



GREEN BIOSYNTHEZED SILVER NANOPARTICLES MEDIATED BY *TRICHOSANTHES CUCUMERINA* EXTRACT AND ITS BIOLOGICAL ACTIVITIES

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ABSTRACT

Biosynthesized Nanoparticles especially from plants are a new research trend nowadays. In this study, green biosynthesis of silver nanoparticles (TCAgNPs) employing *Trichosanthes cucumerina* methanol leaf extract was realized. The antioxidant and anti-inflammatory activities of both the methanol extract of *T. cucumerina* and TCAgNPs were carried out and compared. Indomethacin and ascorbic acid were employed as a positive control for these activities respectively. Fourier transform infrared spectroscopy (FT-IR) scanning electron microscopy (SEM) and Ultraviolet-visible spectroscopy were employed to characterize the formation of TCAgNPs. The TCAgNPs showed absorption bands at around 340-440 nm which is a distinctive band for Ag Nanoparticles and functional groups accountable for Ag nanoparticles synthesized by plants were noticed in the FTIR. TCAgNPs were spherical in morphology as seen from the SEM images. TCAgNPs displayed better antioxidant and anti-inflammatory activities at 400 ug/ml (9.79 ± 0.58) as compared with its polar extracts (7.74 ± 0.58) but both the polar part (52.06 ± 1.27) and TCAgNPs (61.72 ± 2.54) were better in anti-inflammatory activity when compared with the positive control indomethacin (antiinflammatory) (48.30 ± 0.23). This study revealed that AgNPs from plants can find germaneness in food, drugs, environmental sciences and drugs.

Keywords: antioxidant; nanoparticles; *T. cucumerina*; FTIR; green biosynthesized; anti-inflammatory activities

INTRODUCTION

Cucurbitaceae, also known as melons, gourds, or cucurbits, is a plant family that includes, squashes, melons (including watermelons) and luffas, cucumbers (including pumpkins). The family is mostly found in the tropics, where edible fruits were among the first cultivated plants in both the Americas and Europe. Snake gourd, or *Tricosanthes cucumerina* as it is technically known, is a well-known plant whose fruit is primarily used as a vegetable. It belongs to the Cucurbitaceae family and is an annual climber. It's also known as viper gourd, long tomato, snake tomato or snake gourd are all names for the same thing (Adebooye, 2008; Ojiako and Igwe, 2008). Due to its high nutritional value, the fruit is commonly consumed as a vegetable. Vitamin A, Vitamin B, and Vitamin C are all abundant in the berry. It stimulates hunger, functions as a tonic and stomachic, and relieves biliousness. Many ayurvedic remedies use the wild bitter varieties. The fruits of cultivated varieties are also therapeutic, helping those with heart disease, psoriasis, rheumatism and high blood pressure. The plant has a variety of chemical elements like as carotenoids, phenolic acids, and flavonoids which provide it pharmacological and therapeutic properties (Bello et al., 2018; 2018b; 2018c).

Nanotechnology is a branch of current synthetic chemistry that focuses on the growth and production of nanoparticles and their hybrids with varying sizes, shapes, and chemical structures that have applications in a variety of sectors, including medicine. The use of green chemistry in the manufacture of nanoparticles is, nevertheless, the present trend. Although chemical (Sodium borohydride) and physical (mechanical attrition) approaches can produce pure, well-defined nanoparticles, they are both costly and possibly hazardous to the environment if discarded improperly (Barbara and Nora, 2005; Hoag et al., 2009; Prasad and Elumalai, 2011). As a result, the plant extract is used in green synthesis as an alternate technique of creating nanoparticles. The primary ingredient in the plant extract, polyphenols, has both reducing and capping properties (Bello et al., 2019; 2020). The study aims at exerting the biological activities of extract of *T. cucumerina*, synthesizing its silver nanoparticle thus comparing the activities of both *T. cucumerina* and its biosynthesized nanoparticle.

Material and Methods

Trichosanthes cucumerin was collected in October around Ilorin metropolis, Kwara State, Nigeria. The leaves were plucked from the plant and air-dried for three weeks, after

drying the leaves were crushed into powder form and kept in a sealed container.

Preparation of Plant Extracts

The powdered leaves of *T. cucumerin* were soaked in N-hexane and methanol, these were left for four days. The hexane and methanol extract was concentrated using a rotary evaporator to give crude extracts for both solvents.

Preparation of Silver Nanoparticle of *T. cucumerin*

The synthesis of silver (Ag) nanoparticles of *T. cucumerin* was carried out by employing a procedure as described by Bello et al. (2019, 2020). The synthesized nanoparticles are labeled TC-AgNPs

Phytochemical Screening

Phytochemical screening was carried out to identify the presence of phenols, saponins, triterpenes, flavonoids, alkaloids, and steroids in the *T. cucumerin* leaf extracts ((Sofowora, 1993; Trease & Evans, 1989).

Characterization of Silver Nanoparticles

TC-AgNPs with the leaves' extract was characterized employing techniques such as Ultra-violet/Visible (UV-vis), Fourier Transform Infrared (FTIR) and Scanning electron microscopy (SEM).

Ultra-Violet/Visible Spectroscopy

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

Fourier Transform Infrared Analysis (FTIR)

The FTIR analysis was done for the nanoparticles formed and the extracts of these plants were viewed employing the equipment and its software reported earlier by Bello et al., (2019, 2020).

Scanning Electron Microscopy (SEM)

The nanoparticles of these plants' extracts were viewed employing the equipment and its software reported earlier by Bello et al., (2019, 2020).

BIOLOGICAL ACTIVITIES

Anti-inflammation Cell Stabilization Membrane (CSM)

The method employed in carrying out the anti-inflammatory activity of these extracts has been reported by both Bello et al., (2019) and Oyedapo et al., (1997; 2004). All tests and analyses were run in triplicate and averaged

Antioxidant Activity

2, 2'-azino-bis-(3-ethyl) benzothiazoline-6- sulfonic acid (ABTS) radical cation scavenging Activity

The ABTS⁺ radicals' activity employed has been previously been reported by some authors though with little adjustment (Shinde et al., 1999; Oguntoye et al., 2018; Bello et al., 2020). All analysis was determined in duplicate.

RESULT AND DISCUSSION

Phytochemical Screening

Phytochemical constituents of the extract of *Trichosanthes cucumerin* is shown in Table 1. On the whole, flavonoids, steroids, triterpenes and polyphenol were identified both in the hexane and methanol extract of the plant. The hexane extract of *T. cucumerin* gave a poor result for most phytoconstituents explored in **Table 1**.

Table 1: Phytochemical Screening

		<i>Trichosanthes cucumerin</i>	
		MeOH	Hexane
1	Polyphenols	+++	+
2	Steroids	++	--
3	Flavonoids	+++	+
4	Triterpenes	++	-
5	Alkaloids	---	---
6	Saponins	+	---

Characterization

UV-Visible Spectroscopy Study

Colour changes when the AgNPs were formed is a physical examination (visual), TCAgNPs formed as indicated by a change in colour as shown in **Table 2**. Many studies have revealed that AgNPs showed such colour variations in polar solution due to AgNPs surface plasmon resonance excitation (SPR), this is the first confirmation that AgNPs were formed (Dana et al., 2002; Kotakadi et al., 2014; Bello et al., 2020). UV – Vis spectroscopy was further employed to examine the AgNPs formed, it was discovered that Figure 3 shows the curve in each spectrum of produced silver nanoparticles that were absorbed in the wavelength range of 420-440 nm for silver nanoparticles but 440 nm for *T. cucumerina*. According to some publications, this peak falls within the nanoparticle specification range (**Figure 1**).

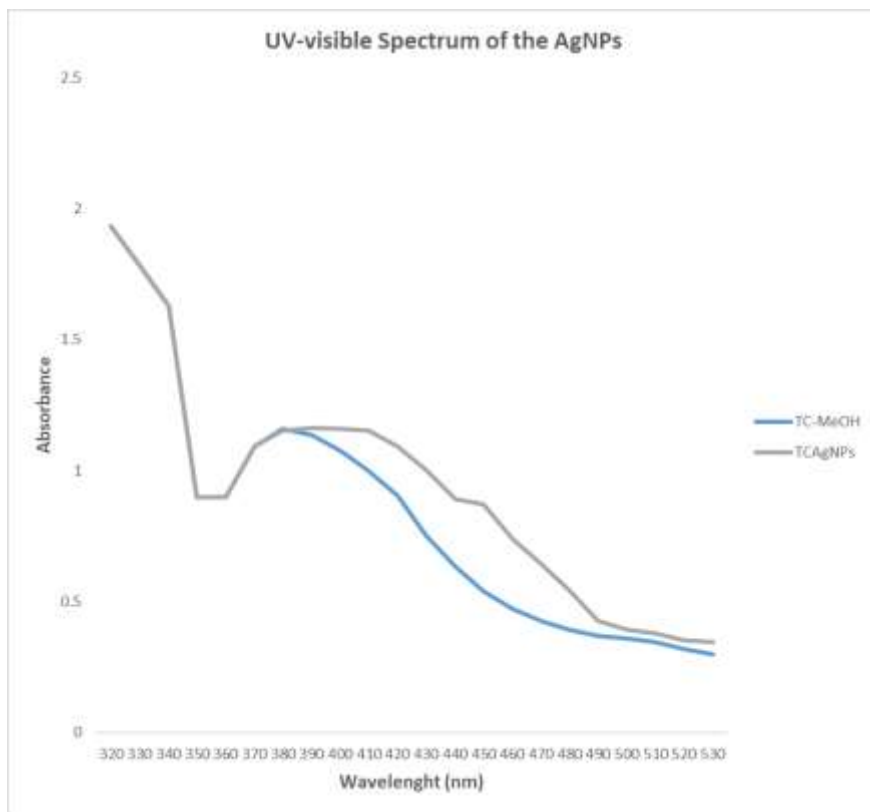


Figure 1: UV-visible spectra of the Synthesized TCAgNPs and TC-MeOH

Table 2: AgNPs Change in Colour Observed

<i>T. cucumerin</i>	Colour Change	
	Initial	Final
Methanol Extract	Black	Brown
Hexane Extract	Light brown	Deep Brown

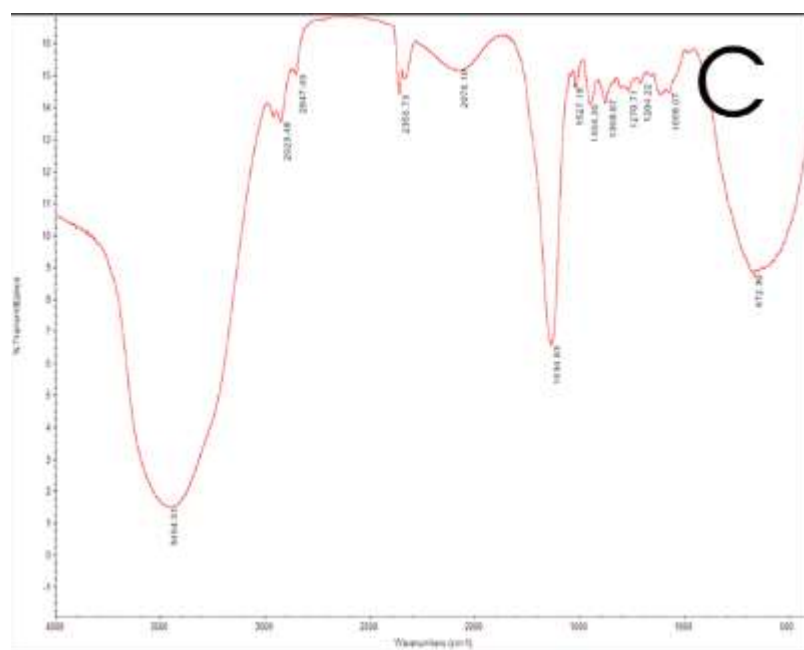


Figure 2: FTIR spectrum for TC-AgNPs

FT-IR Spectroscopy Study

FTIR spectroscopy studies were utilized to identify and describe the biological reduction functional group, which will reveal the likely group of organic compounds found in these underappreciated vegetables that are responsible for the reduction of Ag^+ ions to elemental Ag^0 and finally resulting in the successful stabilization of the silver nanoparticles. The FTIR spectra of the synthesized AgNPs of the *T. cucumerina* are shown in Figure 2. The Infrared spectrum of TCAGNPs revealed the existence of the OH functional group with broadband at 3429.41 cm^{-1} . The TCAGNPs spectra displayed a very sharp absorption band at 1631.64 cm^{-1} which was assigned to C=O stretch and a medium intensity at a wavelength of 1600.16 cm^{-1} which is for C=C.; $1451.19\text{-}1384.70$ (C-H, bending), 1141.42 (C-O) for the TCAGNPs synthesized (Figure 2) The broad and penetrating bands detected at roughly 3400 cm^{-1} are due to OH stretching, indicating that flavonoids and polyphenols are likely to present in the nanoparticles generated. This indicates that flavonoids, alkaloids, and other compounds may be present in this plant extract. As shown in Figure 2, the spectra revealed distinct functional groups of phytocompounds,

such as phyosterols, tannins, phenolic acids, alkaloids, flavonoids, and coumarins, all of which could be responsible for the capping and reduction of the AgNPs that were generated (Zhang et al., 2016; Bello et al., 2020)

Scanning Electron Microscope (SEM)

To quantify the concentration of silver generated in the nanoparticles, SEM detects the physiognomies of the AgNPs' surface, its shape, and the distribution of TCAGNPs described on the SEM micrograph (Figure 3a and 3b). Due to the surface plasma resonance phenomena, nanoparticles produced from silver usually have a distinct absorbance characteristic peak at around 3 keV (Filippo et al., 2010; Zayed et al., 2012). The image indicated uneven dispersion and holes, indicating that Ag nanoparticles might be employed as both for toxicant removal (adsorbent) and antibacterial material. The nanoparticles synthesized by *T. cucumerina* were highly agglomerated for TCAGNP showed a brief scattered morphology (Figure 3) (Bello et al., 2019). MubarakAli et al. (2011) attribute this cluster revealed by TCAGNPs to a dehydration-induced combination of Ag nanoparticles.

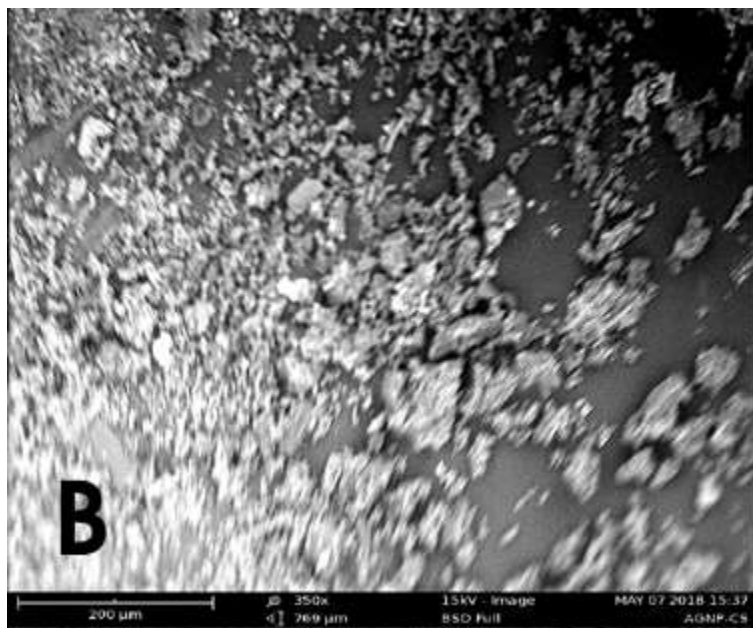


Figure 3a: SEM for picture for TCAGNPs

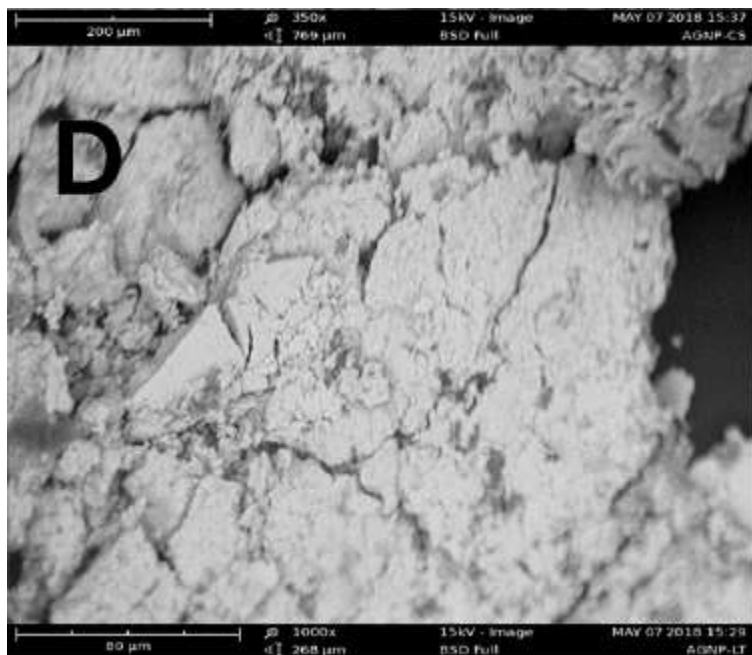


Figure 3b: SEM for picture for TCMeOH

Table 3: Antioxidant and anti-inflammatory activities of TC-AgNP and MeOH extract of *T. cucumerin*

<i>T. cucumerin</i>						
ug/ml	ABTS			CSM		
	Ascorbic acid	TCAgNPs	MeOH	TCAgNPs	MeOH	Indomethacin
100	19.65±0.60	3.15±2.56	2.64±2.71	54.22±1.25	54.85±0.62	28.02±0.56
200	22.61±0.38	6.72±0.72	4.74±1.72	55.22±0.36	50.15±0.34	44.84±1.62
300	27.11±6.03	7.74±1.04	5.31±3.18	56.79±0.56	46.20±0.87	45.76±0.71
400	28.34±4.96	8.89±1.55	6.25±3.22	58.18±1.06	51.47±0.54	46.32±1.26
500	23.55±0.15	9.79±0.58	7.74±0.58	61.72±2.54	52.06±1.27	48.30±0.23

Biological Activities

Antioxidant Activity

The methanolic extract of *T. cucumerina* with its biosynthesized silver nanoparticles was assessed and compared using cell-based assays for their antioxidant activity as shown in Table 3. The TCAgNPs and the methanol extract for the plant were assessed for *in vitro* activity employing ABTS assay only. The results are expressed in terms of IC₅₀ (the concentration that caused a 50 % inhibition) and presented in Table 3. These were carried out with *in vitro* method at various concentrations (100, 200, 300....500 µg/mL) of the extract. The TCAgNPs and the extracts apt to show a major antioxidant activity at the dose 100 µg/ml concentration, this was similar to the ascorbic acid employed as the positive control. The antioxidant activity climax as the concentration surge high, the highest antioxidant activity was a witness at 400 µg/mL as shown in Table 3. This meant both the extract and the TCAgNPs are dose-dependent and dose-related. Though the nanoparticle and its extract cannot be favourably compared with the positive control but the TCAgNPs showed a better activity when compared with its polar extract.

Anti-inflammation Activity

The methanolic extract of *T. cucumerina* with its biosynthesized silver nanoparticles was assessed and compared using cell-based assays for their anti-inflammatory activity as shown in Table 3. The TCAgNPs and the methanol extract for the plant were assessed for *in vitro* activity employing the Human Red Blood Cell Membrane Stabilization method only. The results are expressed in terms of IC₅₀ (the concentration that caused a 50 % inhibition) and presented in Table 3. These were carried out with *in vitro* method at various concentrations (100, 200, 300....500 µg/mL) of the extract. The TCAgNPs and the methanol extracts are apt to show a major anti-inflammatory activity at the dose 100 µg/ml concentration, this was similar to the indomethacin employed as a positive control. The anti-inflammatory activity climax as the concentration surge high, the highest anti-inflammatory activity was a witness at 400 µg/mL as shown in Table 3. This meant both the extract and the TCAgNPs are dose-dependent and dose-related. Though the nanoparticle and its extract cannot be favourably compared with the positive control but the TCAgNPs showed a better activity when compared with its polar extract.

Future Direction and Conclusion

Future research will focus on isolating and elucidating bioactive compounds in the active fractions of this plant utilizing chromatographic techniques, spectroscopic techniques, and mass spectrometry (MS). Identification of the exact receptors on which these potent plant extracts and their corresponding manufactured AgNPs may have anti-inflammatory actions. In this study, it was discovered that the synthesis of AgNPs using a simple, cost-effective, non-toxic, and repeatable green chemistry approach results in higher antioxidant and anti-inflammation value. The produced AgNPs were found to be stable, and the FTIR results revealed that phytochemicals may have played a role in AgNP stabilization and reduction. The study has demonstrated the viability of replacing sodium borohydride with plant extract, which acts as a reducing and capping agent. These qualities are thought to be the green plants' advantage over the poisonous sodium borohydride.

Conflict of Interest

No conflict of interest among the authors based.

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