


Biogenic Synthesis of Silver Nanoparticles using *Cola millenii*: Structural Characterization, Antioxidant Properties and Inhibitory Activity Against Enzymes Linked to Diabetes Mellitus

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Abstract: Biogenic synthesis of nanoparticles is one of the novel approaches for the formulation of drugs for tackling human diseases. In this study, aqueous extracts of *Cola millenii* were used as the reductant for the reduction of silver nitrate and synthesis of the corresponding silver nanoparticles (hereinafter designated *Cm*-AgNPs). The synthesis of *Cm*-AgNPs was verified by a color change from brown to reddish. The *Cm*-AgNPs were characterized by energy dispersive spectroscopy (EDS), Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), scanning electron microscopy (SEM), and X-ray diffraction (XRD). The FTIR analysis showed the presence of functional groups typical of phytochemicals that are associated with nanoparticles as capping agents. TEM and SEM analysis revealed that the *CM*-AgNPs were small in size and mostly spherical. About 70% of the *Cm*-AgNPs had a diameter of less than 20 nm. The crystalline structure of the *Cm*-AgNPs was confirmed by XRD analysis. *In vitro* studies based on 2,2-diphenyl-picrylhydrazyl (DPPH) radical inhibition and Total Antioxidant Power assays showed that the *Cm*-AgNPs possessed antioxidant properties. Furthermore, the *Cm*-AgNPs exhibited an inhibitory effect against carbohydrate hydrolyzing enzymes linked to diabetes, namely, alpha-amylase and alpha-glucosidase. Based on the results of this study, *Cm*-AgNPs could be potentially used for managing diabetes and other diseases in which oxidative stress is implicated in its pathology.

Keywords: *Cola millenii*; *Cm*-AgNPs; nanoparticles; antioxidants; diabetes.

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1. Introduction

Nanotechnology involves a fusion of techniques utilized in developing, fabricating, characterizing, and applying structures, materials, devices, and systems by manipulating matter at the atomic and molecular levels [1]. At this nanoscale level, the interaction between atoms and molecules confers special properties that are quite different from the general properties of matter at a macroscopic dimension. Therefore, nanotechnology seeks to harness the unique chemical and physical properties that matter exhibits at such small scales to create new and

innovative solutions across different industries, such as engineering, food packaging technologies, robotics, and medicine [2].

With the ever-growing health challenges facing mankind, new and effective methods are required to investigate the pathophysiological basis of diseases. Similarly, there is a need for more effective ways of diagnosing/treating diseases and also improving prognosis. Nanotechnology offers innovative solutions to these medical challenges [3]. The application of nanotechnology in medicine is called ‘nanomedicine’, a relatively new area in medicine. Nanomedicine uses nano-sized tools to make the processes of disease diagnosis, treatment, and health monitoring effective, with the ultimate goal of improving the quality of life [4]. Several successes recorded in nanomedicine include using nanoparticles to rid blood vessels of obstructions, destroying cancer cells, and synthesizing artificial tissues and organs [5,6]. Recently, nanomedicine has also curtailed the devastating complications of chronic diseases such as diabetes mellitus [7,8].

Diabetes mellitus is a metabolic abnormality that is usually characterized by the persistent presence of increased levels of blood glucose resulting from abnormalities in the secretion of insulin from the pancreatic beta-cells and/or its action on peripheral tissues [9]. Broadly speaking, the majority of diabetic cases fall under two etiopathogenetic categories. The first category is type 1 diabetes mellitus (T1DM), also known as insulin-dependent diabetes mellitus (IDDM). T1DM occurs as a result of the complete auto-immune destruction of the beta cells of the pancreas, resulting in the complete depletion of insulin secretion [10]. The second category is type 2 diabetes mellitus (T2DM), also known as non-insulin-dependent diabetes mellitus (NIDDM). This results from both resistance of peripheral tissues to the action of insulin and an inadequate compensatory insulin-secretory response [11]. The classic symptoms of untreated diabetes are unexplained weight loss, increased urination, hunger, and thirst. Other symptoms associated with diabetes include partial or complete loss of vision, tiredness, and chronic foot ulcers [12]. Diabetes represents a global health threat due to its tendency to damage the micro- and macro-vascular systems [13]. Preventing chronically high blood glucose levels plays a leading role in curtailing these complications and improving the quality of life of people with diabetes. Currently, pharmacological management of diabetes employs the use of oral hypoglycaemic agents (such as biguanides, sulfonylureas, thiazolidinediones, meglitinides, inhibitors of carbohydrate-metabolizing enzymes) and insulin therapy [14]. It has been observed that these pharmacological agents are not able to address all the pathophysiological aspects of the disease effectively, and a number of undesirable complications come with their use [15]. Hence, alternative interventions are sought.

The latest advancements in diabetes treatment have been directed towards nanoparticles. These nanoparticles are made using different techniques such as chemical reduction, co-precipitation, and microemulsion methods [16-18]. While these methods are widely used, they often require the use of toxic chemicals, such as reducing agents, surfactants, or metal salts. These chemicals can pose potential risks to human health and the environment. Alternatively, efforts have been made to develop greener and more sustainable methods. A plant-based approach, also known as “green synthesis,” is now being used to synthesize nanoparticles with reduced energy consumption and minimized use of toxic chemicals. The stability and versatility of nanoparticles are enhanced when sourced from plants, resulting in a greater diversity in shape and size [19]. AgNPs have been documented to exhibit diverse pharmacological activities such as antipathogenic, antioxidant, and anticancer activities

[20,21]. Each plant possesses a unique set of phytochemicals that are capable of achieving bioreduction and, hence, synthesis of nanoparticles [22].

Cola millenii K. Schum (family: *Sterculiaceae*) is among the numerous species of the genus- *Cola* [23]. Various parts of this plant have been reported to possess therapeutic potential [24]. It has been documented that in the North-Central geopolitical zone of Nigeria and some parts of Cameroon, natives enjoy eating the plant's fruits. Other primates, such as baboons and monkeys, also like eating fruits, which could have inspired the name 'Monkey Cola' [25]. The leaves of *Cola millenii* have a long history of being used in traditional medicine to treat a number of conditions, such as infections and lung diseases [26]. The ability of the leaf extract and fractions of *C. millenii* to scavenge free radicals have also been reported by Oghenerobo *et al.* [27]. The study of Orisakeye *et al.* [28] also revealed the antimicrobial and free-radical scavenging potentials of fractions of different plant parts. Odugbemi [29] reported that the leaves of *C. millenii* are used to treat bacterial, fungal, and viral infections.

Considering the abundance of various phytochemicals present in this plant and its numerous folkloric applications, it was selected for this study. To the best of our knowledge, AgNPs have never been synthesized from *C. millenii*; therefore, this study aimed to synthesize AgNPs from the leaves of *C. millenii* and also evaluate their antioxidant and antidiabetic effects in vitro.

2. Materials and Methods

2.1. Collection of plant samples, processing, and extraction.

Fresh leaves of *Cola millenii* were collected from a forest in Ankpa Local Government Area of Kogi State, Nigeria. The plant material was identified and authenticated at the Department of Plant Science and Biotechnology, Prince Abubakar Audu University, Anyigba, Kogi State. The leaves were air-dried and subsequently pulverized using an electric blender.

Aqueous extract of the pulverized *Cola millenii* leaves was obtained by soaking in water for 48 hours at room temperature. Exactly 100 g of plant material was soaked in 1000 mL of water. After the extraction period, the mixture was filtered using Whatman number 2 filter paper, and the resulting filtrate was concentrated by evaporation using a water bath.

2.2. Quantitative phytochemical analysis.

The concentration of total phenol, flavonoid, alkaloid, and tannin was determined using the method reported by Mythili *et al.* [30]. Saponin was estimated spectrophotometrically using the method of Rajana *et al.* [31], while cardiac glycosides were estimated using the method described by Tofighi *et al.* [32].

2.3. Synthesis of nanoparticles.

A portion of 100 mL of the plant extract was mixed with 900 mL of 1 mM solution of silver nitrate. The mixture was stirred at a low speed (10 rpm) for six (6) hours at room temperature. The initial color of the mixture transitioned from brown to reddish color after six (6) hours, confirming the formation of nanoparticles. The synthesis of nanoparticles was monitored by UV-spectra measurement in the 190-1000 nm range. The nanoparticles were recovered by centrifugation, and the pellet was stored for further analysis.

2.4. Structural characterization of nanoparticles.

Elemental analysis of *Cm*-AgNPs was performed using energy dispersive spectroscopy (EDS). Functional groups were identified by Fourier transform infrared spectroscopy (FT-IR) using a Nicolet iS10 Spectrometer at a Wavenumber range of 4000-400 cm^{-1} at a resolution of 4 cm^{-1} . X-ray diffraction studies were carried out with the aid of a Rigaku Miniflex D/Max-111C Diffractometer. The sample's morphology was determined using a JOEL-JSM 7600 fielded emission scanning electron microscope, while the size was determined using a JEM-ARM200F atomic resolution analytical electron microscope.

2.5. In vitro antioxidant assays.

The in vitro antioxidant activity of the samples was determined using two methodologies: total antioxidant power (TAP) and 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity assays. The total antioxidant capacity of the extract and nanoparticles was determined using a standard method described by Atanu *et al.* [33]. Similarly, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity of both the extract and nanoparticles was assessed using the method Shah *et al.* [34] as reported by Atanu *et al.* [35]. Vitamin C was used as standard. The EC_{50} for TAP and IC_{50} for DPPH inhibitory activity were determined from a plot of absorbance against the concentration of the samples.

2.6. Biological evaluation.

2.6.1. α -amylase inhibition assay.

The α -Amylase inhibition assay was carried out according to Oboh *et al.*'s description [36]. Briefly, 500 μL various concentrations of the sample (plant extract, *Cm*-AgNPs, or Acarbose) were mixed separately with 500 μL of 0.5 mg/ml pancreatic α -amylase (EC 3.2.1.1) prepared in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl). Starch, 1% (500 μL) was added, and the reaction mixture was incubated at 25°C for 10 min. Thereafter, the reaction was stopped by the addition of 1.0 mL of dinitrosalicylic acid color reagent. The mixture was boiled for 5 minutes, followed by a cooling step, and the volume was made up to 10 mL with distilled water. Absorbance was read at 540 nm, and IC_{50} (the extract concentration inhibiting 50% of the α -amylase activity) was calculated.

2.6.2. α -glucosidase inhibition assay.

α -Glucosidase inhibition assay was carried out according to the method reported by Oboh *et al.* [36]. Briefly, 100 μL of α -glucosidase solution (1.0 U/mL; in 0.1 M phosphate buffer pH 6.9) was placed in a water bath set to a temperature of 25°C for 10 min, followed by the addition of 50 μL of 5mM p-nitrophenyl- α -D-glucopyranoside solution in the presence of various concentration of the sample. The reaction mixture was incubated for 5 min for an additional 25°C before measuring absorbance at 405 nm, and IC_{50} (the extract concentration inhibiting 50% of the α -amylase activity) was calculated.

3. Results and Discussion

3.1. Phytochemical screening and in vitro antioxidant activity of *Cola millenii*.

Phytochemical analysis was conducted on the aqueous extract of *Cola millenii* to determine the presence of phytochemicals quantitatively. The results of the phytochemical analysis presented in Table 1 show that *Cola millenii* is a rich reservoir of phenolic compounds, flavonoids, alkaloids, tannins, saponins, and cardiac glycosides. Phytochemicals have great biomedical importance with respect to the prevention and treatment of diseases as well as the modulation of metabolic processes. Some of the drugs used in animal and human medicine have plant origins, and there continues to be a rise in the number of drug leads from plants in clinical trials [37,38]. Consequent upon this is the need to obtain pure bioactive compounds from plants and fully characterize their mechanism of action, which in recent times has been found to include enzyme inhibition, up/down-regulation of specific genes, anti-oxidation, etc. [39,40]. Oxidative stress is implicated in many disease conditions, such as diabetes, cancer, neurological disorders, etc; this understanding has led to the search for natural antioxidant sources [41-43]. In the current studies, the antioxidant properties of *Cola millenii*'s aqueous extract were evaluated by its total antioxidant power (TAP) and DPPH radical scavenging activity in vitro. Results of the in vitro antioxidant studies presented in Table 2 show that the plant extracts possess antioxidant activity, although significantly lower than the reference compound Vitamin C. The order of antioxidant potency was Vitamin C > Aqueous extract > *Cm*-AgNPs. The IC₅₀ for DPPH inhibition was 3.00±0.00, 42.49±0.35 and 102.1±0.43 µg/mL for Vitamin C, Aqueous extract, and *Cm*-AgNPs, respectively. Similarly, the EC₅₀ for total antioxidant power of the samples was 0.082±0.01, 5.41±0.21 and 19.52±1.18 µg/mL for Vitamin C, aqueous extract, and *Cm*-AgNPs respectively.

Table 1. Phytochemical composition of aqueous extracts of *Cola millenii*.

Phytochemical	Composition
Total phenol (mg GAE/g)	44.42±0.51
Total flavonoids (mg QE/g)	21.11±0.26
Alkaloids (mg AE/g)	3.39±0.04
Tannins (mg TAE/g)	4.46±0.03
Saponins (mg DE/g)	11.76±0.06
Cardiac glycosides (mg SE/g)	2.45±0.01

GAE= gallic acid equivalent; QE= quercetin equivalent; AE= atropine equivalent; TAE= tannic acid equivalent; DE= diosgenin; SE= securidaside.

Table 2. In vitro antioxidant activities of aqueous extracts of *Cola millenii* and its silver nanoparticles.

Sample	Inhibition of DPPH Radical (IC ₅₀ µg/mL)	Total Antioxidant Power (EC ₅₀ mg/mL)
AqEx	42.49±0.35 ^a	5.41±0.21 ^a
<i>Cm</i> -AgNPs	102.1±0.43 ^b	19.52±1.18 ^b
Vitamin C	3.00±0.00 ^c	0.082±0.01 ^c

Values with different superscript and in the same column are statistically significantly different at p<0.05.

AqEx= aqueous extract; *Cm*-AgNPs= *Cola millenii* silver nanoparticles.

3.2. Characterization of silver nanoparticles obtained using *Cola millenii*.

Due to the pharmacokinetic challenges many drugs face, several new strategies are being utilized to improve pharmacokinetic profiles. Cardinal among the challenges is low bioavailability and drug absorption. These challenges have been significantly mitigated by the use of Nano-based drugs, which have gained acceptance in the last few decades. Several methods for generating nano-based products are currently used, each with its pros/cons. Nanoparticles of biomedical importance produced by reduction using plants are generally regarded as eco-friendly and safe. In the present studies, silver nitrate was reduced to its nanoparticles using *Cola millenii*. Literature abounds for the use of other plants for similar biogenic synthesis [44-46]. It is asserted that phytochemicals participate in two important ways in the synthetic process. First, it is used as a reductant for the nanoparticles and, second, as a capping agent for stabilizing nano-crystal structures [47]. The success of the synthesis of *Cola millenii* (*Cm*-AgNPs) was confirmed by UV-Spectra peak at a wavelength of 465 nm (Figure 1).

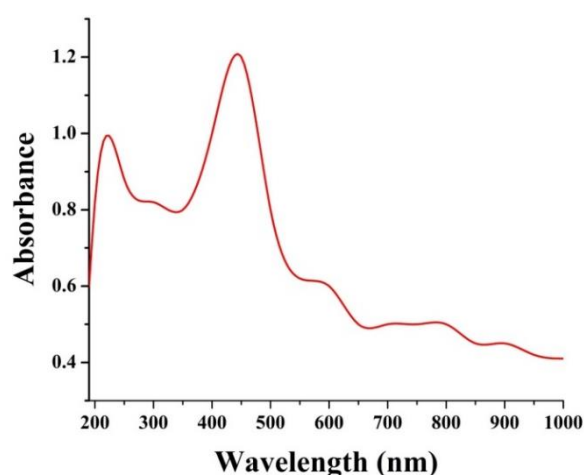


Figure 1. UV-visible absorption spectrum of *Cm*-AgNPs.

The chemical composition of the synthesized nanoparticles was determined using energy dispersion spectroscopy (EDS). The results revealed the nanoparticles contained 65.20% silver alongside other elements of varied compositions, accounting for 34.80%, as shown in Figure 2. Notably, the composition of Iron in the nanoparticles of *Cola millenii* (*Cm*-AgNPs) was 9.20%. This is presumably due to the fact that the plant material used for the study is rich in iron, which is abundant in the photosynthetic apparatus of plants.

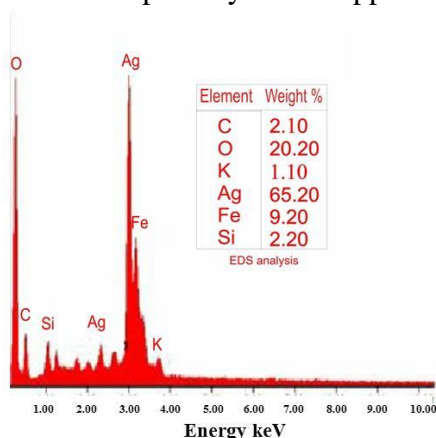


Figure 2. EDS analysis of *Cm*-AgNPs.

To further probe the association of phytochemicals from *Cola millenii* with the *Cm*-AgNPs, Fourier transform infrared spectroscopic (FT-IR) analysis was performed. The FT-IR spectra were recorded to ascertain the type of functional groups associated with the phytochemicals in the *Cm*-AgNPs. The *Cm*-AgNPs showed peaks at 3471 cm^{-1} and 3430 cm^{-1} , which corresponds to amine N-H stretch, 3419 and 2966 cm^{-1} is strong alcohol O-H stretch, 2149 cm^{-1} , alkyne C≡C stretch, absorption band 1686 cm^{-1} corresponds to C=O of carboxylic groups. The absorption bands 1626 cm^{-1} and 1417 cm^{-1} show the presence of C=C stretch of alkene groups, whereas 1026 cm^{-1} corresponds to the C-O group (See Fig 3). The diversity of these functional groups may also reflect the class of phytochemicals involved in reducing silver nitrate to AgNPs.

Additionally, the pharmacological properties are, to a large degree, attributable to functional groups. Functional groups such as -OH, COOH, and NH are most notable, which can extinguish free radicals by donating electrons to complete the electron shell of radicals [48]. The absorption band at 477 cm^{-1} is indicative of Ag-O. This is very close and consistent with the Ag-O peaks reported in the literature, thereby confirming the presence of silver in the nanoparticles, as shown in Figure 3.

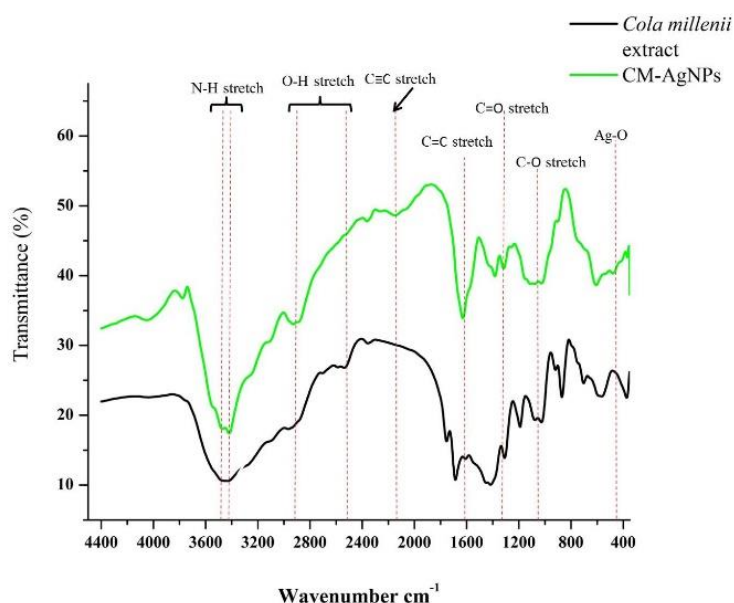


Figure 3. FT-IR spectroscopic analysis of *Cm*-AgNPs and *Cola millenii* extracts.

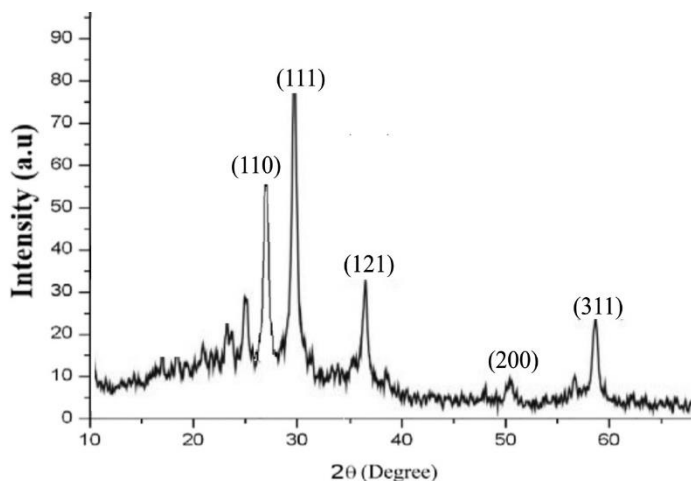


Figure 4. XRD analysis of *Cm*-AgNPs.

The crystallinity of *Cm*-AgNPs was tested by X-ray diffraction (XRD). Figure 4 shows the XRD results, which demonstrated the crystallinity of Ag produced using an aqueous extract of *Cola millenii*. The result is indicative of intense diffraction peaks corresponding to (110), (111), (121), (200), and (311) appearing at 2θ angles owing to the formation of *Cm*-AgNPs. The peaks indicate the crystalline nature of *Cm*-AgNPs, which is in agreement with the joint committee's database on powder diffraction standards.

The morphology of *Cm*-AgNPs was examined using scanning electron microscopy (SEM). The SEM image shown in Figure 5 reveals small-sized nanoparticles polygonal in shape. Transmission Electron Microscopy (TEM) was used to obtain information on the size of the nanoparticles. The TEM image presented in Figure 6 shows that the *Cm*-AgNPs are monodisperse and do not appear aggregated. This indicates the appropriateness of the phytochemicals from *Cola millenii* as a capping/stabilizing agent for the nanoparticles. Data analysis revealed that about 70% of *Cm*-AgNPs have a diameter of less than 20 nm.

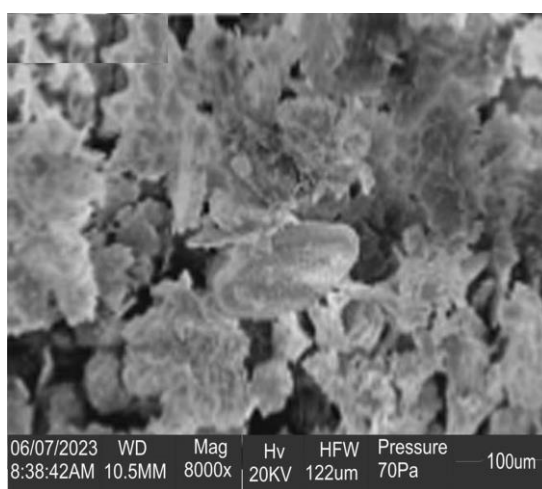


Figure 5. SEM image of *Cm*-AgNPs.

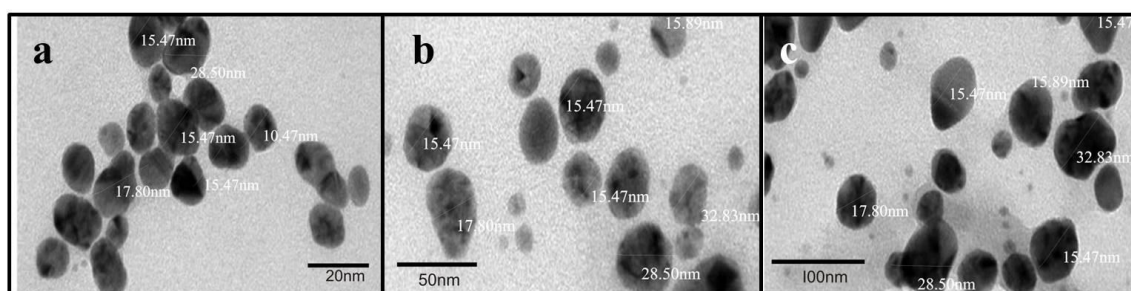


Figure 6. TEM images of *Cm*-AgNPs at (a) 20nm; (b) 50nm; (c) 100nm scale of magnification.

3.3. *In vitro* antidiabetic activity of silver nanoparticles synthesized using *Cola millenii*.

The antidiabetic activity of the *Cm*-AgNPs was determined through an *in vitro* inhibition assay of α -amylase and α -glucosidase. Both enzymes have carbohydrate hydrolyzing activity and are hence necessary for the utilization of complex carbohydrates. The sequential action of α -amylase from the pancreas and α -glucosidase from the small intestine causes the release of monosaccharide units from polysaccharides, leading to spikes in postprandial blood glucose concentration [49]. Chronically elevated blood glucose is the major underlying determinant for the development of diabetes [50]. Hence, inhibiting these two enzymes has served as an effective strategy against hyperglycemia and, by extension, diabetes. Unfortunately, many of the standard inhibitors in the market have attendant toxicity/side

effects, hence justifying the need for potent inhibitors, which added benefits of biosafety [51-55]. The *in vitro* inhibition assay results presented in Table 3 show that *Cm*-AgNPs had a significantly higher inhibitory effect on both α -amylase and α -glucosidase than the aqueous extract of *Cola millenii*. However, the reference drug acarbose exhibited a stronger inhibitory effect than *Cm*-AgNPs. The higher inhibitory effect of the *Cm*-AgNPs over its crude plant extract may be interpreted by the fact that the nanoparticles may have preferentially associated with the pharmacologically active constituents of the plant. Remember that the plant material is composed of both pharmacologically active phytochemicals and nutrients. Additionally, the *Cm*-AgNPs' activity is attributable to phytochemicals from *Cola millenii* (Table 1), whose functional groups have been identified by FT-IR (Figure 3).

Table 3. *In vitro* α -amylase and α -glucosidase inhibitory activities of aqueous extracts of *Cola millenii* and its silver nanoparticles.

Sample	Inhibition of α -amylase (IC ₅₀ μ g/mL)	Inhibition of α -glucosidase (IC ₅₀ μ g/mL)
AqEx	372.60 \pm 4.39 ^a	183.20 \pm 1.41 ^a
<i>Cm</i> -AgNPs	74.06 \pm 1.12 ^b	68.50 \pm 0.51 ^b
Acarbose	29.96 \pm 0.12 ^c	34.10 \pm 0.01 ^c

Values with different superscript and in the same column are statistically significantly different at $p < 0.05$.

AqEx= aqueous extract; *Cm*-AgNPs= *Cola millenii* silver nanoparticles.

4. Conclusions

In this study, we have shown that *Cola millenii* is rich in phytochemicals and effectively reduces silver in its nanoparticles. The *Cm*-AgNPs were small, crystalline, and associated with phytochemicals with characteristic nucleophilic functional groups. Finally, the *Cm*-AgNPs had antioxidant properties and strong inhibitory activity against carbohydrate hydrolyzing enzymes linked to diabetes. Therefore, the *Cm*-AgNPs may be further developed as a nano-based formulation for managing diabetes and other diseases in which oxidative stress is implicated in its etiology.

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Conflicts of Interest

The authors declare no conflict of interest.

References

- 1 Gökçay, B.; Berna, A. Nanotechnology, nanomedicine; ethical aspects. *Rev. Rom. Bioet.* **2015**, *13*.
- 2 Ling, T.S.; Chandrasegaran, S.; Xuan, L.Z.; Suan, T.L.; Elaine, E.; Nathan, D.V.; Chai, Y.H.; Gunasekaran, B.; Salvamani, S. The Potential Benefits of Nanotechnology in Treating Alzheimer's Disease. *BioMed Res. Int.* **2021**, *2021*, 5550938, <https://doi.org/10.1155/2021/5550938>.

- 3 Song, N.; Zhang, J.; Zhai, J.; Hong, J.; Yuan, C.; Liang, M. Ferritin: A Multifunctional Nanoplatfrom for Biological Detection, Imaging Diagnosis, and Drug Delivery. *Acc. Chem. Res.* **2021**, *54*, 3313-3325, <https://doi.org/10.1021/acs.accounts.1c00267>.
- 4 Freitas, R.A., Jr. The future of nanofabrication and molecular scale devices in nanomedicine. *Stud. Health Technol. Inform.* **2002**, *80*, 45-59.
- 5 van der Meel, R.; Sulheim, E.; Shi, Y.; Kiessling, F.; Mulder, W.J.M.; Lammers, T. Smart cancer nanomedicine. *Nat. Nanotechnol.* **2019**, *14*, 1007-1017, <https://doi.org/10.1038/s41565-019-0567-y>.
- 6 Sun, M.; Sen Gupta, A. Vascular Nanomedicine: Current Status, Opportunities, and Challenges. *Semin. Thromb. Hemost.* **2020**, *46*, 524-544, <https://doi.org/10.1055/s-0039-1692395>.
- 7 Lemmerman, L.R.; Das, D.; Higueta-Castro, N.; Mirmira, R.G.; Gallego-Perez, D. Nanomedicine-Based Strategies for Diabetes: Diagnostics, Monitoring, and Treatment. *Trends Endocrinol. Metab.* **2020**, *31*, 448-458, <https://doi.org/10.1016/j.tem.2020.02.001>.
- 8 Luo, X.-M.; Yan, C.; Feng, Y.-M. Nanomedicine for the treatment of diabetes-associated cardiovascular diseases and fibrosis. *Adv. Drug Deliv. Rev.* **2021**, *172*, 234-248, <https://doi.org/10.1016/j.addr.2021.01.004>.
- 9 Alberti, K. G.; Zimmet, P. Z. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabetic medicine : a Journal of the British Diabetic Association* **1998**, *15*(7), 539-553. [https://doi.org/10.1002/\(SICI\)1096-9136\(199807\)15:7<539::AID-DIA668>3.0.CO;2-S](https://doi.org/10.1002/(SICI)1096-9136(199807)15:7<539::AID-DIA668>3.0.CO;2-S)
- 10 Cloete, L. Diabetes mellitus: an overview of the types, symptoms, complications and management. *Nursing Standard* **2021**, *37*, 61-66., <https://doi.org/10.7748/ns.2021.e11709>.
- 11 Faselis, C.; Katsimardou, A.; Imprialos, K.; Deligkaris, P.; Kallistratos, M.; Dimitriadis, K. Microvascular Complications of Type 2 Diabetes Mellitus. *Curr. Vasc. Pharmacol.* **2020**, *18*, 117-124, <https://doi.org/10.2174/1570161117666190502103733>.
- 12 Ramachandran, A. Know the signs and symptoms of diabetes. *Indian J. Med. Res.*, **2014**, *140* (5), 579-81.
- 13 Mansour, A.; Mousa, M.; Abdelmannan, D.; Tay, G.; Hassoun, A.; Alsafar, H. Microvascular and macrovascular complications of type 2 diabetes mellitus: Exome wide association analyses. *Front. Endocrinol.* **2023**, *14*, 1143067. <https://doi.org/10.3389/fendo.2023.1143067>.
- 14 Padhi, S.; Nayak, A.K.; Behera, A. Type II diabetes mellitus: a review on recent drug based therapeutics. *Biomed. Pharmacother.* **2020**, *131*, 110708, <https://doi.org/10.1016/j.biopha.2020.110708>.
- 15 Flory, J.; Lipska, K. Metformin in 2019. *Jama* **2019**, *321*, 1926-1927, <https://doi.org/10.1001/jama.2019.3805>.
- 16 Taranath, T.C.; Patil, B.N. Limonia acidissima L. leaf mediated synthesis of zinc oxide nanoparticles: A potent tool against Mycobacterium tuberculosis. *Int. J. Mycobacteriol.* **2016**, *5*, 197-204, <https://doi.org/10.1016/j.ijmyco.2016.03.004>.
- 17 Pulit-Prociak, J.; Banach, M. Silver nanoparticles – a material of the future...? **2016**, *14*, 76-91, <https://doi.org/10.1515/chem-2016-0005>.
- 18 Jamdagni, P.; Khatri, P.; Rana, J.S. Green synthesis of zinc oxide nanoparticles using flower extract of *Nyctanthes arbor-tristis* and their antifungal activity. *J. King Saud Univ. Sci.* **2018**, *30*, 168-175, <https://doi.org/10.1016/j.jksus.2016.10.002>.
- 19 Rouhi, J.; Mahmud, S.; Naderi, N.; Ooi, C.H.R.; Mahmood, M.R. Physical properties of fish gelatin-based bio-nanocomposite films incorporated with ZnO nanorods. *Nanoscale Res. Lett.* **2013**, *8*, 364, <https://doi.org/10.1186/1556-276X-8-364>.
- 20 Premanathan, M.; Karthikeyan, K.; Jeyasubramanian, K.; Manivannan, G. Selective toxicity of ZnO nanoparticles toward Gram-positive bacteria and cancer cells by apoptosis through lipid peroxidation. *Nanomed.: Nanotechnol. Biol. Med.* **2011**, *7*, 184-192, <https://doi.org/10.1016/j.nano.2010.10.001>.
- 21 Pandurangan, M.; Kim, D.H. In vitro toxicity of zinc oxide nanoparticles: a review. *J. Nanoparticle Res.* **2015**, *17*, 158, <https://doi.org/10.1007/s11051-015-2958-9>.
- 22 Makarov, V.V.; Love, A.J.; Sinitsyna, O.V.; Makarova, S.S.; Yaminsky, I.V.; Taliansky, M.E.; Kalinina, N.O. "Green" Nanotechnologies: Synthesis of Metal Nanoparticles Using Plants. *Acta naturae* **2014**, *6*, 35-44.
- 23 Ratsch, C.; Hofmann, A. The Encyclopedia of Psychoactive Plants: Ethnopharmacology and Its Applications. Simon and Schuster, **2005**.
- 24 Sonibare, M.; Soladoye, M.; Esan, O.; Sonibare, O. Phytochemical and antimicrobial studies of four species of *Cola* Schott & Endl.(Sterculiaceae). *Afr. J. Tradit. Complement. Altern. Med.* **2009**, *6*, 518-525. <https://doi.org/10.4314/ajtcam.v6i4.57182>.

- 25 Ogbu, J.U.; Umeokechukwu, C.E. Aspects of Fruit Biology of Three Wild Edible Monkey Kola Species Fruits (Cola spp: Malvaceae). *Annu. Rev. Cell Biol.* **2014**, *4*, 2007-2014, <https://doi.org/10.9734/ARRB/2014/8066>.
- 26 Engel, N.; Oppermann, C.; Falodun, A.; Kragl, U. Proliferative effects of five traditional Nigerian medicinal plant extracts on human breast and bone cancer cell lines. *J. Ethnopharmacol.* **2011**, *137*, 1003-1010, <https://doi.org/10.1016/j.jep.2011.07.023>.
- 27 Oghenerobo, V.I.; Falodun, A. Antioxidant activities of the leaf extract and fractions of *Cola lepidota* K. Schum (sterculiaceae). *Nig. J. Biotech.* **2013**, *25*, 31-36.
- 28 Juárez Olguín, H.; Rivera Espinosa, L.; Calderón Guzmán, D. Clinical consequences of anxiolytics binded to plasma proteins. *Afr. J. Pharm. Pharmacol.* **2013**, *7*, 3019-3025, <https://doi.org/10.5897/AJPP2013.3416>.
- 29 Odugbemi, T. *Outlines and Pictures of Medicinal Plants from Nigeria*; University of Lagos Press: **2008**.
- 30 Mythili, K.; Reddy, C.U.; Chamundeeswari, D.; Manna, P.K. Determination of Total Phenol, Alkaloid, Flavonoid and Tannin in Different Extracts of *Calanthe Triplicata*. *J. Pharmacogn. Phytochem.* **2014**, *2*, 40-44.
- 31 Singh, R.; Mendhulka, V.D. *Abutilon indicum* (Linn.) Sweet leaves, a Natural source of Saponin: a Spectrophotometric assay. *Int. J. Pharm. Tech. Res.* **2015**, *8*, 725-729.
- 32 Tofighi, Z.; Ghazi saeidi, N.; Hadjiakhoondi, A.; Yassa, N. Determination of cardiac glycosides and total phenols in different generations of *Securigera securidaca* suspension culture. *Res. J. Pharmacogn.* **2016**, *3*, 23-31.
- 33 Atanu, F.O.; Idih, F.M.; Nwonuma, C.O.; Hetta, H.F.; Alamery, S.; El-Saber Batiha, G. Evaluation of Antimalarial Potential of Extracts from *Alstonia boonei* and *Carica papaya* in *Plasmodium berghei*-Infected Mice. *Evid.-Based Complementary Altern. Med.* **2021**, *2021*, 2599191, <https://doi.org/10.1155/2021/2599191>.
- 34 Shah, M.D.; D'Souza, U.J.A.; Iqbal, M. The potential protective effect of *Commelina nudiflora* L. against carbon tetrachloride (CCl₄)-induced hepatotoxicity in rats, mediated by suppression of oxidative stress and inflammation. *Environ. Health Prev. Med.* **2017**, *22*, 66, <https://doi.org/10.1186/s12199-017-0673-0>.
- 35 Atanu, F.O.; Ikeojukwu, A.; Owolabi, P.A.; Avwioroko, O.J. Evaluation of chemical composition, *in vitro* antioxidant, and antidiabetic activities of solvent extracts of *Irvingia gabonensis* leaves. *Heliyon* **2022**, *8*, e09922, <https://doi.org/10.1016/j.heliyon.2022.e09922>.
- 36 Oboh, G.; Ademosun, A.O.; Ademiluyi, A.O.; Omojokun, O.S.; Nwanna, E.E.; Longe, K.O. *In Vitro* Studies on the Antioxidant Property and Inhibition of α -Amylase, α -Glucosidase, and Angiotensin I-Converting Enzyme by Polyphenol-Rich Extracts from Cocoa (*Theobroma cacao*) Bean. *Pathol. Res. Int.* **2014**, *2014*, 549287, <https://doi.org/10.1155/2014/549287>.
- 37 Atanasov, A.G.; Waltenberger, B.; Pferschy-Wenzig, E.-M.; Linder, T.; Wawrosch, C.; Uhrin, P.; Temml, V.; Wang, L.; Schwaiger, S.; Heiss, E.H.; Rollinger, J.M.; Schuster, D.; Breuss, J.M.; Bochkov, V.; Mihovilovic, M.D.; Kopp, B.; Bauer, R.; Dirsch, V.M.; Stuppner, H. Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnol. Adv.* **2015**, *33*, 1582-1614, <https://doi.org/10.1016/j.biotechadv.2015.08.001>.
- 38 Henkin, J.M.; Ren, Y.; Soejarto, D.D.; Kinghorn, A.D. The Search for Anticancer Agents from Tropical Plants. In *Progress in the Chemistry of Organic Natural Products* 107, Kinghorn, A.D., Falk, H., Gibbons, S., Kobayashi, J.I., Asakawa, Y., Liu, J.-K., Eds.; Springer International Publishing: Cham, **2018**; Volume 107, pp. 1-94, https://doi.org/10.1007/978-3-319-93506-5_1.
- 39 Pérez-Pérez, A.; Vilarinho-García, T.; Guadix, P.; Dueñas, J.L.; Sánchez-Margalet, V. Leptin and Nutrition in Gestational Diabetes. *Nutrients* **2020**, *12*, 1970, <https://doi.org/10.3390/nu12071970>.
- 40 Silva, M.L.; Bernardo, M.A.; Singh, J.; de Mesquita, M.F. Cinnamon as a Complementary Therapeutic Approach for Dysglycemia and Dyslipidemia Control in Type 2 Diabetes Mellitus and Its Molecular Mechanism of Action: A Review. *Nutrients* **2022**, *14*, 2773, <https://doi.org/10.3390/nu14132773>.
- 41 Islam, M.T. Oxidative stress and mitochondrial dysfunction-linked neurodegenerative disorders. *Neurosci. Res.* **2017**, *39*, 73-82, <https://doi.org/10.1080/01616412.2016.1251711>.
- 42 Senoner, T.; Dichtl, W. Oxidative Stress in Cardiovascular Diseases: Still a Therapeutic Target?. *Nutrients* **2019**, *11*, 2090, <https://doi.org/10.3390/nu11092090>.
- 43 Kang, Q.; Yang, C. Oxidative stress and diabetic retinopathy: Molecular mechanisms, pathogenetic role and therapeutic implications. *Redox Biol.* **2020**, *37*, 101799, <https://doi.org/10.1016/j.redox.2020.101799>.

- 44 Parvataneni, R. Biogenic synthesis and characterization of silver nanoparticles using aqueous leaf extract of *Scoparia dulcis* L. and assessment of their antimicrobial property. *Drug Chem. Toxicol.* **2020**, *43*, 307–321, <https://doi.org/10.1080/01480545.2018.1505903>.
- 45 Lu, H.; Zhang, X.; Khan, S.A.; Li, W.; Wan, L. Biogenic Synthesis of MnO₂ Nanoparticles With Leaf Extract of *Viola betonicifolia* for Enhanced Antioxidant, Antimicrobial, Cytotoxic, and Biocompatible Applications. *Front. Microbiol.* **2021**, *12*, 761084. <https://doi.org/10.3389/fmicb.2021.761084>.
- 46 Zinsaz, P.; Jafarizadeh-Malmiri, H.; Anarjan, N.; Nekouiefard, A.; Javadi, A. Biogenic synthesis of gold nanoparticles using *Artemia urumiana* extract and five different thermal accelerated techniques: fabrication and characterization. *Zeitschrift für Naturforschung C* **2022**, *77*, 395-402, <https://doi.org/10.1515/znc-2021-0323>.
- 47 Gami, B.; Bloch, K.; Mohammed, S.M.; Karmakar, S.; Shukla, S.; Asok, A.; Thongmee, S.; Ghosh, S. *Leucophyllum frutescens* mediated synthesis of silver and gold nanoparticles for catalytic dye degradation. *Front. Chem.* **2022**, *10*, 932416. <https://doi.org/10.3389/fchem.2022.932416>.
- 48 Ji, Z.; Sheardy, A.; Zeng, Z.; Zhang, W.; Chevva, H.; Allado, K.; Yin, Z.; Wei, J. Tuning the Functional Groups on Carbon Nanodots and Antioxidant Studies. *Molecules* **2019**, *24*, 152, <https://doi.org/10.3390/molecules24010152>.
- 49 Ceriello, A.; Prattichizzo, F.; Phillip, M.; Hirsch, I.B.; Mathieu, C.; Battelino, T. Glycaemic management in diabetes: old and new approaches. *Lancet Diabetes Endocrinol.* **2022**, *10*, 75-84, [https://doi.org/10.1016/S2213-8587\(21\)00245-X](https://doi.org/10.1016/S2213-8587(21)00245-X).
- 50 Cisneros-Yupanqui, M.; Lante, A.; Mihaylova, D.; Krastanov, A.I.; Rizzi, C. The α -Amylase and α -Glucosidase Inhibition Capacity of Grape Pomace: A Review. *Food Bioprocess Technol.* **2023**, *16*, 691-703, <https://doi.org/10.1007/s11947-022-02895-0>.
- 51 Ayua, E.O.; Nkhata, S.G.; Namaumbo, S.J.; Kamau, E.H.; Ngoma, T.N.; Aduol, K.O. Polyphenolic inhibition of enterocytic starch digestion enzymes and glucose transporters for managing type 2 diabetes may be reduced in food systems. *Heliyon* **2021**, *7*, e06245, <https://doi.org/10.1016/j.heliyon.2021.e06245>.
- 52 Thunell, J.; Chen, Y.; Joyce, G.; Barthold, D.; Shekelle, P.G.; Brinton, R.D.; Zissimopoulos, J. Drug therapies for chronic conditions and risk of Alzheimer's disease and related dementias: A scoping review. *Alzheimers Dement.* **2021**, *17*, 41-48, <https://doi.org/10.1002/alz.12175>.
- 53 Liakos, A.; Karagiannis, T.; Avgerinos, I.; Malandris, K.; Tsapas, A.; Bekiari, E. Management of type 2 diabetes in the new era. *Hormones* **2023**, *22*, 677-684, <https://doi.org/10.1007/s42000-023-00488-w>.
- 54 Koutentakis, M.; Kuciński, J.; Świczkowski, D.; Surma, S.; Filipiak, K.J.; Gąsecka, A. The Ketogenic Effect of SGLT-2 Inhibitors—Beneficial or Harmful?. *J. Cardiovasc. Dev. Dis.* **2023**, *10*, 465, <https://doi.org/10.3390/jcdd10110465>.
- 55 Cipriani, C.; Lauriero, G.; Tripepi, G.; Ferrari, S.; Bover, J.; Ravera, M.; Barbuto, S.; Cianciolo, G.; De Nicola, L.; Brandi, M.L.; Minisola, S.; Mereu, M.C.; Corrao, G.; Del Vecchio, L.; Fusaro, M. Effect of Antidiabetic Drugs on Bone Health in Patients with Normal Renal Function and in Chronic Kidney Disease (CKD): Insight into Clinical Challenges in the Treatment of Type 2 Diabetes. *J. Clin. Med.* **2023**, *12*, 7260, <https://doi.org/10.3390/jcm12237260>.