# Phytobiological Facilitated Production of Silver Nanoparticles from Selected Non-Cultivated Vegetables in Nigeria and Their Biological Potential.

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**Abstract**

Plant-mediated synthesis (Silver (Ag) to form nanoparticles) is progressively becoming well accepted in many scientific and pharmaceutical fields. This study is aimed at synthesizing Ag nanoparticles using air-dried leaves of four (4) neglected vegetables i.e. *Cerathoteca sesamoides, Ceiba pentandra, Crassocephalum crepidioides, Launaea taraxacifolia*. Ultraviolet–visible spectroscopy, Fourier transform infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM) were used for characterization. Cell Stabilization Membrane (CSM) and lipoxidase assays, DPPH and ABTS+ assays were used to assess the anti-inflammatory and antioxidant activities of these AgNPs. *L. taraxacifolia* Ag nanoparticles (LT-AgNPs), *C. sesamoides* Ag nanoparticles (CS-AgNPs), *C. pendandra* Ag nanoparticles (CP –AgNPs) and *C. crepidioides* Ag nanoparticles (CC-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 nanoparticles. The SEM image of the AgNPs formed displayed were spherical in morphology. CC-AgNPs exhibited the most significant inhibitory activity against HRBC ($IC_{50}$: 32.2) while CS-AgNPs displayed the most significant inhibitory activity against lipoxygenases ($IC_{50}$: 32.8). CP-AgNPs exhibited the most significant antioxidant effect against both ABTS and DPPH ($IC_{50}$: 5.5 and 6.4) when compared to ascorbic acid ($IC_{50}$: 4.7 µg/mL).

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

**Introduction**

From time memorial, cultures around the world have continuously employed and taken the advantages of edible but non-cultivated plants to give sufficient nutrition, food security and wealth creation (Antia et al., 2006; Dhellot et al., 2006a; Omoti and Okyi, 1987). These non-cultivated plants supply necessary and essential component of human diet supplying the body with various body minerals, protein and certain precursors of human hormones beside helping in the build-up of energy (Edmonds and Chweya, 1997; Fleuret, 1979; Onyenuga and Fetuga, 1995**).**

Some of the plants studied here are non-cultivated plants but eaten by the locals due to it being tagged as "poor man" vegetable. *Ceratotheca sesamoides* (Burkill, 1985) belongs to the Family Pedaliaceae, it is mostly found in Africa and it grows as a wild and non-cultivated plant. Though in some parts of Africa, it is being cultivated, because its similarities with the common sesame (*Sesamum indicum*), some call it false sesame (Van Wyk and Gericke, 2000; Vanderjagt et al., 2000). Literatures available on the plant and its consumption though widely taken as a delicacy in most West African Countries, are scanty and not sufficient (Grubben and Denton, 2004). *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 (Bedigian, 2003, Bedigian and Adetula, 2004**,** Grubben and Denton, 2004, Bello et al., 2017). In northern Nigeria, *C. sesamoides* seeds are used to relieve circumcision pains.

*Ceiba pentandra* (Burkill, H.M. 1985) belongs to the family Malvaceae, it is native to the Caribbean, Central America, Northern South America, Mexico and to tropical West Africa. Beside, 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. treatment against headache, diuretic, aphrodisiac, diabetes. Its use as one of the main ingredients in a hallucinogenic drink has been acclaimed (Adebisi, 2000; Bello et al., 2018c).

*Crassocephalum crepidioides* (Burkill, 1985) 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 (Burkill, 1995; Robert, 1955). In Southern Nigeria, *C. crepidioides'* leaves have been reported to be valuable in the management of indigestion, stomach ache, fresh wound (in Uganda), 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 application to stop nose bleeding and aid in sleeping (Arawande et al., 2013).

*Launaea taraxacifolia* (synonymous to *Lactuca taraxacifolia*) (Burkill, 1985) is a greenish leafy vegetable that is mainly eaten in the Western part of Nigeria. Most countries in Africa eat this vegetable either cooked or as salad i.e. Dahomey, Ghana, Senegal, Sierra-Lone (Arawande et al., 2013). Most people in West Africa call *L. taraxacifolia* by the name African lettuce or wild lettuce (Lydia, 2012). 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 its leaves concoction on the limbs of toddlers to facilitate walking (Adebisi, 1966; Bello et al., 2018b).

Many studies have reported the green synthesis of leafy vegetables extracts employing various metals i.e. the green synthesis of copper nanoparticles using *Ocimum sanctum* (Sathiraju et al., 2014), green synthesis of palladium nanoparticles employing *Origanum vulgare* leaf extract (Mohammed et al., 2017), lemon fruits were used, turmeric powder was used to steadied the green synthesis employing manganese nanoparticles (MnNPs) (Jayandran et al., 2015), the synthesis of silver nanoparticles from *Curcuma longa* (Ramar, 2015) and *Calotropis* . Beside their nutritional benefits, leafy and non-cultivated vegetables (**Fig. 4**) have been known to possess therapeutic uses (Bello et al., 2017; Bello et al., 2017; Oguntoye et al., 2018). 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.

**Table 1**: Ethnomedicinal Importance of the Non-Cultivated Vegetables

| S/N | Plant Name | Other Names | Country found | Ethnomedicine | Biological Activities | Phytochemical Present | References |
|---|---|---|---|---|---|---|---|
| **1** | *C. crepidioides* | Thickhead, fireweed, red flower ragleaf (English); Okinawa spinach (Igbo); Efo Ebolo or Ebire (Yoruba), Ssekkoteka Ekyakiragala (Southern Uganda) | Uganda, West Africa 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 | β-Cell protection, antidiabetic, antioxidant, anticholinesterases | polyphenolic, Pyrrolizidine Alkaloid, tannin, dihydroisocoumarins, monoterpenes, | Asada et al., 1985; Rozhon et al., 2018; Aniya et al., 2005; Musa et al., 2011; Tomimori et al., 2012; Adedayo et al., 2015; Ssegawa and Kasenene, 2007 |
| **2** | *C. sesamoides* | Eku (Yoruba-Western Nigeria); Bungu (Nigeria); Tchaba-laba (Guinea Bissau); Lalu-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, tumour, relieve circumcision pains, malaria, aphrodisiac, jaundice, snake bites and skin ailments | Antiviral**,** Antidiarrhoeal, Antiplasmodial, Antiplasmodial, Antioxidant, Hyaluronidase, Phospholipase A2, Proteolytic | Flavonoids Saponins Alkaloids Tannins Phenols Phenolics | Obi et al., 2006; Toyin et al., 2012; Benoit-Vical et al., 2008; Konan et al.,2014; olander et al., 2014; Nadembega et al., 2011; Diarra et al., 2015; Abubakar et al., 2007; Fasola and Ogunsola, 2014 |
| **3** | *L. taraxacifolia* | Yarin/Yamurin/Odundun-Odo (*Yoruba*); Nononbarya, namijin dayii (*Hausa*); Ugu (Igbo); Yantotoé/ yantoto (*Fon*); Lantoto/ yantotoé (*Mahi*); Odôdô/ Odôdôlodôdô (*Idaacha)* | 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 | Amujoyegbe et al., 2015; Owoeye et al., 2017; Obi et al., 2006; Thomford et al., 2016; Adetutu et al., 2016; Bello et al., 2017; Bello et al., 2018b; Adinortey et al., 2012; Dairo et al., 2015; Gbadamosi et al., 2012; Olugbenga |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | | | et al., 2015; Ruffina et al., 2016; Soelberg et al., 2015; |
| **4** | *C. pentandra* | 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 Africa 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, anti-inflammatory, anti-allergic, antiviral, antioxidant, antimicrobial, anti-diarrhoeal, | Naphthaquinone, Flavonoids, linoleic acids, fatty acids | Bamishaiye et al., 2011; Jimoh et al., 2010; Adeniyi et al., 2012; Aloke et al., 2010; Anosike et al., 2014; Bello et al., 2018c; Cowan, 1999; Anigo et al., 2012; Enechi et al., 2013 |

## Material and Methods

Fresh green plants of *Crassocephalum crepidioides* (I.U. 0345), *Ceratotheca sesamoide* (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 on latitude 10$^{o}$ 00' North of the Equator and longitude 8o 00' East of the Greenwich Meridian. The plants were identified and authenticated at Plant Biology Department, University of Ilorin and voucher numbers collected. The authenticated plant materials were air-dried at ambient temperature for two 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

Pestle and mortal, extraction jar, rotary evaporator, centrifuging machine, ultraviolet-visible spectrophotometer and Fourier transform infrared spectrophotometer. The reagents include; n-hexane, methanol, silver nitrate, ferric chloride, potassium ferricyanide, chloroform, sulphuric 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. crepidioides* were macerated in 3 L of n-hexane in extraction jar such that the level of the solvent was above that of the plant materials. The macerated mixtures were then left for 72 hrs at ambient temperature. The extracts were filtered out from the macerated mixture using Whatman 185 µm filter paper. The n-Hexane extracts were concentrated in a vacuum Rotary Evaporator under reduced pressure and suitable temperature, transferred to appropriately labelled 250 mL beaker 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 deionized water. The various tests were carried out following the procedures described below: Standard techniques of screening and detecting secondary metabolites in plants was used (Sofowora, 1993; Trease & Evans, 1989). The metabolites tested for were alkaloids, anthraquinones, cardiac glycosides, carbohydrates, flavonoids, saponins, steroids, phenolics, tannins and triterpenes.

**Synthesis of Silver Nanoparticles**

The synthesis of silver nanoparticles was carried out according to the method that has been previously described in our previous study (Dada et al., 2018). 10 mL of the leaf extract was measured and poured into a clean 250 mL beaker and reacted with 100 mL of 0.01 M $AgNO_3$ (prepared from stock $AgNO_3$ - 0.1 M of $AgNO_3$) from a burette (titration method) using $AgNO_3$ as the titrant and the extracts as the titrant at ambient temperature. A colour change to yellow was observed. The synthesized mixture was left for 24 hours and then separated by centrifugation using centrifuging machine. Clear liquid was decanted and the settled layer (nanoparticles) was stored in a 5 mL plastic sample vial and labelled accordingly. The following nomenclature was given to the synthesized nanoparticles: *L. taraxacifolia* silver nanoparticles (LT-AgNPs), *C. sesamoides* silver nanoparticles (CS-AgNPs), *C. pendandra* silver nanoparticles (CP –AgNPs) and *C. crepidioides* silver nanoparticles (CC-AgNPs).

**Characterization of Silver Nanoparticles**

The characterization of both LT-AgNPs, CS-AgNPs, CP-AgNPs and CC-AgNPs was done using a combination of analytical and spectroscopic techniques vis-à-vis UV-VIS, FTIR and SEM.

- ***Ultraviolet- Visible Spectroscopy***

The optical properties of the AgNPs of both plants were determined by UV- visible spectroscopy on Biochrom Libra PCB 1500 UV-VIS spectrophotometer. The wavelength with the highest absorbance was determined. The absorbance of silver nanoparticle 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 min interval, then 90 minutes and after 24

hours. Varying the wavelength from 320 nm to 620 nm for *L. taraxacifolia* and 320 nm to 670 nm for *C. crepidioides*, 320 nm to 620 nm for *C. sesamoides* and 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. pendantra* which were responsible for capping and efficient stabilization of the synthesized AgNPs were determined using SHIMADZU FTIR model IR8400s spectrophotometer. The solutions were dried at 75 $^{o}$ C and the dried powders were characterized in the range 4000–400 $cm^{-1}$ using KBr pellet method.

- ***Scanning Electron Microscopy***

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

**Biological Activities**

**Anti-inflammatory Activity**

- ***Cell Stabilization Membrane (CSM)***

The anti-inflammatory activity of these extracts was tested by *in-vitro* (HRBC) Human Red Blood Cell Membrane Stabilization method. The reaction mixtures (4.5 mL) will consist of 2 mL hypotonic saline solution, phosphate buffer (pH 7.4) and 1 mL 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 560 $^{0}$C for 30 min cooled under running water and centrifuged while the absorbance of the supernatants were read at 560 nm. Percentage membrane stabilizing activity was calculated as follows:

% Stabilization = (100 – O. D. of drug sample/O. D. of control) × 100

The control represents 100 % lysis. The result was compared with STD (100 μg/ml) treated samples (Oyedapo et al., 1997; 2004).

- ***Lipoxidase Assay***

The inhibitory activity against lipoxygenases 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 added 0.25 mL of lipoxidase enzyme solution (20, 000 U/mL) and incubated for 5 min at 250C. After which, 1.0 mL of lenoleic acid solution (0.6 mM) was added, mixed well and absorbance was measured at 234 nm. Indomethacin was used as reference standard. The percent inhibition was calculated from the following equation,

% inhibition= [{Abs control- Abs sample}/Abs control] x 100

All tests and analyses were run in triplicate and averaged (Shinde et al., 1999; Stenhilder, 1995).

**Antioxidant Activity**

- ***2, 2-diphenyl-1-picrylhydrazyl (DPPH) Activity*.**

The method employed was the one reported by Oguntoye et al., (2018) though with slight modifications (Atolani et al., 2012). 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 2,2′-azinobis-3-ethylbenzothiazoline-6- sulfonate, ABTS radical cation decolourization assay based on the scavenging of ABTS·+ radicals by antioxidants component of the extracts was used. The assay follows the procedure of Oguntoye et al., (2018), with slight modifications (Atolani et al., 2012). All analysis was determined in duplicate.

**Result and Discussion**

**Phytochemical Screening**

Phytochemical constituents of the extracts of *C. crepidioides*, *C. sesamoides*, *C. pendranta* and *L. taraxacifolia* are shown in Table **2**. On the whole, polyphenol, flavonoids, triterpenes and steroids were identified in all plants' extracts. Alkaloids and saponnins are absent in most of these plants except for methanol extract of *C. crepidioides* and hexane extract of *C. pendranta.*

The hexane extracts of *C. sesamoides* gave a poor result for most groups of secondary metabolites investigated as showed in Table **2**. The phytochemical screening reveals that flavonoids are present in the various extracts.

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**Table 2**: Phytochemical Screening Result.

| | *C. crepidioides* | | *C. sesamoides* | | *L. taraxacifolia* | | *C. pentandra* | |
|---|---|---|---|---|---|---|---|---|
| | **MeOH** | **Hexane** | **MeOH** | **Hexane** | **MeOH** | **Hexane** | **MeOH** | **Hexane** |
| **Polyphenol** | +++ | + | +++ | - | ++ | + | - | + |
| **Flavonoids** | +++ | + | +++ | - | +++ | + | ++ | + |
| **Triterpenes** | ++ | ++ | ++ | ++ | - | +++ | +++ | + |
| **Saponnins** | - | - | - | - | - | + | - | +++ |
| **Alkaloids** | +++ | - | ++ | - | ++ | - | - | - |
| **Steroids** | ++ | ++ | +++ | - | + | - | ++ | ++ |
| **Phenols** | ++ | ++ | +++ | ++ | +++ | ++ | +++ | ++ |

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

**Table 3:** AgNPs Colour Changes observed

| | **Plant Name** | **Colour Change** | |
|---|---|---|---|
| | | **Initial** | **Final** |
| **1** | *C. crepidioides* | Black | brown |
| **2** | *Ceratotheca sesamoides* | Blackgreenish | Yellow |
| **3** | *Ceiba pentandra* | deep brown | Yellow |
| **4** | *L. taraxacifolia* | light yellow | reddish brown |

**Table 4:** Antioxidant Activity of the Synthesized AgNPs and Extracts of the Plant Species

| **µg/mL** | ***C. crepidioides*** | | | | ***C. sesamoides*** | | | | ***L. taraxacifolia*** | | | | ***C. pentandra*** | | | | ***Ascorbic Acid*** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **ABTS** | | **DPPH** | | **ABTS** | | **DPPH** | | **ABTS** | | **DPPH** | | **ABTS** | | **DPPH** | | |
| | **CC-AgNPs** | **Me-CC** | **CC-AgNPs** | **Me-CC** | **CS-AgNPs** | **Me-CS** | **CS-AgNPs** | **Me-CS** | **LT-AgNPs** | **Me- LT** | **LT-AgNPs** | **Me-LT** | **CP-AgNPs** | **Me-CP** | **CP-AgNPs** | **Me-CP** | |
| **100** | 11.4±2.1 | 13.4±1.5 | 15.4±3.1 | 13.4±1.5 | 12.4±0.1 | 14.7±1.6 | 9.4±0.1 | 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±2.1 | 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±0.00 | 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±0.01 | 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 C. crepidioides; Me-CS=Methanol Extract of C. sesamoides; Me-LT= Methanol Extract of L. taraxacifolia; Me-CP= Methanol Extract of C. pentandra: The IC50 are means of three replicates (N=3 ± SD).*

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**Table 5**: Anti-inflammatory Activity of the Synthesized AgNPs and Extracts of the Plant Species

| µg/mL | *C. crepidioides* | | | | *C. sesamoides* | | | | *L. taraxacifolia* | | | | *C. pentandra* | | | | *Indomethacin* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CSM | | LIP | | CSM | | LIP | | CSM | | LIP | | CSM | | LIP | | |
| | **CC-AgNPs** | **Me-CC** | **CC-AgNPs** | **Me-CC** | **CS-AgNPs** | **Me-CS** | **CS-AgNPs** | **Me-CS** | **LT-AgNPs** | **Me-LT** | **LT-AgNPs** | **Me-LT** | **CP-AgNPs** | **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 C. crepidioides; Me-CS=Methanol Extract of C. sesamoides; Me-LT= Methanol Extract of L. taraxacifolia; Me-CP= Methanol Extract of C. pentandra: The $IC_{50}$ are means of three replicates (N=3 ± SD).*

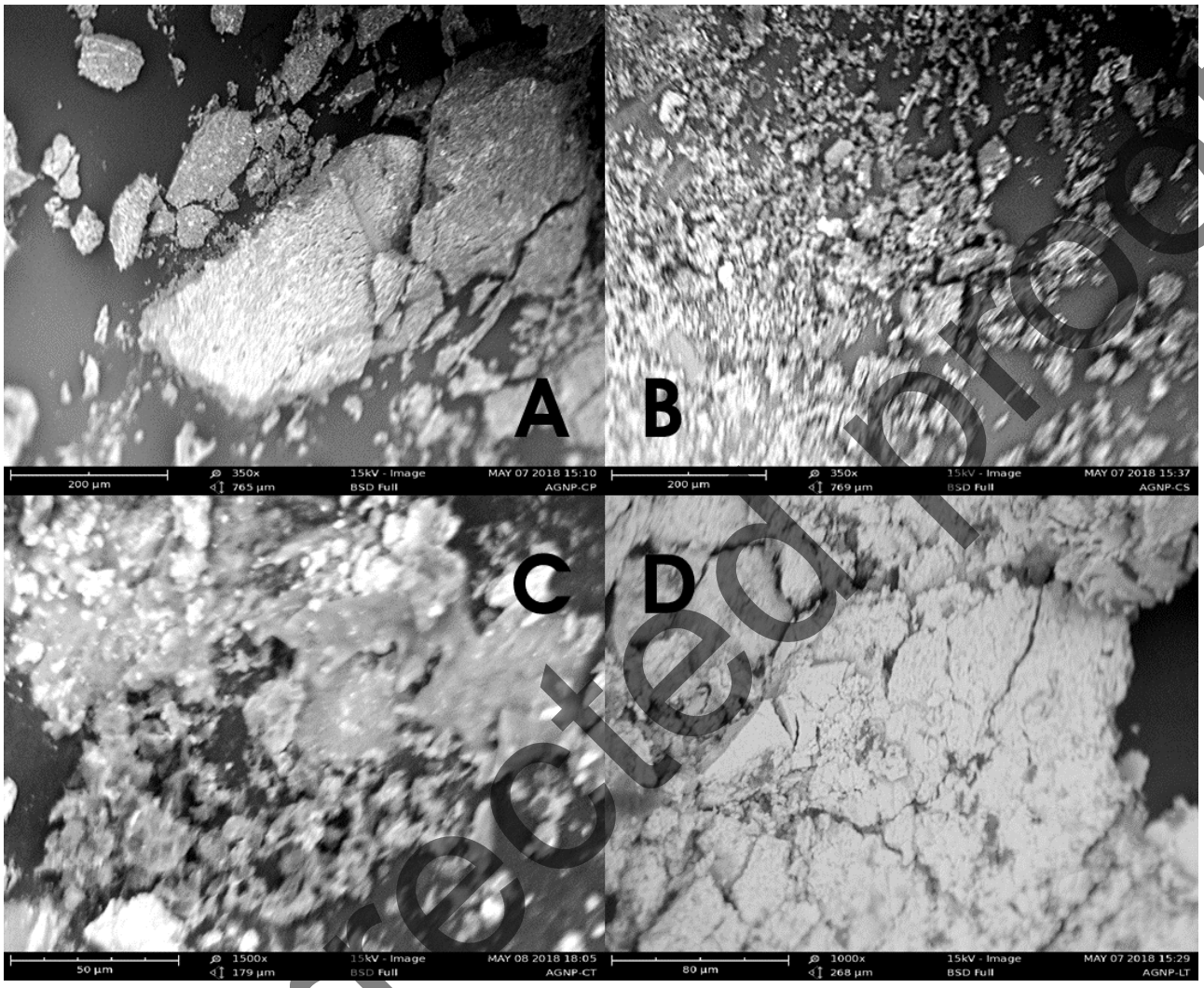


**Figure 1:** Scanning electron microscope picture A= *CC-AgNPs*; B= *CS-AgNPs*; C= CP–*AgNPs*; D= *LT-AgNPs*

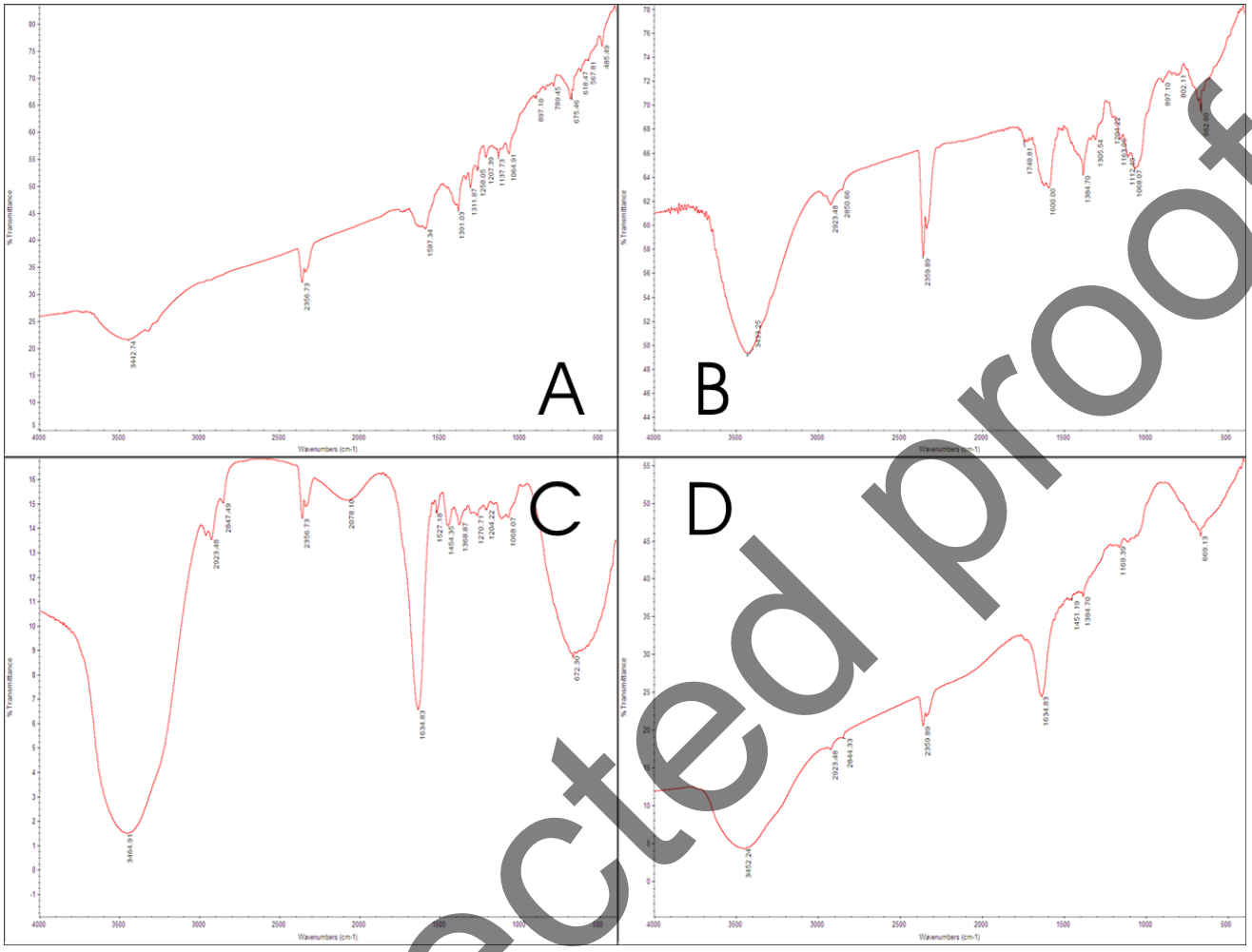


**Figure 2:** FTIR spectrum of A= *CC-AgNPs*; B= *CS-AgNPs*; C= CP–*AgNPs*; D= *LT-AgNPs*

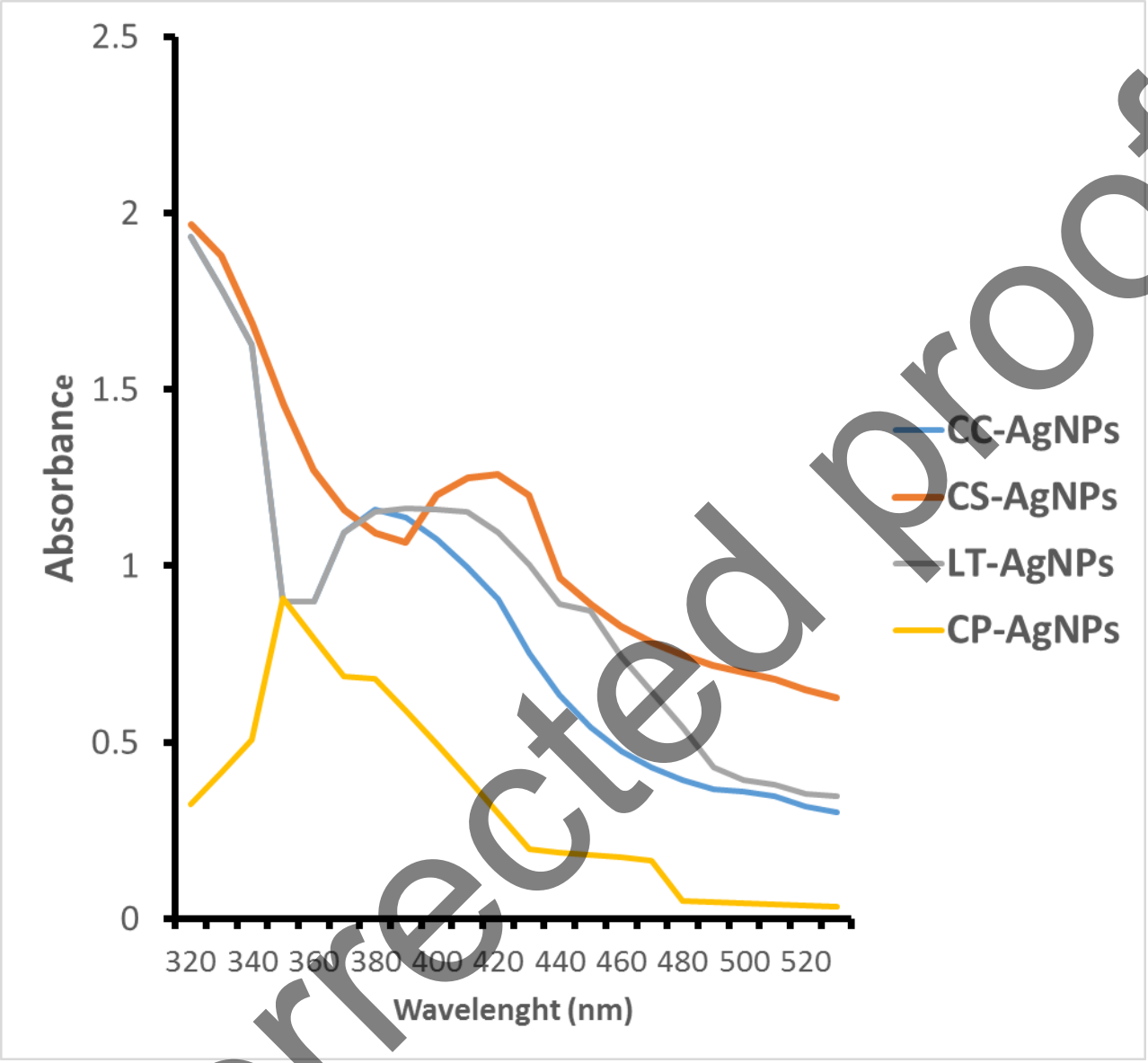


**Figure 3:** UV-visible spectra of the Synthesized AgNPs


**Figure 4:** The leaves of A= *C. crepidioides*; B= *C. sesamoides*; C= *C. pendranta*; D= *L. taraxacifolia*

**Characterization**

***UV-Visible spectroscopy study***

Visual inspection showed that colour changes were observed. The colour changes that were witnessed indicate the formation of *C. crepidioides*, *C. sesamoides*, *C. pendranta* and *L. taraxacifolia* AgNPs as shown in **Table 3**. Many studies have shown that AgNPs displayed these colour changes in aqueous solution due to the excitation of surface plasmon resonance (SPR) of AgNPs, this was the first confirmation test that silver nanoparticles have been formed (Kotakadi et al., 2014; Kuppusamy et al., 2014; Sharma et al., 2013; Stephen and Seethalakshmi, 2013;

Zhang et al., 2016). The AgNPs formed were examined further by the use of UV–Vis spectroscopy which is an imperative and popular tool used for characterization**.**

It was discovered that the aqueous extract of *C. sesamoides* and *C. pentandra* were able to reduce silver nitrate to silver nanoparticles at 450 nm being the surface plasmon absorbance peak. among others. Looking at **Fig. 3**, the curve in each spectrum of synthesized silver nanoparticles that have been absorbed in the wavelength range 380-440 nm of silver nanoparticle of *C. sesamoides* and *C. pentandra*. The absorption spectra showed a surface plasmon resonance and peaks which were observed at 380 nm in case of *L. taraxacifolia* (**Fig. 3**) whereas the bands for *C. crepidioides* were observed at 410 nm as shown in (**Fig. 3**). This peak falls within the range of specification for nanoparticles as reported by some authors (Sigamoney et al., 2016; Fafal et al., 2017)

***FT-IR spectroscopy study***

FTIR spectroscopy measurements was 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 $Ag^{+}$ ions to elemental $Ag^{0}$ and the ensuing capping resulting into efficient stabilization of the AgNPs formed (Kumar et al., 2014). The FTIR spectra of the synthesized AgNPs of the four vegetables i.e. CC-AgNPs; B= CS-AgNPs; C= CP–AgNPs; D= LT-AgNPs are shown in **Fig. 2**. The Infrared spectrum of CP–AgNPs showed the present of O-H functional group with a broadband at 3464.94 $cm^{-1}$, the IR spectrum of CP–AgNPs further revealed C=C structure with medium intensity at wavenumber of 1634.33 $cm^{-1}$ which is $sp^2$ carbon. The IR spectrum of CS-AgNPs shows a very broad band at 3433.48 $cm^{-1}$ which was assigned to -OH stretch. It shows a very sharp absorption band at 1748.81 $cm^{-1}$ which was assigned to C=O stretch, there is present of C=C functional group at wavenumber of 1600 $cm^{-1}$. 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), 1169.39 (C-O) for the LT-AgNPs synthesized (**Fig. 2)**. The intense and broad bands observed at around 3452 $cm^{-1}$ 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 $cm^{-1}$ in both the synthesized nanoparticles was attributed to the –C=C–

stretching. The peaks at 1451 $cm^{-1}$ correspond to C–H stretching of the aromatic compounds (**Fig. 2**). 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), 1258.05-1064.91 (C-O, stretching). This give a hint about the presence of alkaloids, flavonoids and others in this plant extract. The peaks at 1587 $cm^{-1}$ correspond to C–H stretching of the aromatic compounds. As shown in **Figure 2**, 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***

Scanning electron microscope identifies the surface characteristics, morphology and the distribution of the CC-AgNPs, CS-AgNPs, CP–AgNPs and LT-AgNPs depicted on the SEM micrograph (**Fig 1**)., to determine the silver concentration of the nanoparticles. AgNPs generally show a typical absorption characteristic peak at approximately 3 keV due to the surface plasma resonance phenomenon (Prasad et al., 2012). The cracked lines in the SEM micrographs (Figs 1 A-D) would enhance a lamina flow indicating the potential of the AgNPs for toxicant removal (Dada et al., 2017; Dada et al., 2015). The nanoparticles synthesized by these non-cultivated vegetables were highly agglomerated except for CC-AgNPs which displayed a scattered morphology (**Fig 1).** MubarakAli et al. (2011) ascribes this cluster to a dehydration-induced combination of Ag nanoparticle. Though, CS-AgNPs, CP–AgNPs and LT-AgNPs showed a trend in term of differences in the dimension and magnitude of the synthesized nanoparticles. The study can be credited to the fact that the bigger and bulkier nanoparticles are possible to hold more Ag.

**Biological Activities**

***Antioxidant Activity***

The methanolic extracts of the four non-cultivated vegetables with their corresponding synthesized nanoparticles 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 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 the non-cultivated vegetables 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 *C. crepiodioides, C. sesamoides, L. taraxacifolia* and *C. pentandara* displayed a better in vitro antioxidant activity ($IC_{50}$: 11.4, 12.4, 11.3, 5.5 µg/mL) with ABTS assay and ($IC_{50}$: 15.4, 9.4, 13.3 and 6.4 µg/mL) using DPPH assay but the methanol extracts of these plants displayed a lower value to 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 µg/mL) while CP-AgNPs and CS-AgNPs displayed the most significant antioxidant activity against DPPH ($IC_{50}$: 6.4 and 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 too (**Table 4**). Higher plants always contain constituents and substances with antioxidant effect. Flavonoids are one of these naturally occurring substances that are widely renowned to exert scavenging ability against superoxide, free and hydroxyl radicals (Chang et al., 2002). In this study, we assess the antioxidant 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 this study. Though, CP-AgNPs display a significant antioxidant activity in both assays employed but CS-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 antioxidant activity. Bello et al., 2018c reported that antioxidant of the leaves of *L. taraxacifolia* and *C. pentandara* (Methanol extracts). These plant

species displayed significant antioxidant activity when ABTS assay was employed as compared with ascorbic acid.

***Anti-inflammatory Activity***

The methanolic extracts of the four 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 each of these plants were evaluated for *in vitro* activity employing the Human Red Blood Cell Membrane Stabilization method and lipoxidase 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 *C. crepiodioides, C. sesamoides, C. pentandara* and *L. taraxacifolia* displayed a better in vitro anti-inflammatory activity ($IC_{50}$: 32.2, 38.5, 56.4, 34.7 µg/mL) against Human Red Blood Cell Membrane (HRBC) and (IC50: 57.6, 32.8, 55.4, 48.5 µg/mL) against lipoxygenases but the methanol extracts of these plants displayed a lower value to the former. AgNPs from *C. crepiodioides, C. sesamoides, L. taraxacifolia* and *C. pentandara* exhibit $IC_{50}$ (32.2, 38.5, 56.4, 34.7 µg/mL) against Human Red Blood Cell Membrane (HRBC) and showed inhibitory activity ($IC_{50}$: 57.6, 32.8, 55.4, 48.5 µg/mL) against lipoxygenases. CC-AgNPs and CP-AgNPs exhibited the most significant inhibitory activity against HRBC ($IC_{50}$: 32.2 and 34.7 µg/mL) while CS-AgNPs and LT-AgNPs displayed the most significant inhibitory activity against lipoxygenases ($IC_{50}$: 32.8 and 48.5 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. CS-AgNPs and LT-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. pendantara* through LOX assay. It was reported that the methanol extract of its leaves displayed an inhibitory activity against LOX with an $IC_{50}$: 102.4 µg/mL when compared with that of the positive control 90.4 µg/mL (Indomethacin) (Bello et al., 2018b), This neglected vegetable (*C. pendantara*) extracts exhibited inhibitory activity against LOX with an $IC_{50}$: 53.6 µg/mL. Lipoxygenases are present in airway and stomach epithelium, leukocytes, gut cells, 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 anti-inflammatory activity at lipoxygenase assay hence inhibiting the biological genesis of leukotriene and 5-HETE. Hence, the search for specific inhibitors of lipoxygenase activity from medicinal plants is on-going and so imperative. Lipoxygenase inhibitors i.e. *C. sesamoides, L. taraxacifolia*, CS-AgNPs and LT-AgNPs could possess some great advantage for the treatment of allergic rhinitis, arthritis, asthma, atherosclerosis, cancer, osteoporosis, and psoriasis (Fukuishi et al., 2001 and Dana et al., 2002).

**Future Direction and Conclusions**

Future studies will be carried out using various chromatographic techniques, spectroscopic techniques and Mass spectrometry (MS) to isolated and elucidate the bioactive compounds in the active fractions of the wild and non-cultivated vegetables. Determination of the specific receptors these active plants' extracts and their corresponding synthesized AgNPs might be acting on to elicit anti-inflammatory effects. There should be *in vivo* testing on small mammals to verify the anti-inflammatory of these compounds in living organisms. Because both AgNPs of *C. crepiodioides* and *C. sesamoides* significantly inhibited inflammatory response, it would be interesting to assay other plants from these families for anti-inflammatory activity. The phytobiological facilitated production of AgNPs from selected non-cultivated vegetables proves to be eco-friendly and successful. In this current research, it has been shown that the synthesis of AgNPs by a simple, cost effective, non-toxic and reproducible way of green chemistry method allows for better antioxidant and anti-inflammation worth. This study reports for the first time the synthesis, characterization, anti-inflammatory 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 vegetable can find relevance and application in health, drugs, food and environmental science. The evidences herein further confirmed their ethnopharmacological applications.

**Conflict of Interest**

No conflict of interest among the authors based**.**

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