Facile synthesis of silver nanoparticles using leaf extract of *Hyptis suaveolens* (L.) Poit for environmental and biomedical applications

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Abstract

Aqueous leaf extract of *Hyptis suaveolens* was investigated to synthesize silver nanoparticles (Hs-AgNPs). Biomolecules such as phenolics, proteins, carbohydrates and esters facilitated bioformation of Hs-AgNPs with λ<sub>max</sub> at 420 nm. The nearly spherical Hs-AgNPs were polydispersed and sized 29.19-52.27 nm with yield of 85.8 %. Hs-AgNPs showed potent inhibition of 75.22-100 % against multidrug resistant bacteria, and fungal growth inhibition of 73.33-100 %. Hs-AgNPs scavenged DPPH and H<sub>2</sub>O<sub>2</sub> by 77.75-83.19, and 54.21-70.11 % respectively. It also effectively inhibited coagulation of blood. The study established the nanobiotechnological importance of *H. suaveolens*, with bioformation of AgNPs that have potent activities for environmental and biomedical applications.

Keywords: Silver nanoparticles; antimicrobial; anticoagulant; antioxidant; *Hyptis suaveolens*

1 Introduction

There has been a continued expansion in the extent of synthesis and applications of nanomaterials (1-100 nm) in the field of nanoscience and nanotechnology relating to diverse areas of human endeavours. Its result has really bridged the gap that has existed between materials science and life sciences. The various efficacious uses of nanoparticles have
engineered a vital and conspicuous role in the search for the production of nanoparticles that are eco-friendly using different materials that are of biological origin. In the traditional mode of chemical synthesis; the application of noxious solvents, consumption of high energy and pressure have been found to be detrimental to the environment. However, the biological synthesis has resulted into the fabrication of nanoparticles with unique attributes, less consumption of energy and avoidance of hazardous procedures that have contributed to their expanding applications.

Due to the rich diversity of microorganisms as well as plants, the efficacy of bioresources in biofabrication of nanoparticles has not been fully realized. Relatively, limited plants from Nigeria have been reported for phytosynthesis of nanoparticles [1-13]. Other novel biological materials used for biosynthesis of various nanoparticles in different investigations comprise of cell-free bacterial extract, enzymes and metabolites of spider and paper wasp [14-23]. The sustained interest in the green synthesis of nanoparticles is consequent upon unfussiness of the process, reduced usage of chemicals and being eco-friendly [24-27]. Additionally, the existence of a lot of macromolecules or substances in biological resources that often act as excellent reducing, capping and stabilizing agents to mediate one-pot biosynthesis of nanoparticles furthermore contributed to the soaring popularity of biosynthesis as demonstrated by several researchers [28-33].

The genus *Hyptis* in the family Lamiaceae consists of about 400 different species. *Hyptis suaveolens* (Labiatae) is an average fragrant shrub that grows annually. It is commonly found both tropics and subtropics of the World, where it grows to reach height of about 2.5. The stem of the plant is quadrate and hairy, while its leaves are simple, entire, opposite, stalked, ovate or obovate and are about 3-5 cm long and 2-4 cm wide with serrulate margins and a long petiole [34]. It is a plant of medical importance with most of its parts exploited in local medicine for the treatment of variety of illnesses. Its leaves have found applications as stimulant, carminative as well as in the treatment of stomach-ache in Indian herbal medicine. In addition, the leaves are also used as anticancer and anti-fertility agents in females, and can play vital role in the management of anemia during pregnancy. The leaves also served as insect (mosquitoes) repellant [35], and used to treat colics, stomach ache, and fever.

The plant possesses significant pharmacological activities, including tumorigenic, mycotoxic, phytotoxic, antispasmodic, hepatoprotective, cytoprotective, anti-inflammatory,
antioxidant activity, and antiseptic in wounds, burns as well as various skin diseases [36-40]. Moreover, suaveol, an isolated compound of this plant showed gastroprotection to fight nausea, indigestion, colds, flatulence, and infection of the gall bladder [41]. The extract of the roots is confirmed to be an appetizer with reports showing the presence of urosolic acid, a natural HIV integrase inhibitor [42, 43]. Chemically, the plant also contains saponins, carbohydrates, phenols, tannins, glycosides and steroids that account for its vast medicinal values [44]. However, only one report exists on its usage for the synthesis of nanoparticles and investigated for larvicidal activities [45]. This work therefore aims at advancing the nanobiotechnological utilization of the plant; to phytosynthesize silver nanoparticles for antimicrobial, radical scavenging and anticoagulant applications with consideration for applications in the environment and healthcare.

2 Materials and methods

2.1 Green synthesis of Hs-AgNPs and characterization

The extract of leaves *H. suaveolens* was prepared as earlier demonstrated [5] by heating 0.1 g of dried leaf sample in 10 ml of distilled water at 60 °C for 1 h. The centrifuged extract was utilized to synthesize Hs-AgNPs as stated previously [5] by reacting 40 ml of 1 mM AgNO₃ with 1 ml of the extract at ambient temperature (30 ± 2 °C). The transformation in colour was monitored, and the synthesis of Hs-AgNPs was assessed through measurement of its absorbance spectrum on a UV-visible spectrophotometer (Cecil, USA) at the range of 300-900 nm. Biomolecules that were involved in the phytosynthesis were resolved by FTIR spectroscopy (IR Affinity-1S spectrophotometer, Shimadzu, UK), while the size, morphology, selected area electron diffraction (SAED) and elemental fingerprints of Hs-AgNPs were revealed by transmission electron microscopic (JEM-1400, JEOL, USA) and energy dispersive X-ray (EDX) analyses.

2.2 Antibacterial actions of biosynthesized Hs-AgNPs

Antibacterial efficacy of Hs-AgNPs was investigated against strains of clinical bacterial isolates using the modified liquid culture method [46]. Multidrug resistant (MDR) clinical bacterial strains of *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Escherichia coli* that were originally sourced from LAUTECH Teaching Hospital, Ogbomoso served as test organisms to assess the potency of the nanoparticles. The isolates were inoculated into peptone water and
incubated at 37 °C for 24 h. Hs-AgNPs (1 ml each of graded concentrations from 5-15 µg/ml) were dispensed into test tubes that contained 9 ml of sterile peptone water. An aliquot of 0.1 ml (1 × 10⁶ cfu/ml) served as inoculum and incubation was done at 37 °C for 24 h. The growth of the bacterial isolates was measured by determining the optical density (OD₆₀₀nm). The hindrance of bacterial growth was estimated accordingly:

\[
\text{Percentage growth inhibition} = \frac{OD_{\text{control}} - OD_{\text{test}}}{OD_{\text{control}}} \times 100 \%
\]

where OD is the optical density.

2.3 Antifungal activity of Hs-AgNPs

Antifungal activity of Hs-AgNPs was assayed using mycelial growth inhibition test [47]. Prepared Hs-AgNPs were included into potato dextrose agar (PDA), and thereafter inoculated with circular agar block of 7 mm obtained from 48 h-old cultures of Aspergillus fumigatus, A. flavus and A. niger. Hs-AgNPs was not included in the control plates. Incubation of the plates was done at 28 ± 2 °C and lasted till 72 h. At the end of 72 h, diameters of growth on plates were measure to calculate fungal growth inhibition as follows:

\[
\text{Percentage growth inhibition} = \frac{D_{\text{control}} - D_{\text{test}}}{D_{\text{control}}} \times 100 \%
\]

where D is the diameter of growth on plate.

2.4 Evaluation of antioxidant activities of Hs-AgNPs

2.4.1 Scavenging of DPPH

The ability of Hs-AgNPs to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich, Germany) was evaluated as stated by William et al. [48]. About 1 ml of different concentrations of Hs-AgNPs was separately reacted with 4.0 ml of methanolic solution of 0.1 mM DPPH in the dark box for 30 min at 30 ± 2 °C. Methanolic DPPH alone served as the control. At the end of the reaction, absorbance readings were taken at 517 nm on UV-vis spectrophotometer (Spectrumlab 752S, UK), and used to subsequently calculate the amount of DPPH scavenged:

\[
\text{Percentage DPPH scavenging activity} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \%
\]

where A is the absorbance value.
2.4.2 Scavenging of hydrogen peroxide (H$_2$O$_2$)

To evaluate the capability of Hs-AgNPs at scavenging H$_2$O$_2$, the methods of Bhakya et al. [49] was employed, whereby 4 ml of graded concentrations of Hs-AgNPs was reacted with 0.6 ml of 40 mM H$_2$O$_2$ (pH 7.4), and held at 30 ± 2 °C for 20 min. The H$_2$O$_2$ solution and distilled water were used as the control and blank respectively. Following the reading of absorbance at 610 nm, the amount of H$_2$O$_2$ scavenged was calculated thus:

\[
\% \text{ H}_2\text{O}_2 \text{ scavenged} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100; \text{ where } A \text{ is the absorbance value.}
\]

2.5 Anticoagulant activity of Hs-AgNPs

The capability of Hs-AgNPs to inhibit coagulation of blood was determined by adding 1 ml of 100 µg/ml of Hs-AgNPs to 0.5 ml of blood unreservedly given by a hale and hearty donor. Three control samples that involved the use of EDTA, _H. suaveolens_ leaf extract (HE), and solution of AgNO$_3$ were assayed for anticoagulation. The reaction mixes were incubated at 30 ± 2 °C for 1 h and subsequently observed both visually and microscopically for development of blood clot [19, 50].

3 Results and discussion

3.1 Phytosynthesis and characteristics of Hs-AgNPs

Leaf extract of _H. suaveolens_ potentiated the bioformation of brown colloidal AgNPs within 2 min, when it was reacted with silver nitrate (Figure 1). The colour of biofabricated Hs-AgNPs stabilized within 10 minutes of formation. It is evident from the phytochemistry of the plant as earlier stated that it was rich in biomolecules that facilitated the reduction Ag$^+$ to Ag$^0$, thereby influencing the frequency of the surface plasmon resonance that led to the development of brown colour. Varying colours of AgNPs that include brown, dark brown, brownish-orange and light yellow exist [4, 15, 34, 51]. Biosynthesized Hs-AgPs had characteristic peak of absorbance at 420 nm (Figure 2), and this is within limit of various values of SPR ascribed to AgNPs by several authors [1, 2, 10, 15]. Elumalai et al. [45] reported SPR of 408 nm for the brown colloidal AgNPs phytosynthesized using the leaf extract of _H. suaveolens_.

FTIR spectrum (Figure 3), indicated the prominence of peaks at 3444.2, 2293.8, 1707.7, 1385.8, 1128.8 and 1109.3 cm$^{-1}$, that implied N-H found in amines or O-H stretch linked to alcohols/phenols, P-H phosphine stretch, C=O stretch of amides or esters; CH$_3$ absorption
bending of alkane, and C-O stretch of ethers, carboxylic acids, esters and alcohols. The incidence of C-H, C-O and C=O have been reported for the extract [45]. The presence of these peaks is a pointer to the richness of the plant extract in different phytochemicals. The TEM micrograph showed nearly spherical-shaped Hs-AgNPs that were polydispersed and varied in size from 29.19 to 52.27 nm (Figure 4a); which are in conformity with existing literature [4, 52]. However, Elumalai et al. [45] reported anisotropic shape of sphere, hexagon, triangle and polyhedral for AgNPs synthesized by leaf extract of *H. suaveolens*. The particles showed high level of dispersity, stability and devoid of aggregation on long storage. The predominance of Ag in EDX spectrum (Figure 4b) to tune of 85.8 % was indicated. Hs-AgNPs depicted the characteristic ring-like SAED pattern (Figure 4c) associated with face-centered cubic crystalline structure of Ag [5, 53].

**Figure 1.** The biosynthesis of Hs-AgNPs using the leaf extract of *H. suaveolens*
Figure 2. UV-visible absorption spectrum of Hs-AgNPs biosynthesized using the leaf extract of *H. suaveolens*

Figure 3. FTIR spectrum of Hs-AgNPs biosynthesized using the leaf extract of *H. suaveolens*
Figure 4. The TEM micrograph (A), EDX spectrum (B) and SAED pattern (C) of Hs-AgNPs biosynthesized by *H. suaveolens*.

### 3.2 Hs-AgNPs action against MDR bacteria

The Hs-AgNPs effectively suppressed growth of MDR strains of *E. coli, S. aureus* and *K. pneumoniae* with percentage growth inhibition of 75.22-100 % (Figure 5a) at concentrations of 5-15 µg/ml, which conforms with some earlier reports [13, 46]. The antibacterial activities shown by Hs-AgNPs can be of tremendous importance in controlling MDR bacteria that thrive in the environment [54-56]. The extremely small-sized nature of AgNPs enhances its infiltration into the bacterial cell, and thereafter hinders metabolism activities through myriad of ways that may include by binding to phosphorus and sulphur molecules proteins, enzymes and DNA that ultimately give rise to death of the cell.

### 3.3 Antifungal Activities of Hs-AgNPs

The Hs-AgNPs greatly inhibited the fungi tested, although to varying extents (Figure 5b). While 100 % growth inhibition of *A. flavus* and *A. fumigatus* was recorded with Hs-AgNPs at concentration of 150 µg/ml, it was 78.57 % for *A. niger*. At 100 µg/ml of Hs-AgNPs, 100 %
inhibition of growth was also obtained for *A. fumigatus*, while it was 73.33 % for both *A. flavus* and *A. niger* at the same concentration. However, massive growths of fungi were observed in the control plates lacking Hs-AgNPs. These results revealed the potency of Hs-AgNPs against fungi of medical and agricultural importance, and thus can be used for their control in several biomedical and environmental applications. AgNPs have been variously reported to inhibit the growth of fungi [5, 47]. The ability of nanoparticles at inhibiting fungal growth is attributable to combination of assault on the fungal wall, and destruction of spores, that culminate into outflow of the constituents of cytoplasm that eventually cause the death of exposed fungi.

3.4 Antioxidant activities of Hs-AgNPs

3.4.1 Scavenging of DPPH

The synthesized Hs-AgNPs scavenged DPPH by 77.75-83.16 %, which was dose-independent at the tested concentrations of 5-40 µg/ml. In general, the free-radical scavenging potentials of AgNPs have been credited to the presence of reducing molecules that capped the nanoparticles thereby increasing the surface areas for antioxidant activity [49]. There are also reports of potentiation of improved antioxidant activities and phytomedicinal contents of plants treated with nanoparticles [57, 58]

![Figure 5](image_url)

**Figure 5.** Antibacterial (A) and antifungal (B) activities of Hs-AgNPs biosynthesized using the leaf extract of *H. suaveolens*
3.4.2 Scavenging of H$_2$O$_2$

The phytosynthesized Hs-AgNPs scavenged H$_2$O$_2$ by 54.21-70.11 % with the spontaneous clearance of turbid solution of H$_2$O$_2$ which was prepared in phosphate buffer. Other researchers have reported H$_2$O$_2$ scavenging activities of 77.00-99.80 % for some biogenic AgNPs [49, 50, 59]. Human exposure to H$_2$O$_2$ is very soaring, because of its use as components of personal care products that include bleaching agents and disinfectants. It can also be encountered in industrial effluents, especially from paper manufacturing industry, where it is used to bleach paper and pulp. H$_2$O$_2$ is capable of initiating the production of strong reactive hydroxyl radicals (OH$^-$) in living organisms [60] that may herald cellular injury and on-set of intestinal disorders, cancers, Alzheimer’s disease, inflammation, aging amongst other debilitating conditions [61]. Environmental sources of H$_2$O$_2$ notably, drinking and wastewater could be treated with nano-based materials to effectively scavenge H$_2$O$_2$. Analytically, AgNPs-based sensors have been reported to detect as well as quantify H$_2$O$_2$ [62-64].

3.5 Hs-AgNPs as anticoagulant agent

The in vitro anticoagulation of human blood was ensured by Hs-AgNPs, simultaneously retaining the form of red blood cells as obtained in the fresh blood as well as the one collected in the EDTA bottle (Figure 6). Blood clot occurred in the control samples treated with extract of H. suaveolens and AgNO$_3$ solution due to their failure to prevent coagulation of blood. Some studies revealed that AgNPs possessed antiplatelet activity [7, 18, 65] and fibrinolytic activity [66], which did not permit blood coagulation. In addition, in vitro anticoagulation properties of AuNPs [20, 22], and Ag-AuNPs [7] have been recently reported. In the same way, AgNPs may inhibit fibrinogen to obstruct the formation of fibrin, thereby ensuring anticoagulation of blood. Additionally, anticoagulant activity of heparin was improved by 118.9 % through coupling with earthworm extract-mediated AuNPs [67]. The growing evidences imply that metallic nanoparticles could be potential sources of anticoagulant, thrombolytic and theranostic agents to manage blood coagulation illnesses [68-70].
4 Conclusion

This study has shown that leaf extract of *H. suaveolens* has the capacity to synthesize AgNPs in an eco-friendly manner. The synthesized Hs-AgNPs were spherical, sized 29.19-52.27 nm, and polydispersed in nature. Hs-AgNPs greatly inhibited MDR bacterial strains by 75.22-100 %, and equally displayed potent action against fungi (73.33-100 %), thus ascertaining the significance of Hs-AgNPs as antimicrobial agent. Correspondingly, Hs-AgNPs scavenged both DPPH and hydrogen peroxide with acceptable outcome, which can be deployed for scavenging free radicals in the environment. Hs-AgNPs also displayed potent blood anticoagulation property. Conclusively, the nanobiotechnological importance of *H. suaveolens*, most essentially, the cost-effective phytosynthesis of Hs-AgNPs has been established in this work. Until now, there is no report on antimicrobial, antioxidant and anticoagulant activities of AgNPs biofabricated by *H. suaveolens*.

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