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The Antibacterial Efficacy of Gold Nanoparticles Derived from *Gomphrena celosioides* and *Prunus amygdalus* (Almond) Leaves on Selected Bacterial Pathogens

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***Abstract*—**Gold nanoparticles (AuNPs) have gained increasing interest in recent times. This is greatly due to their special features, which include unusual optical and electronic properties, high stability and biological compatibility, controllable morphology and size dispersion, and easy surface functionalization. In typical synthesis, AuNPs were produced by reduction of gold salt $AuCl_4$ in an appropriate solvent. A stabilizing agent was added to prevent the particles from aggregating. The antibacterial activity of different sizes of gold nanoparticles was investigated against *Staphylococcus aureus, Salmonella typhi* and *Pseudomonas pneumonia* using the disk diffusion method in a Müeller–Hinton Agar. The Au-NPs were effective against all bacteria tested. That the Au-NPs were successfully synthesized in suspension and were used to study the antibacterial activity of the two medicinal plants against some bacterial pathogens suggests that Au-NPs can be employed as an effective bacteria inhibitor and may be an effective tool in medical field. The study clearly showed that the Au-NPs exhibiting inhibition towards the tested pathogenic bacteria in vitro could have the same effects *in vivo* and thus may be useful in the medical field if well researched into.

***Keywords*—**Gold Nanoparticles, *Gomphrena celesioides, Prunus amygdalus*, Pathogens.

I. Introduction

NANOTECHNOLOGY deals with processes that take place on the nanometer scale, that is, from approximately 1 to 100nm. Properties of metal nanoparticles are different from those of bulk materials made from the same atoms. In recent years, so much attention is being given to research into noble metal nanoparticles which may be due to their unique optical, electronic, mechanical, magnetic, and chemical properties that are significantly different from those of bulk materials [1]. These special and unique properties could be attributed to their small sizes and large surface areas. For these reasons, metallic nanoparticles have found uses in many applications in different fields, such as catalysis, photonics, and electronics. Preparation of silver nanoparticles has attracted particularly considerable attention due to their diverse properties and uses, like magnetic and optical polarizability, electrical conductivity [2], catalysis, antimicrobial and antibacterial activities [3], [4], DNA sequencing [5], and surface-enhanced Raman scattering (SERS) [6] as cited by [7].

It is believed that due to their large surface areas nanoparticles have more penetration powers into microorganisms and if the active plant extracts can be delivered into the 'interior' of the microbes more activity could be recorded. Many chemical methods exist for synthesis of nanoparticles but have been found to be toxic since nanoparticles could be used in humans and other animals or plants which may eventually end up in human system [8]-[10]. It therefore, became extremely important to focus on the biological methods for the production of nanoparticles. Biological methods of nanoparticle synthesis using microorganisms [11]-[13], enzymes [14], fungus [15], and plants or plant extracts [16]-[18] have been suggested as possible ecofriendly alternatives to chemical and physical methods. In the opinion of [16], 'sometimes the synthesis of nanoparticles using plants or parts of plants can prove advantageous over other biological processes by eliminating the elaborate processes of maintaining microbial cultures'.

Microorganisms are responsible for infectious diseases in plants animals and humans. The microbes *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas pneumonia* are bacterial pathogens that cause human diseases like opportunistic skin infection, typhoid fever and pneumonia disease in children as well as in the elderly.

In this present research, gold nanoparticles synthesized from the two plants, *Gomphrena celosioides* and *Prunus amygdalus,* were used against the three bacterial pathogens to determine their susceptibility to the particles and to ascertain if this can useful in therapy formulation against the diseases caused by these pathogens.

II. Materials and Methods

A. Preparation of Stock Solution of Gold Chloride from $Aucl_4$

To make 50ml of 10mM stock solution the following calculations were made:

Molecular weight of AuCl4 = 393.83g
1M =393.83g in 1
To convert this to mM = 0.39383g/mM
To convert g to mg = 393.83mg/mM /L

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10mM = 10 × 393.85mg/L = 3938.5mg/L

$$50\text{ml} = \frac{3938.5mg \times 50ml}{1000ml} = 196.925\text{mg}$$

This (196.925mg $AuCl_4$) was then dissolved in 50ml distilled water and stored in a dark brown bottle and labeled 10mM $AuCl_4$

Now the working solution of 2.5mM was prepared using the formula,

$$RV/O$$

where R = required concentration, V = volume, O = original concentration

Now, R =2.5mM

V = 50ml

O = 10Mm

Then

$$\frac{2.5mM\ x\ 50ml}{10Mm} = 12.5\text{ml}$$

Twelve point five (12.5ml) millilitre of stock solution taken and made up to 50ml with distilled water.

B. Plant Synthesis of Gold Nanoparticles (AuNPs)

Mature leaves of the plants *Gomphrena celosioides* and *Prunus amygdalus* were collected from around Bosso Campus of Federal university of Technology, Minna, Nigeria. The plants leaves were thoroughly washed and air-dried after which they were crushed in a mortar.

One gram (1g) each of the powdered leaves of the plants was weighed and placed in a beaker into which 10ml of distilled water was added, mixed and filtered with Whatman filter paper.

Two milliliter (2ml) each of the filtrate was pipetted into five (5) test tubes. The pH of each test tube was adjusted to 3, 4, 7 and 8 using appropriate buffer solutions, the 5th test tube served as the control.

Into each test tube 1ml of 2.5mM gold chloride was added (except the control). The test tube were mixed thoroughly and observed for color change.

Ultraviolet light (UV) was used to carry out the spectrophotometric analysis to determine the wavelength. Gold has Plasmon resonance peak at 500–600nm.

C. Antibacterial Assay of the AuNPs on the Test Organisms

1. AuNps Discs

The Whatman filter paper was perforated with paper perforator to obtain discs the size of standard antibiotic discs. About 25 pieces of the discs were counted into McCartney bottles into which 5ml of the standardized AuNPs from each of the plant was added, thoroughly mixed to give even distribution of the nanoparticles. This was allowed to fix and kept for further assay.

2. Preparation of Test Culture

The test organisms which include *Staphylococcus aureus, Salmonella typhi* and *Pseudomonas pneumonia* were obtained from Microbiology Department of National Institute for Pharmaceutical Research Development (NIPRD) Abuja and maintained on Nutrient Agar 37°C. The colonies of each pure culture were inoculated into 5ml sterile broth in a McCartney bottle and incubated over night at 37°C.

3. Antimicrobial Assay

The antimicrobial assay was conducted according to the method of [19]. The sterile paper discs (6mm) impregnated with gold nanoparticles derived from the two plants leaf extracts were suspended in sterile distilled water and were left to dry at 37°C for 24h in a sterile condition. The bacterial suspension was prepared by making a saline suspension of isolated colonies selected from nutrient agar plate, the agar plates were grown for 18 hours. Five milliliters (5ml) of sterile nutrient broth was inoculated with a loop-full of test organism and incubated for 24hrs. 0.2ml from the overnight culture of the organisms were dispensed into 19ml of sterile nutrient broth and incubated for 3-5hrs by using McFarland turbidity standard using the spectrophotometer of 600 nm to standardize the culture to 10^6cfu/ml. The surface of Muller Hinton Agar was completely inoculated using a sterile swab. The impregnated discs were then placed on the inoculated agar and incubated at 37°C for 24 hours. After incubation, the diameter of the growth inhibition zones was measured. Chloramphenicol (30µg) and Streptomycin (30µg) were used as the positive standards control. All tests were conducted in triplicate.

III. Results

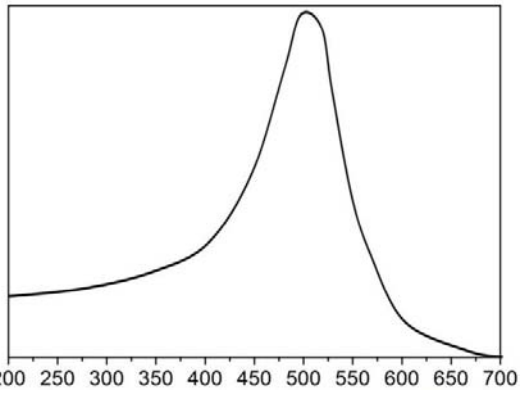

Fig. 1 UV- Vis absorption spectrum of gold nanoparticles (AuNPs) in aqueous solution of *Gomphrena celosioides* and *Prunus amygdalus* leaf extracts

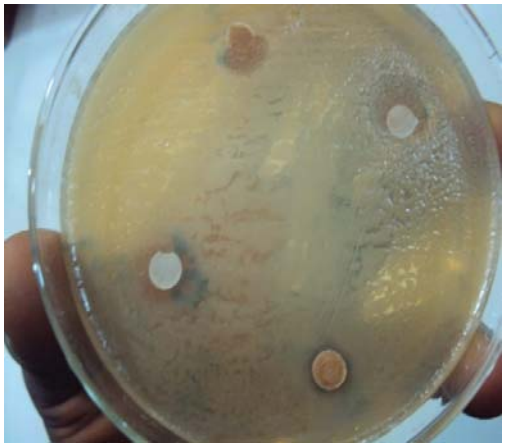

Fig. 2 Susceptibility testing of AuNPs against one of the test organisms

IV. Discussion

When each of the leaf extracts was mixed with the aqueous solution of the AuCl4, it began to change the color slowly. It was seen that the gold ion is being reduced to gold which indicates the formation of stable gold nanoparticles. The formation of gold nanoparticles was monitored periodically using UV-Vis spectroscopy. Gold has Plasmon resonance peak at 500–600nm therefore from Fig. 1 the peak of 500nm can be said to be gold nanoparticles. Reference [20] obtained similar results in their experiments on nanoparticles therefore the present gold nanoparticle synthesis in this research in agreement with theirs.

The *in vitro* antibacterial activity of the samples was evaluated by utilizing the disc diffusion method using Müeller–Hinton Agar (MHA) with determination of inhibition zones in millimeter (mm), which conform to recommended standards of the [21]. *Staphylococcus aureus, Salmonella typhi* and *Pseudomonas pneumonia* were used for the antibacterial effect assay. The results as shown on table one suggest that all three bacteria were susceptible to the nanoparticles of the two plants extracts. Extracts of *G. celesioides* showed more activities on the test organisms compared to *P. amygdalus.*

The bacterium, *P. pneumonia* was most susceptible to the extracts followed by *S. aureus* while the least susceptible was *S. typhi*. The zones of inhibition created by the gold nanoparticles of the plants extracts compared favorably with those of the two standard antibiotics employed as control in this study. That these nanoparticles have activity against these organisms is a welcome development. Reference [22] revealed the activities of gold and silver nanoparticles against *Mycobacterium tuberculosis* and *Escherichia coli* and suggested that these particles could be used to treat tuberculosis faster than the conversional drugs.

The results of the present research could give a lead way to the use of gold nanoparticles from these plants to treat a lot of human ailments that have hitherto evaded other orthodox medications. We suggest more and extensive work is carried out on these plants and a host of other African herbs. Who knows, the solution to human disease problems is now on the horizon with the use of nanotechnology.

TABLE I
Average Inhibition Zone and Standard Deviation for AuNPs Derived from *Gomphrena celosioides* and *Prunus amygdalus*

	Zones of inhibition (mm)		Control	
Bacteria	*G. celosioides*	*P. amygdalus*	CTX	STX
S. aureus	16.65 ± 0.50	14.78 ± 0.10	18.64 ± 0.20	16.62 ± 0.30
S. typhi	14.67 ±0.30	12.44±0.30	18.51 ± 0.49	16.62 ± 0.30
P. pneumoniae	17.56 ±0.30	16.71 ± 0.10	15.64 ± 0.30	18.62 ± 0.30

CTX, Chloramphenicol; STX, Streptomycin

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