cardiac glycosides, carbohydrates, flavonoids, saponins, steroids, phenolics, tannins, and triterpenes.

#### Synthesis of silver nanoparticles

The synthesis of AgNPs was carried out according to the method described in our previous study.<sup>31</sup> Ten milliliters of the leaf extract was measured and poured into a clean 250 mL beaker and reacted with 100 mL of 0.01 M  $\text{AgNO}_3$  (prepared from stock  $\text{AgNO}_3$ -0.1 M of  $\text{AgNO}_3$ ) from a burette (titration method) using  $\text{AgNO}_3$  as the titrant and the extracts as the titrant at ambient temperature. A color change to yellow was observed. The synthesized mixture was left for 24 h and then separated by centrifugation using a centrifuging machine. Clear liquid was decanted and the settled layer (NPs) was stored in a 5 mL plastic sample vial and labeled accordingly. The following nomenclature was given to the synthesized NPs: *L. taraxacifolia* (LT)-AgNPs, *C. sesamoides* (CS)-AgNPs, *C. pentandra* (CP)-AgNPs, and *C. crepidioides* (CC)-AgNPs.

#### Characterization of silver nanoparticles

The characterization of LT-AgNPs, CS-AgNPs, CP-AgNPs, and CC-AgNPs was done using a combination of analytical and spectroscopic techniques, namely UV-Vis, FTIR, and scanning electron microscopy (SEM).

#### Ultraviolet-visible spectroscopy

The optical properties of the AgNPs of both plants were determined by UV-Vis spectroscopy on a Biochrom Libra PCB 1500 UV-VIS spectrophotometer. The wavelength with the highest absorbance was determined. The absorbance of AgNPs dispersed in a quartz cuvette with a 1 cm optical path was measured by withdrawing a small aliquot from the reaction mixture and a wavelength scan was taken every 60 min, then 90 min, and after 24 h. The wavelength was varied from 320 nm to 620 nm for *L. taraxacifolia*, from 320 nm to 670 nm for *C. crepidioides*, from 320 nm to 620 nm for *C. sesamoides*, and from 320 nm to 620 nm for *C. pentandra*.

#### Fourier transform infrared spectroscopy

The functional groups present in the methanolic extract of *L. taraxacifolia*, *C. crepidioides*, *C. sesamoides*, and *C. pentandra*, which were responsible for capping and efficient stabilization of the synthesized AgNPs, were determined using a Shimadzu FTIR model IR8400s spectrophotometer. The solutions were dried at 75°C and the dried powders were characterized in the range 4000-400  $\text{cm}^{-1}$  by KBr pellet method.

#### Scanning electron microscopy

NPs of these plants' extracts were viewed using an Ultra Plus FEGSEM (Carl Zeiss, Germany) and the size and shape of the NPs were determined using the Smart SEM Ver. 5 software (Carl Zeiss, Germany).

#### Biological activities

##### Antiinflammatory activity

##### Cell stabilization membrane

The antiinflammatory activity of these extracts was tested by *in vitro* human red blood cell (HRBC) membrane stabilization method. The reaction mixtures (4.5 mL) consisted of 2 mL hypotonic saline solution, phosphate buffer (pH 7.4), and 1 mL of test solution in normal saline; 0.5 mL of 10% rabbit RBC in normal saline was added. For control tests, 1 mL of isotonic solution was used. The mixtures were incubated at 56°C for 30 min, cooled under running water, and centrifuged, while the absorbance of the supernatants was read at 560 nm. Percentage membrane stabilizing activity was calculated as follows:

$$\% \text{ stabilization} = (100 - \text{O.D. of drug sample} / \text{O.D. of control}) \times 100$$

The control represents 100% lysis. The result was compared with STD (100  $\mu\text{g}/\text{mL}$ ) treated samples.<sup>32,33</sup>

##### Lipoxidase assay

The inhibitory activity against lipoxygenases (LOXs) was studied using linoleic acid as substrate and lipoxidase as enzyme. Test samples were dissolved in 0.25 mL of 2 M borate buffer pH 9.0 and 0.25 mL of lipoxidase enzyme solution (20,000 U/mL) was

added followed by incubation for 5 min at 250°C. After that, 1.0 mL of lenoleic acid solution (0.6 mM) was added followed by thorough mixing and absorbance was measured at 234 nm. Indomethacin was used as reference standard. The percent inhibition was calculated from the following equation:

$$\% \text{ inhibition} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

All tests and analyses were run in triplicate and averaged.<sup>34,35</sup>

#### Antioxidant activity

##### 2,2-Diphenyl-1-picrylhydrazyl (DPPH) activity

The method employed was the one reported by Oguntoye et al.<sup>28</sup> but with slight modifications.<sup>36</sup> Mean  $\pm$  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 (ABTS) activity

The ABTS radical cation decolorization assay based on the scavenging of ABTS + radicals by antioxidant components of the extracts was used. The assay follows the procedure of Oguntoye et al.<sup>28</sup> with slight modifications.<sup>36</sup> All analyses were performed in duplicate.

#### Statistical analysis

Mean  $\pm$  standard error of the mean of two independent experiments run in duplicate was used to present the results. The results are reported as mean  $\pm$  standard deviation.

## RESULTS AND DISCUSSION

#### Phytochemical screening

The phytochemical constituents of the extracts of *C. crepidioides*, *C. sesamoides*, *C. pentandra*, and *L. taraxacifolia* are shown in Table 2. On the whole, polyphenol, flavonoids, triterpenes, and steroids were identified in all the plants' extracts. Alkaloids and saponins are absent in most of these plants except for methanol extract of *C. crepidioides* and hexane extract of *C. pentandra*. The hexane extracts of *C. sesamoides* gave poor results for most groups of secondary metabolites investigated as shown in Table 2. The phytochemical screening reveals that flavonoids are present in the various extracts.

**Table 2. Phytochemical screening results**

	<i>Crassocephalum crepidioides</i>		<i>Ceratotheca sesamoides</i>		<i>Launaea taraxacifolia</i>		<i>Ceiba pentandra</i>	
	MeOH	Hexane	MeOH	Hexane	MeOH	Hexane	MeOH	Hexane
Polyphenol	+++	+	+++	-	++	+	-	+
Flavonoids	+++	+	+++	-	+++	+	++	+
Triterpenes	++	++	++	++	-	+++	+++	+
Saponins	-	-	-	-	-	+	-	+++
Alkaloids	+++	-	++	-	++	-	-	-
Steroids	++	++	+++	-	+	-	++	++
Phenols	++	++	+++	++	+++	++	+++	++

+++; Very good, ++=Good, += Fair, -: Not present, MeOH: Methanol

#### Characterization

##### UV-Vis spectroscopy study

Visual inspection showed color changes. The color changes that were witnessed indicate the formation of *C. crepidioides*, *C. sesamoides*, *C. pentandra*, and *L. taraxacifolia* AgNPs as shown in Table 3. Many studies have shown that AgNPs displayed these color changes in aqueous solution due to the excitation of surface plasmon resonance (SPR) of AgNPs, and this was the first confirmation test to show that AgNPs were formed.<sup>37-41</sup> The AgNPs formed were examined further by the use of UV-Vis spectroscopy, which is an important and popular tool used for characterization.

It was discovered that the aqueous extracts of *C. sesamoides* and *C. pentandra* were able to reduce silver nitrate to AgNPs at 450 nm, being the surface plasmon absorbance peak among others. Figure 2 shows the curve in each spectrum of synthesized AgNPs absorbed in the wavelength range 380-440 nm of AgNPs of *C. sesamoides* and *C. pentandra*. The absorption spectra showed SPR and peaks at 380 nm in the case of *L. taraxacifolia* (Figure 2), whereas the bands for *C. crepidioides* were observed at 410 nm as shown in Figure 2. This peak falls within the range of specification for NPs reported by previous authors.<sup>42,43</sup>

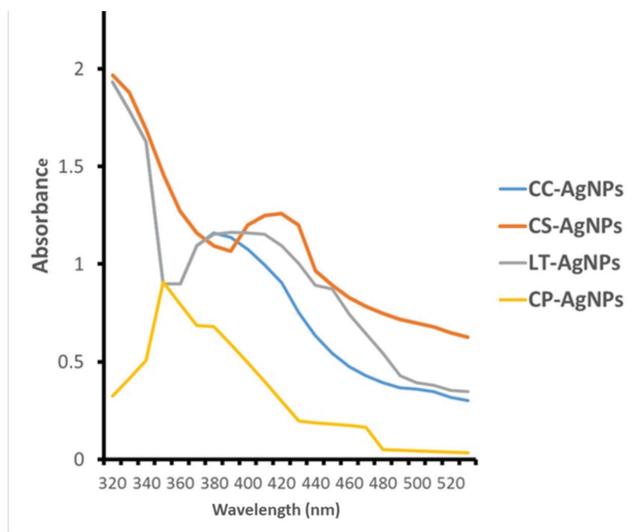
##### Fourier transform infrared spectroscopy study

FTIR spectroscopy measurements were employed to recognize and identify the biological reducing functional group, which will give a hint about the likely group of organic compounds present in these wild and non-cultivated vegetables responsible for the reduction of the Ag<sup>+</sup> ions to elemental Ag<sup>0</sup> and the ensuing capping resulting in efficient stabilization of the AgNPs formed.<sup>44</sup> The FTIR spectra of the synthesized AgNPs of the

**Table 3. AgNPs' color changes observed**

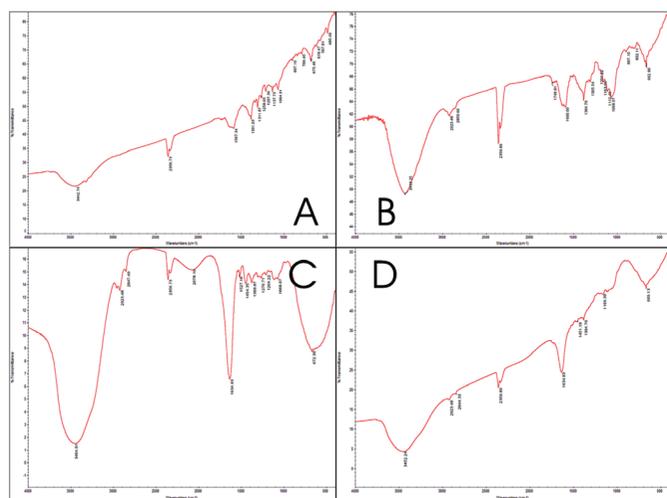
	Plant name	Color change	
		Initial	Final
1	<i>Crassocephalum crepidioides</i>	Black	Brown
2	<i>Ceratotheca sesamoides</i>	Black greenish	Yellow
3	<i>Ceiba pentandra</i>	Deep brown	Yellow
4	<i>Launaea taraxacifolia</i>	Light yellow	Reddish brown

AgNPs: Silver nanoparticles



**Figure 2.** UV-visible spectra of the synthesized AgNPs

UV: Ultraviolet, AgNPs: Silver nanoparticles, CC: *Crassocephalum crepidioides*, CS: *Ceratotherca sesamoides*, LT: *Launaea taraxacifolia*, CP: *Ceiba pentandra*



**Figure 3.** FTIR spectra of A=CC-AgNPs; B=CS-AgNPs; C=CP-AgNPs; D=LT-AgNPs

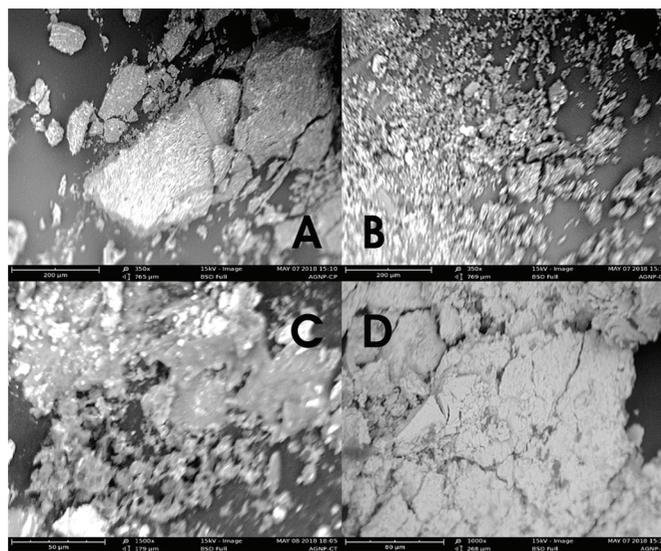
FTIR: Fourier transform infrared, CC: *Crassocephalum crepidioides*, CS: *Ceratotherca sesamoides*, LT: *Launaea taraxacifolia*, CP: *Ceiba pentandra*, AgNPs: Silver nanoparticles

four vegetables, i.e. A=CC-AgNPs, B=CS-AgNPs, C=CP-AgNPs, and D=LT-AgNPs, are shown in Figure 3. The infrared spectrum of CP-AgNPs showed the presence of an O-H functional group with a broad band at 3464.94  $\text{cm}^{-1}$ , while the IR spectrum of CP-AgNPs further revealed a C=C structure with medium intensity at a wave number of 1634.33  $\text{cm}^{-1}$ , which is  $\text{sp}^2$  carbon. The IR spectrum of CS-AgNPs showed a very broad band at 3433.48  $\text{cm}^{-1}$ , which was assigned to a -OH stretch. It showed a very sharp absorption band at 1748.81  $\text{cm}^{-1}$ , which was assigned to a C=O stretch, while there was a C=C functional group at a wave number of 1600  $\text{cm}^{-1}$ . Clear and broad absorbance bands were observed at 3452.24 (-OH), 2923.48-2844.33 (C-H, stretching), 1634.83 (C=C, stretching), 1451.19-1384.70 (C-H, bending), and 1169.39 (C-O) for the LT-AgNPs synthesized (Figure 3). The intense and broad bands observed at around 3452  $\text{cm}^{-1}$  for all the

AgNPs was due to the O-H stretching, which gives an indication for the presence of polyphenols. A medium band observed at around 1634  $\text{cm}^{-1}$  in both the synthesized NPs was attributed to -C=C- stretching. The peaks at 1451  $\text{cm}^{-1}$  correspond to C-H stretching of the aromatic compounds (Figure 3). The IR spectrum of CC-AgNPs showed an intense and broad band at 3442.74 (-OH, stretching), 1587.34 (C=C, stretching), 1391.03-1311.87 (N=O, stretching), and 1258.05-1064.91 (C-O, stretching). This indicated the presence of alkaloids, flavonoids, and others in this plant extract. The peaks at 1587  $\text{cm}^{-1}$  correspond to C-H stretching of the aromatic compounds. As shown in Figure 3, most of these spectra proved distinctive functional groups of compounds, i.e. alkaloids, coumarins, flavonoids, and phenolic acids, which may all have had an active role in the reduction and capping of the synthesized AgNPs.

### Scanning electron microscope

The scanning electron microscope identifies the surface characteristics, morphology, and the distribution of the CC-AgNPs, CS-AgNPs, CP-AgNPs, and LT-AgNPs depicted in the SEM micrograph (Figure 4), to determine the silver concentration of the NPs. AgNPs generally show a typical absorption characteristic peak at approximately 3 keV due to the surface plasma resonance phenomenon.<sup>45</sup> The cracked lines in the SEM micrographs (Figure 1A-1D) would enhance laminar flow, indicating the potential of the AgNPs for toxicant removal.<sup>46,47</sup> The NPs synthesized by these non-cultivated vegetables were



**Figure 4.** Scanning electron microscope picture: A=CC-AgNPs; B=CS-AgNPs; C=CP-AgNPs; D=LT-AgNPs

CC: *Crassocephalum crepidioides*, CS: *Ceratotherca sesamoides*, LT: *Launaea taraxacifolia*, CP: *Ceiba pentandra*, AgNPs: Silver nanoparticles

highly agglomerated except for CC-AgNPs, which displayed a scattered morphology (Figure 1). MubarakAli et al.<sup>48</sup> ascribes this cluster to a dehydration-induced combination of Ag NPs. However, CS-AgNPs, CP-AgNPs, and LT-AgNPs showed a trend in terms of differences in the dimensions and magnitude of the synthesized NPs. This can be accredited to the fact that the bigger and bulkier NPs can hold more Ag.

**Table 4. Antioxidant activity of the synthesized AgNPs and extracts of the plant species**

µg/mL	<i>Crassocephalum crepidioides</i>			<i>Ceratotherca sesamoides</i>			<i>Launaea taraxacifolia</i>			<i>Ceiba pentandra</i>			Ascorbic acid				
	ABTS	DPPH	Me-CC	ABTS	DPPH	Me-CS	ABTS	DPPH	Me-LT	ABTS	DPPH	Me-CP					
100	11.4±2.1	15.4±3.1	13.4±1.5	12.4±0.1	14.7±1.6	13.3±1.6	11.3±0.9	18.5±4.3	13.3±1.9	16.4±2.3	5.5±18.2	27.9±6.5	6.4±1.2	14.9±0.5	4.7±0.6		
200	13.9±0.2	24.2±1.8	18.9±0.2	24.2±0.2	13.4±0.1	17.2±2.2	10.4±0.1	13.9±2.1	17.6±0.2	28.8±0.2	16.6±1.2	19.1±1.2	7.6±17.9	29.2±5.9	6.7±1.9	15.2±1.9	5.6±0.5
300	16.8±0.2	34.3±1.3	21.2±0.2	34.3±1.3	16.4±1.1	35.3±1.3	11.4±1.1	14.1±1.3	11.7±1.8	26.5±3.4	16.7±2.8	20.4±2.4	14.6±16.1	29.6±5.8	7.6±1.1	15.5±0.8	7.1±6.1
400	15.3±1.7	38.3±0.4	21.8±1.7	38.3±1.4	15.4±0.0	34.8±1.1	11.5±0.0	15.1±2.2	22.3±1.1	32.9±1.9	20.3±0.1	21.9±1.5	16.6±17.1	28.7±4.8	8.6±1.1	18.5±1.8	8.3±4.9
500	14.9±1.8	38.5±0.6	20.5±0.6	38.5±0.6	17.4±0.0	35.2±2.6	16.4±0.1	17.2±3.1	24.7±3.9	33.4±0.7	23.7±1.9	23.4±0.7	17.9±17.6	31.4±7.3	9.2±5.6	19.5±2.5	13.6±0.2

Me-CC: Methanol extract of *Crassocephalum crepidioides*, Me-CS: Methanol extract of *Ceratotherca sesamoides*, Me-LT: Methanol extract of *Launaea taraxacifolia*, Me-CP: Methanol extract of *Ceiba pentandra*, The IC<sub>50</sub> values are means of three replicates (N=3 ± standard deviation), ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), DPPH: 2,2-diphenyl-1-picryl-hydrazyl-hydrate, AgNPs: Silver nanoparticles

**Table 5. Antiinflammatory activity of the synthesized AgNPs and extracts of the plant species**

µg/mL	<i>Crassocephalum crepidioides</i>			<i>Ceratotherca sesamoides</i>			<i>Launaea taraxacifolia</i>			<i>Ceiba pentandra</i>			Indomethacin				
	Me-CC	CC-AgNPs	LIP	Me-CC	CS-AgNPs	LIP	Me-LT	LT-AgNPs	LIP	Me-CP	CP-AgNPs	Me-CP					
100	32.2±0.1	39.1±0.1	57.6±0.1	63.1±0.1	38.5±0.1	62.9±1.1	32.8±0.1	51.9±1.1	56.4±2.1	59.2±0.1	55.4±1.1	59.2±0.1	34.7±1.0	53.1±0.1	48.5±1.0	53.0±0.1	28.1±0.0
200	32.5±0.1	39.9±1.1	55.5±0.1	59.9±1.1	43.2±1.1	58.3±2.1	33.8±2.1	58.3±2.1	58.2±1.1	59.2±0.1	52.6±2.1	59.2±0.1	34.9±2.1	55.5±2.6	51.1±2.1	58.3±1.6	34.4±0.0
300	33.1±0.1	38.4±2.1	49.1±0.1	58.4±2.1	44.2±1.2	61.1±1.2	34.5±1.2	61.1±1.2	57.1±1.9	61.3±1.1	47.1±1.1	61.3±1.1	41.2±1.1	56.2±1.1	47.8±1.2	61.1±0.1	34.8±0.0
400	34.4±0.1	43.3±1.0	49.4±0.1	63.3±1.0	46.6±0.2	64.5±0.1	34.7±0.1	64.5±0.1	58.9±2.2	59.3±2.1	52.3±1.0	59.3±2.1	42.3±1.2	57.5±0.2	37.7±1.1	64.5±0.1	37.3±0.0
500	35.9±0.1	45.4±1.3	45.9±0.1	61.4±1.3	51.2±1.3	63.6±0.1	35.1±1.1	63.6±0.1	61.2±0.0	57.2±0.1	54.2±0.0	57.2±0.1	43.2±0.1	57.8±1.1	31.2±0.1	63.7±1.1	36.3±0.0

Me-CC: Methanol extract of *Crassocephalum crepidioides*, Me-CS: Methanol extract of *Ceratotherca sesamoides*, Me-LT: Methanol extract of *Launaea taraxacifolia*, Me-CP: Methanol extract of *Ceiba pentandra*, The IC<sub>50</sub> values are means of three replicates (N=3 ± standard deviation), CSM: Cell stabilization membrane, LIP: Lamprey immune protein, AgNPs: Silver nanoparticles

## Biological activities

### Antioxidant activity

The methanolic extracts of the four non-cultivated vegetables with their corresponding synthesized NPs were evaluated and compared employing two different assays for their antioxidant activity as shown 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 50% inhibition) and are presented in Table 4. These were obtained by *in vitro* method at various concentrations (100, 200, 300...500  $\mu\text{g/mL}$ ) of the extracts and AgNPs formed. The synthesized AgNPs of the non-cultivated vegetables and the extracts tend to display significant antioxidant activity at the dose 100  $\mu\text{g/mL}$  concentration; this was noted with the positive control as well. The higher the concentration the lower the antioxidant effect that was observed, although there was a climax at 400  $\mu\text{g/mL}$  as shown in Table 4. Table 4 shows that there is an obvious trend: the synthesized AgNPs displayed better activity when compared to the extracts of these plants, i.e. AgNPs from *C. crepidioides*, *C. sesamoides*, *L. taraxacifolia*, and *C. pentandra* displayed better *in vitro* antioxidant activity ( $IC_{50}$ : 11.4, 12.4, 11.3, and 5.5  $\mu\text{g/mL}$ ) with the ABTS assay and ( $IC_{50}$ : 15.4, 9.4, 13.3, and 6.4  $\mu\text{g/mL}$ ) using the DPPH assay but the methanol extracts of these plants displayed values lower than those of the former. CP-AgNPs, CC-AgNPs, and LT-AgNPs exhibited the most significant antioxidant effect against ABTS ( $IC_{50}$ : 5.5, 11.3, and 11.4  $\mu\text{g/mL}$ ), while CP-AgNPs and CS-AgNPs displayed the most significant antioxidant activity against DPPH ( $IC_{50}$ : 6.4 and 9.4  $\mu\text{g/mL}$ ) when compared to the positive control used, ascorbic acid ( $IC_{50}$ : 4.7  $\mu\text{g/mL}$ ). Most of the AgNPs formed showed the most significant result at 100  $\mu\text{g/mL}$ , although the positive control gave the best result at this dose as well (Table 4). Higher plants always contain constituents and substances with antioxidant effects. Flavonoids are among these naturally occurring substances that are widely renowned to exert scavenging ability against superoxide, free, and hydroxyl radicals.<sup>49</sup> In the present study, we assessed the antioxidant effects of the AgNPs of the non-cultivated vegetables and their methanolic 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 method. As a result of this, the generally accepted assays, i.e. DPPH and ABTS methods, were used in the present study. CP-AgNPs displayed significant antioxidant activity in both assays employed, but CS-AgNPs only showed good antioxidant activity in the DPPH assay only. The DPPH and ABTS antioxidant assays proved that these neglected vegetables with their synthesized AgNPs show antioxidant activity. Bello et al.<sup>15</sup> examined the antioxidant effects of the leaves of *L. taraxacifolia* and *C. pentandra* (methanol extracts). These plant species displayed significant antioxidant activity when the ABTS assay was employed as compared with ascorbic acid.

### Antiinflammatory activity

The methanolic extracts of the four non-cultivated vegetables with their corresponding synthesized NPs were evaluated and

compared using cell-based assays for their antiinflammatory activity as shown in Table 5. The AgNPs and the methanol extract for each of these plants were evaluated for *in vitro* activity employing the HRBC membrane stabilization method and lipoxidase assay. The results are expressed in terms of  $IC_{50}$  (the concentration that caused 50% inhibition) and are presented in Table 5. These were carried out with an *in vitro* method at various concentrations (100, 200, 300...500  $\mu\text{g/mL}$ ) of the extract. The extract tends to display a significant antiinflammatory activity at 100  $\mu\text{g/mL}$  concentration; this was noted with the positive control as well. The higher the concentration the lower the antiinflammatory effect that was seen, although there was a climax at 400  $\mu\text{g/mL}$  as shown in Table 5. Table 5 shows that there is an obvious trend: the synthesized AgNPs displayed better activity when compared to the extracts of these plants, i.e. AgNPs from *C. crepidioides*, *C. sesamoides*, *C. pentandra*, and *L. taraxacifolia* displayed better *in vitro* antiinflammatory activity ( $IC_{50}$ : 32.2, 38.5, 56.4, and 34.7  $\mu\text{g/mL}$ ) against HRBC membrane and ( $IC_{50}$ : 57.6, 32.8, 55.4, 48.5  $\mu\text{g/mL}$ ) against LOXs but the methanol extracts of these plants displayed values lower than those of the former. AgNPs from *C. crepidioides*, *C. sesamoides*, *L. taraxacifolia*, and *C. pentandra* exhibited  $IC_{50}$  of 32.2, 38.5, 56.4, and 34.7  $\mu\text{g/mL}$  against HRBC membrane and showed inhibitory activity ( $IC_{50}$ : 57.6, 32.8, 55.4, and 48.5  $\mu\text{g/mL}$ ) against LOXs. CC-AgNPs and CP-AgNPs exhibited the most significant inhibitory activity against HRBC ( $IC_{50}$ : 32.2 and 34.7  $\mu\text{g/mL}$ ), while CS-AgNPs and LT-AgNPs displayed the most significant inhibitory activity against LOXs ( $IC_{50}$ : 32.8 and 48.5  $\mu\text{g/mL}$ ) when compared to the positive control using indomethacin ( $IC_{50}$ : 28.1  $\mu\text{g/mL}$ ). Most of the AgNPs formed showed the most significant result at 100  $\mu\text{g/mL}$ , although the positive control gave the best result at this dose as well. CS-AgNPs and LT-AgNPs displayed good activity against the LOX assay employed; they could serve as good LOX inhibitors. It was very surprising that they displayed moderate activity in the other assay used. Some authors have reported the antiinflammatory activity of *C. pentandra* through the LOX assay. It was reported that the methanol extract of its leaves displayed inhibitory activity against LOX with an  $IC_{50}$  of 102.4  $\mu\text{g/mL}$  when compared with that of the positive control, 90.4  $\mu\text{g/mL}$  (indomethacin).<sup>22</sup> This neglected vegetable's (*C. pentandra*) extracts exhibited inhibitory activity against LOX with an  $IC_{50}$  of 53.6  $\mu\text{g/mL}$ . LOXs are present in the airway and stomach epithelium, leukocytes, and gut cells, and they aid in the introduction of an oxygen molecule to the 5-position of arachidonic acid to give the intermediate (5S)-hydroxy-(6E,8Z,11Z,14Z)-eicosatetraenoic acid or 5-HETE. This is an important aspect of antiinflammatory activity in the LOX assay, hence inhibiting the biological genesis of leukotriene and 5-HETE. Hence, the search for specific inhibitors of LOX activity from medicinal plants is ongoing and imperative. LOX inhibitors, i.e. CS-AgNPs and LT-AgNPs, could possess some great advantages for the treatment of allergic rhinitis, arthritis, asthma, atherosclerosis, cancer, osteoporosis, and psoriasis.<sup>50,51</sup>

## CONCLUSION

Future studies will be carried out using various chromatographic techniques, spectroscopic techniques, and mass spectrometry to isolate and elucidate the bioactive compounds in the active fractions of wild and non-cultivated vegetables. The specific receptors these active plants' extracts and their corresponding synthesized AgNPs might be acting on to elicit antiinflammatory effects will be determined. There should be *in vivo* testing on small mammals to verify the antiinflammatory effects of these compounds in living organisms. Because AgNPs of both *C. crepidioides* and *C. sesamoides* significantly inhibited inflammatory response, it would be interesting to assay other plants from these families for antiinflammatory activity. The phybiological facilitated production of AgNPs from selected non-cultivated vegetables proves to be ecofriendly and successful. In the current research, it has been shown that the synthesis of AgNPs by a simple, cost-effective, nontoxic, and reproducible green chemistry method allows for better antioxidant and antiinflammation worth. This study reports for the first time the synthesis, characterization, and antiinflammatory and antioxidant activities of CS-AgNPs, CP-AgNPs, and LT-AgNPs. The synthesized AgNPs were found to be stable and the FTIR evidence suggested that the phytochemicals might have played an important role in the reduction and stabilization of AgNPs. This work showed that the synthesized AgNPs from non-cultivated vegetables can find relevance and application in health, drugs, food, and environmental science. The evidence herein further confirmed their ethnopharmacological applications.

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