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In-vitro Antihypertensive, Antifungal and Antioxidant properties of Green Synthesized Gold Nanoparticles from *Strophantus hispidus* leaf aqueous extract

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Abstract

Nanotechnology, the science of manipulating matter at atomic level has paved way for many innovative ideas in all areas. In this research, the green synthesis of gold nanoparticles from Strophanthus hispidus and some of its biomedical applications were evaluated. The aqueous extract of Strophanthus hispidus was employed in the mediation of gold nanoparticles, the reaction resulted in deep purple colour colloidal suspension formation after 15 minutes of photo-activation. The colloidal suspension was subjected to characterization using UV-Vis spectroscopy, FTIR, EDX and TEM. The UV-Vis spectrum of the AuNPs displayed strong peak at 552 nm. The FTIR showed broad peaks at 3417.98, 2918.40, 2359.02, 2000.25, 1766.85, 1622.19, 1384.94 and 1097.53 cm^{-1} which are attributed to the involvement of sugar, alkaloids and proteins in the AuNPs synthesis and stabilization. Gold was the prominent metal observed in the EDX analysis while the TEM micrograph showed spherical particles whose sizes are between 10.90 and 34.63 50nm.The AuNPs showed antifungal activities of 87.6, 70.1, 72.5, 82.8 and 51.8% against Aspergillus flavus, Aspergillus niger, Fusarium poae, Fusarium solani and Penicillium avenatum respectively at 50 µl /ml. The AuNPs showed significant antioxidant properties of 90.96, 88.09, 85.23 and 84.47% at 50, 100, 150 and 200µl/ml against DPPH. The AuNPs showed a significant angiotensin converting enzyme (ACE) inhibitory activities of 60.88 ± 3.100% at concentration of 50µg/ml. Conclusively, this study has established the relevance of Strophanthus hispidus leaf aqueous extract in the bio-fabrication of eco-friendly gold nanoparticles. The nanoparticles showed significant antifungal properties which could be useful in the production of eco-friendly fungicides, and also its antihypertensive properties have proven its drug improvement potentials, also the antioxidant activity of the gold nanoparticles could be useful in combating against oxidative stress.

Keywords: Eco-friendly, gold nanoparticles, antioxidant, antifungal, anti-hypertensive.

Introduction

Since the birth of nanotechnology, the science of manipulating matter at atomic level, latent innovations in areas of technology and bio-medicine are being emancipated beyond the confines of imagination. Nanoparticles, the minutest of all particles now holds the key to many lasting solutions in science and technology (Kolahalam *et al.,* 2019; Milan *et al.*, 2022). Medicinal plants in the synthesis of metallic nanoparticles have proven effective over the years, as the bioactive molecules react with the precursor by reducing and stabilizing it, they are increased in reactivity, permeability, and surface area (Oladipo & Ogunsona, 2019).*Strophanthus hispidus* is a medicinal plant to reckon with when it comes to phytochemicals constitution. *Strophanthus hispidus* belongs to the family Apocynaceae, popularly known as poison arrow vine (Balde *et al*., 2015). In western Africa, Nigeria, it locally called "*Isagere*". It is very useful in folk medicine as it is used in the treatment of arthritis, constipation, diabetes, hypertension, inflammatory, gonorrhea, ulcer and rheumatism to mention a few (Ezuruike and Prieto, 2014; Agbaje & Fageyinbo, 2014; Taofik *et al*., 2015). *Strophanthus hispidus* contain a great deal of alkaloids, saponins and flavonoids (Mensah *et al*., 2019; Owoola, 2021). On the evidence of *Strophanthus hispidus* phytochemicals, the research is aimed at synthesizing gold nanoparticles mediated with the aqueous extract of *Strophanthus hispidus* and evaluating its in-vitro antihypertensive, free radical scavenging and mycelial inhibitory properties.

Materials and Methods

Synthesis and Characterization of Gold nanoparticles

The synthesis of the gold nanoparticles was mediated with the

aqueous extract of *Strophanthus hispidus* leaf. The air dried and pulverized leaf sample of *Strophanthus hispidus* was obtained and subjected to aqueous extraction in water bath at 60°C by weighing 1 g in 100 ml of distilled water.0.5 ml of the filtered and centrifuged aqueous leaf extract was mediated with 10 ml of 1mM of gold chloride at room temperature. At stabilization, the gold nanoparticles were subjected to characterization with UV-vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM) and Energy dispersive X-ray (EDX) analysis.

In-vitro Antihypertensive Assay

Angiotensin I-Converting-Enzyme (ACE) Inhibitory Activity

Employing the method of Jimsheena and Gowda (2010) the Angiotensin I-Converting-Enzyme (ACE) Inhibitory activity of the synthesized gold nanoparticles was evaluated through the measurement of Hippuric acid (HA) release from the substrate Hippuryl-L-Histidyl-L-Leucine (HHL). The assay mixture constitutes 0.3M NaCl,0.05ml of 5MmHHL and 0.025ml of ACE (2.5MU) in 0.125ml of a 0.05M Sodium bromate buffer(pH8.2) which was pre-incubated with the nanoparticles concentrations of 50, 100, 150and 200 µg/ml. After incubation of the mixture at 37°C for 30 minutes, the reaction was stopped by the addition of 0.2ml of 1MHCl pyridine (0.4ml) followed by 0.2ml of Benzene Sulphonyl Chloride (BSC), the addition of the reagents was critically monitored, and mixed by inversion of 1 minute then cooled on ice. Absorbance wavelength for Uv-Vis spectroscopy was at 410nm in a microtiter plate. The degree of ACE inhibition (%) was calculated with the formula:

$$\text{ACE inhibition } \% = \frac{A1-A2}{A1-A3} \times 100$$

A1=Absorbance of the ACE solution without an inhibitor
A2=Absorbance of the tested sample
A3= Absorbance of HHL solution (a buffer was added instead of ACE solution and sample)

Anti-radical Activities of Biosynthesized Gold Nanoparticles

2, 2-diphenyl-1-picryhydrazyl (DPPH) scavenging Potentials

The antioxidant activity of the nanoparticles was evaluated by using the antioxidant assay method of Oladipo *et al*. (2020). To determine the antioxidant activity of the nanoparticles, 0.04g of DPPH was dissolved into 1000ml of methanol, different concentrations (50, 100, 150 and 200µl/ml) of the gold nanoparticles were reacted with 4ml of the prepared methanolic DPPH, and the mixtures were kept in the dark cupboard to enhance the scavenging of the gold nanoparticles for DPPH radicals, after 30 minutes, the absorbance was measured at 517 nm using UV-visible spectrophotometer. The scavenging percentage was calculated using the formula below:

$$\text{DPPH scavenging (\%)} = \frac{\text{Absorbance control} - \text{Absorbance of sample}}{\text{Absorbance control}} X\ 100$$

Antifungal Activity

The method of Oladipo *et al.* (2020) was employed to carry out the antifungal assay. The lowest concentration of the gold nanoparticles i.e. 50 µl /ml was incorporated into potato dextrose agar plates, which were then inoculated with 8mm agar plug of 48-h old cultures of *Aspergillus flavus, Aspergillus niger, Fusarium poae, Fusarium solani and Penicillium avenatum*. The control was the fungal plugs inoculated on PDA plates without the incorporation of the gold nanoparticles. The plates were incubated at room temperature for 72 hours. The diameters of fungi mycelial growths in all the plates were measured and used in evaluating the percentage growth inhibitions as follows:

$$\frac{\text{Dcontrol} - \text{Dtest}}{\text{Dcontrol}} X\ 1\ 00\%$$

Where D is the diameter of fungal growth on the PDA plates.

Statistical Analysis

The statistical analysis of data was done with Statistical Package for Social Sciences (SPSS) 21.1. The data were expressed as mean ± SEM and analyzed using One-way Analysis of Variance (ANOVA). The difference between concentrations of control and test groups was determined using Duncan test and considered at p value < 0.05.

Result and Discussion

Biofabrication and Characterization of the Gold Nanoparticles

Strophanthus hispidus leaf aqueous extract engineered the bio-reduction, bio-fabrication and stabilization of the gold nanoparticles as shown in Figure 1. The reaction was catalyzed through exposure to sunlight and the synthesis and stabilization of the gold nanoparticles occurred within 15 minutes. Light purple was observed at the beginning of the reaction then deep purple coloration was observed at stabilization of the reaction as no further color change was observed. Change in colouration has always marked the biofabrication of gold nanoparticles as many researchers have reported several colour change like red (Milanezi *et al.*, 2019), pink (Oladipo *et al.,* 2020), ruby red, blue black and purple (Abirami *et al.,* 2016; Alaa *et al.,* 2018).

As the gold nanoparticles was biofabricated through the reduction of $AuCl_4$ by the aqeouse extract of *Strophanthus hispidus*, the Uv-Vis spectroscopy localized surface plasmon resonance (LSPR) characteristic band was observed at 552 (Figure 2) nm as supported by Abdulrahman *et al.* (2019) who reported size range of 534nm for green synthesized gold nanoparticles and Milanezi *et al.* (2019) which reported size range of 520nm for green synthesized gold nanoparticles. The UV-Vis spectrum for the gold nanoparticles absorption maximum (λmax) was 552 nm and it is within the range of the visible spectrum 500–600 nm characteristic λmax for spherical AuNPs (Prevo *et al.*, 2008; Oladipo *et al.*, 2020)

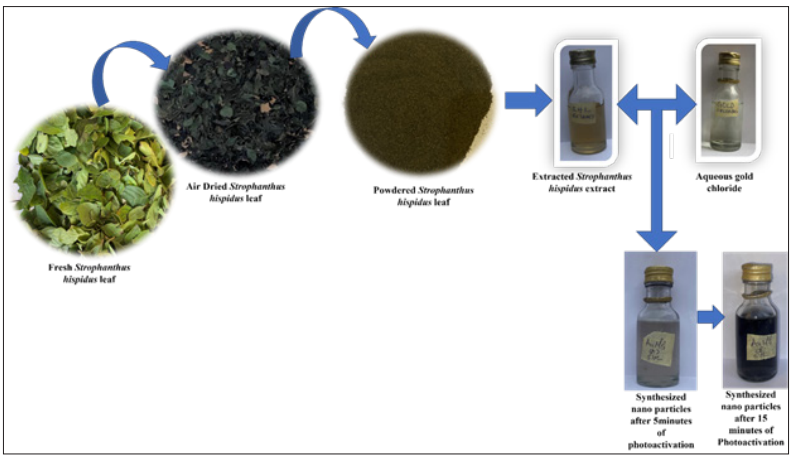

Figure 1: Sample preparation and green synthesis of gold nanoparticles from *Strophanthus hispidus* leaf

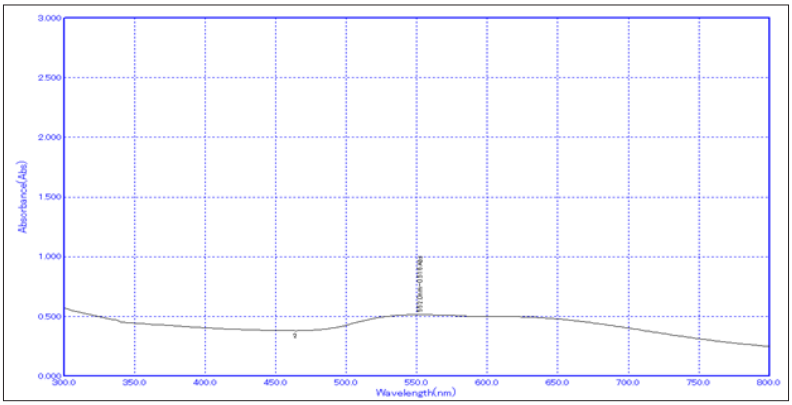

Figure 2: Ultraviolet-Visible spectrum of the biosynthesized gold nanoparticles from *Strophantus hispidus* leaf extract

The gold nanoparticles FTIR spectrum showed prominent broad peaks at 3417.98, 2918.40, 2359.02, 2000.25, 1766.85, 1622.19, 1384.94 and 1097.53 cm^{-1} (Figure 3). The vibrational stretch around 3417 cm^{-1} is ascribed to (O=H stretching. The peak at 2918.40 is assigned to C-H stretch of the alkyl group, the peak around 2359.02 cm^{-1} correspond to C≡C stretching and the peak at 1622.19 to 1097.53 cm^{-1} is ascribed to the conjugation of C=O stretching of N-H binding of protein or carbonyl groups (Pal *et al*., 2013; Hamed *et al.*, 2019). All these functional groups acertain the presence of monomers to macromolecules like sugars, proteins and phytochemicals which are involved in the fabrication and stabilization of the gold nanoparticles. Furthermore, *Strophanthus hispidus* contain alkaloids, saponins and flavonoids as reported by Mensah *et al*. (2019) and Owoola (2021), these feat of phytochemicals have made the plant a good bio-reducing agent which has helped in the reduction of Au^{3+} ions to Au^{0} (Abirami *et al.*, 2016).

The Energy Dispersive X-ray (EDX) analysis (Figure 4) showed that gold is the most prominent metal in the nanoparticles colloidal suspension. The transmission electron microscope (Figure 5) micrograph of the *Strophanthus hispidus* mediated gold nanoparticles showed that the nanoparticles morphology is spherical with the sizes between 10.90 and 34.63nm, this is in support with the reports of Chun-Tao *et al.* (2016).

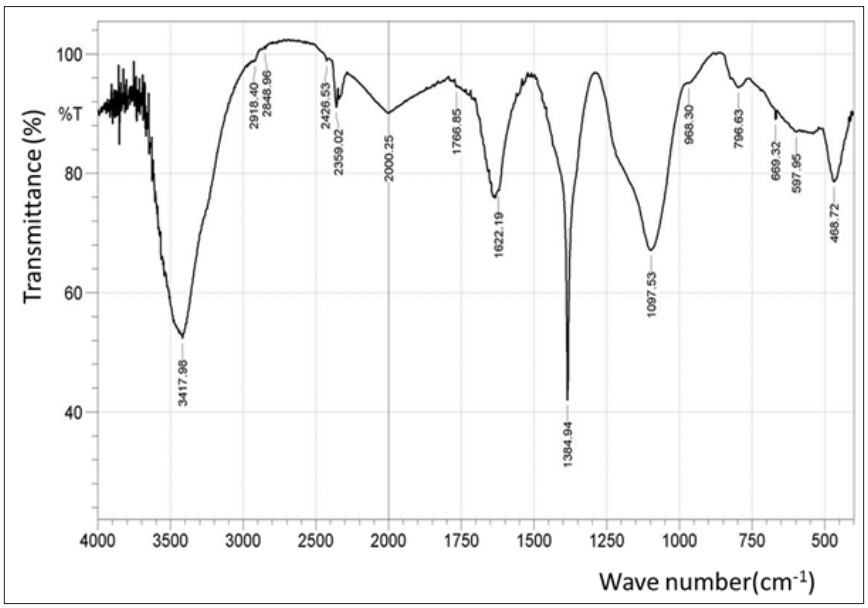

Figure 3: Fourier transform infrared spectrum of the synthesized gold nanoparticles from *Strophantus hispidus* leaf extract

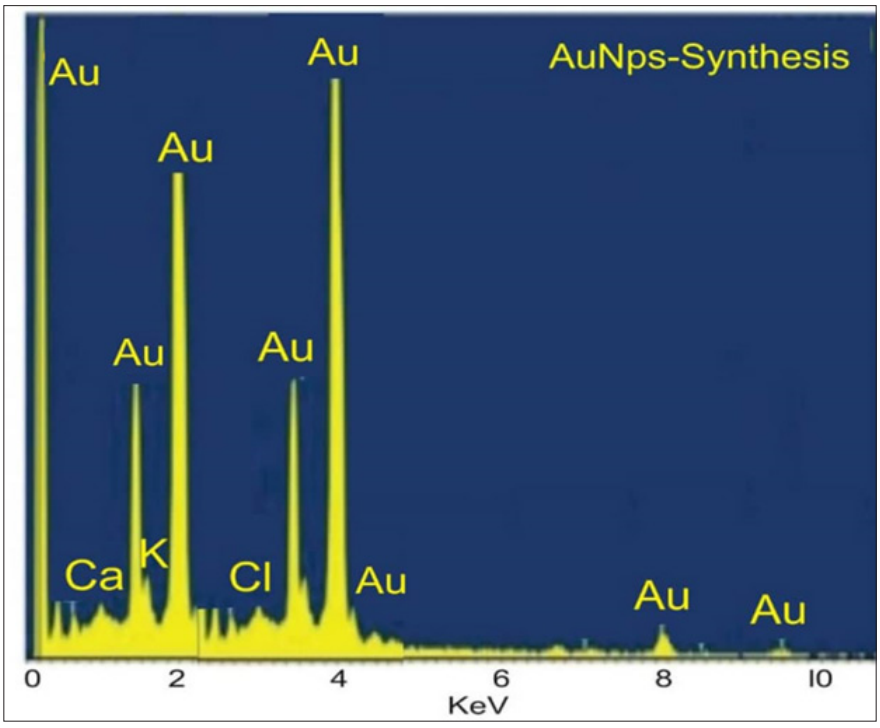

Figure 4: Energy dispersive x-ray spectrum of the biosynthesized gold nanoparticles from *Strophanthus hispidus* leaf extract

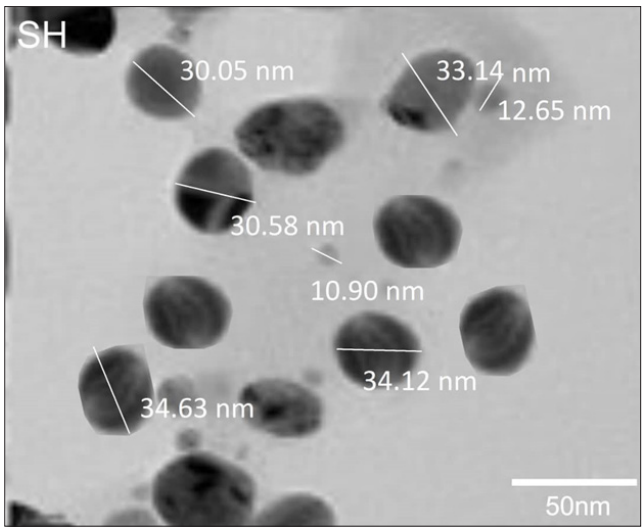

Figure 5: Transmission electron microscope micrograph of the synthesized nanoparticle from *Strophanthus hispidus* leaf extract

According to WHO (2020) and Zhou *et al*.(2021) hypertension or high blood pressure has been linked to over 8 million deaths through its complications leading to stroke, vascular diseases, renal disease and ischaemic heart disease worldwide (Olsen *et al*., 2016; WHO, 2020; Zhou *et al.*, 2021).One of the most effective medications for the treatment of high blood pressure is angiotensin converting enzyme inhibitors. Meanwhile, many medicinal plants have been used for managing high blood pressure; extracts of *Strophanthus huspidus* is known to have antihypertensive properties (Ezuruike & Prieto, 2014; Agbaje & Fageyinbo, 2014; Taofik *et al.*, 2015).Therefore, improving this extract through nanobiotechnology can be important avenue to develop new drug candidates to combat high blood pressure (Kouchmeshky, 2012).

According to this study, significant angiotensin converting enzyme inhibition activities of the synthesized gold nanoparticle was observed at the lowest concentration; the lower the concentration of the nanoparticles, the better the inhibitory activities (Table 1). The standard on the other hand performed better at higher concentration. This is in agreement with the previous work of Oladipo *et al*. (2023) who reported high ACE inhibition activities at low concentration of AuNPs synthesized from aqueous leaf extract of *Icacina trichantha*. Similar result was also reported by Hassani *et al.* (2020) with magnesium orotate nanoparticles suggesting that, less dosage of the nanoparticles could be administered to hypertensive patients.

Concentration (µg/ml)	AuNPs (%)	ACE Standard (%)
50	60.88± 3.100	48.15 ± 0.5694
100	42.98 ± 0.4755	57.86 ± 0.8716
150	37.80 ± 0.1077	80.14 ± 0.5457
200	36.66 ± 0.04436	85.90 ± 2.856

Table 1: Antihypertensive Activities of biosynthesized Gold nanoparticles from leaf aqueous extract of *Strophantus hispidus*

The antioxidant properties of the gold nanoparticles against 2, 2- diphenyl-1-picryhydrazyl (DPPH) was effective by 90.96, 88.09, 85.23 and 84.47% at 50, 100, 150 and 200µl/ml (Table 2) respectively. The result gotten in this research work was lent credence to by Milanezi *et al.* (2019) who evaluated 20µl of quercetin capped gold nanoparticles against the radicals of DPPH (Milanezi *et al.,* 2019). In their research, it was observed that the lower concentration of 50 µl/ml displayed highest antioxidant properties against the radical of DPPH than the other concentrations of 100, 150 and 200. The antioxidant properties of the gold nanoparticles could be attributed to the biomolecules that stabilized and coated the nanoparticles, this fact is important for therapeutic applications of gold nanoparticles as also confirmed by Pal *et al.* (2013) and Milanezi *et al.* (2019).

Samples	DPPH Free Radical Scavenging (%)
Ascorbic acid	77.53
Concentration of AuNps (µl/ml)	
50	90.96
100	88.09
150	85.23
200	84.47
Extract	85.23

Table 2: 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) activities of the synthesized nanoparticles from *Strophanthus hispidus* leaf extract

The gold nanoparticles were effective against the mycelial growth of *Aspergillus flavus, Aspergillus niger Fusarium solani, Fusarium poae, and Penicillium avenatum* (Table 3).The gold nanoparticles showed antifungal activity of 87.6%, 70.1%, 72.5 %, 82.8% and 51.8% against *Aspergillus flavus, Aspergillus niger Fusarium solani, Fusarium poae*, and *Penicillium avenatum* respectively at 50 µl /ml which are in contrast with the control plate that was not incorporated with the gold nanoparticles. This result established the efficacy of gold nanoparticles against mycelial growth and exospore production by toxigenic fungi, this was confirmed by Milanezi *et al.* (2019) who used quercetin mediated gold nanoparticles against three clinical strains of *Aspergillus fumigatus* and Oladipo *et al.* (2020) who also used *Datura stramonium* mediated gold nanoparticles against *Aspergillus niger, Aspergillus flavus* and *Fusarium solani*. The mycotoxigenic molds are known to produce mycotoxin as one of their secondary metabolite which have been reported to be toxic to man and detrimental to food crops especially seeds (Makun *et al.*, 2018; UKFSA, 2018), even some have been reported to be carcinogenic when consumed in great amount (Naseem *et al.*, 2019), so it is of great necessity to check the growth of these molds and that is what the *Strophanthus hispidus* mediated gold nanoparticles has proffered lasting solution to.

Organisms	Antifungal activities of AuNPs (%)
F. *solani*	72.5
F. *poae*	82.8
A. *niger*	70.1
A. *flavus*	87.6
P. *avenatum*	51.8

Table 3: Antifungal activities of the synthesized gold nanoparticles from *Strophantus hispidus* leaf extract

Conclusion and Recommendation

This study has established the relevance of *Strophanthus hispidus* leaf aqueous extract in the bio-fabrication of low cost, eco-friendly, safe, reliable and stable gold nanoparticles with its biotechnological applications. The biosynthesized gold nanoparticles showed significant anti-mycelial properties which could be useful agent in the production of eco-friendly fungicides, and also its antihypertensive properties have proven its drug improvement potentials in that area. The anti-radical activity of the gold nanoparticles against the radicals of 2,2-Diphenyl-1-Picryhydrazyl (DDPH) has proven useful in preventing or slowing the progress of various oxidative stress-related diseases.

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