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Bio-Fabrication, Spectroscopic Investigation and Antibacterial Potency of Ag-Co Bimetallic Nanoparticles Synthesized from the Root Extract of *Borassus aethiopum* (Palmyra Palm)

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Abstract

In this study, silver-cobalt bimetallic hybrid nanoparticles were synthesized using green method from $AgNO_3$ and $CoCl_2$ metal precursors as well as the locally available root extract of *Borassus aethiopum* acting as the reducing agent. The formation of bimetallic nanoparticles was first noticed by a color change of the reaction mixture from light pink to light brown as the result of Surface Plasmon absorptions. The optical measurements using UV-Vis showed the maximum absorption wavelength at 420nm while the functional group identification using FT-IR revealed some replacements in the absorption of functional groups, disappearance, and appearance of some others in the spectra of the BMNPs relative to that of the root extract indicating that those involved in the bio-reduction process. In vitro antibacterial potency was investigated against five clinically isolated bacteria. The outcome of the result suggested that they inhibit the tested bacteria especially against *Salmonella typhi*, *Bacillus subtilis* and *Klebsiella pneumoniae*. Thus, it can be developed as a bio-control agent for the treatment of diseases caused by these bacterial pathogens.

1. Introduction

Green is a term associated with a renewable product or non-toxic process whose introduction in the society has no harm or toxicity and a general favorable life cycle analysis and is non-persistant **(Lucia, 2016)**. Owing to the rich biodiversity of plants and their potential secondary constituents, plants and plant parts have gained attention in recent years as medium for nanoparticles' syntheses **(Roopan *et al.*, 2013)**. The main objective of the green chemistry is to decrease hazards associated with the processes and products that are important to the economy of the world and to maintain the good quality of life that we enjoy through chemistry **(Ismail *et al.*, 2018)**.

Recently, nanotechnology is developing and expanding and is used in many areas including health, nutrition, environmental health and agriculture **(Parang and Moghadamnia, 2018)**. Cobalt nanoparticles have catalytic, magnetic, optical, antibacterial and biomedical properties **(Igwe and Ekebo, 2018)**. High performance permanent magnetic properties and possess biomedical and cytotoxic activity **(Kuchekar *et al.*, 2018)**. Research has shown that nano sized silver particles have the ability to penetrate through the cell membrane, **(Chikkanna and Neelagund, 2018)**. Evidence suggests that germ cells and embryonic fibroblasts in rats are toxic with silver nanoparticles **(Parang and Moghadamnia, 2018)**.

Biosynthesis of bimetallic nanoparticles method is a good, low-cost and nontoxic method compared to physical and chemical methods which showed a high bioactive efficiency**, (Abd-Elsalam *et al.*, 2016)**. Bimetallic nanoparticles show better optical, electrical and medical applications due to their peculiar mixing patterns and synergistic effects of two metal nanoparticles that form bimetallic **(Mazhar *et al.*, 2017)**. In a study by **Parang and Moghadamnia (2018)**, Ag-Co NPs were synthesized using chemical reduction method and were found to have antifungal properties. **Cheng *et al.*, (2012)** reported the synthesis of Ag-Co bimetallic star shaped NPs and found out that they have the ability to be used in spectroscopy and catalytic applications. **Nelli *et al.*, (2002)** synthesized Ag-Co nanocomposite and studied the spectroscopic investigations using X-Ray spectrometry, TEM and energy loss spectroscopy.

Borassus aethiopum belongs to the family trees commonly known as Palm trees or simply Palms. It is a monotypic family in the order Arecales. The family contains several commercially important species such as coconuts, area nuts and date palms. Palms are well known for their great heights, exclusive foliages, conspicuous inflorescences and big seeds. Palms are predominantly perennial species and remaining green throughout the year **(Basu *et al.*, 2014)**. *Borassus aethiopum is* economically and medicinally useful. It is used as a vegetable, a beverage called arrack, crude sugar called jiggery. It has high transpiration rate thus has the potential to humidify the atmosphere and thereby having direct impact to cloud formation and rainfall **(Abdulrahman and Oladele, 2009)**.

This research is aimed at the green synthesis of Ag-Co bimetallic nanoparticles using aqueous root extract of *Borassus aethiopum*, its optical measurement using UV-Visible and functional groups

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determination using FT-IR spectrophotometers. Moreover, its bacterial potency was evaluated against five bacterial pathogens.

2. Material and methods

2.1 Plant Sample collection

Fresh roots of *Borassus aethiopum* were dug and collected from open area in Kaltungo Local Government Area, Gombe State and were brought to the Chemistry Laboratory, Gombe State University. They were identified by a botanist in the Botany Laboratory, Department of Biological Science, Gombe State University.

2.2 Sample preparation

Extract Preparation: Fresh roots of *Borassus aethiopum* were washed with tap water twice and then rinsed with de-ionized water so that contamination was minimized. They were then cut into smaller units and transferred into a crucible and were ground. A 30g of it was weighed and mixed with 200ml de-ionized water and warmed to 80˚C for 30 minutes and was allowed to cool. The supernatant was filtered through Whatman number 1 filter paper and used immediately for the synthesis of the hybrid nanoparticles.

2.3 Synthesis of Silver-Cobalt Bimetallic Nanoparticles

A 100 ml of the prepared *Borassus aethiopum* root extract was mixed with 500ml of the mixture of the two metal precursors containing 250ml each of centimolar $AgNO_3$ and $CoCl_2$ (1:5 v/v) gradually while heating at 80˚C for 30 minutes in a 600 ml beaker. Reduction of silver (I) and Cobalt (II) ion to their zero oxidation states were visually noticed by change in color from light pink to deep light brown. The solution was allowed to stay for 24 hours after which the nanoparticles settled at the bottom of the beaker. The supernatant was decanted and the residue was dried by evaporation.

2.4 Ultraviolet-Visible Spectrophotometer

The supernatant liquid was characterized using UV-Visible Spectrophotometer model 6705 for the wavelength range of 250 to 800 nm. Maximum absorption wavelength was determined by placing each aliquot sample in quartz cuvette operated at a resolution of 1 nm, using de-ionized water as the blank or reference solvent. The samples were placed in 1 x 1 cm quartz cell.

2.5 Fourier Transform Infrared Spectrophotometer

The synthesized silver-cobalt bimetallic nanoparticles and dried root extract samples were characterized using Fourier Transform Infrared Spectroscopy to determine the various functional groups involved in the bio-reduction process (PerkinElmer Spectrum Version 10.03.09 was used).

2.6 Determination of zone of inhibition using agar well diffusion method

Antibacterial activity of synthesized nanoparticles was assessed by the well plate agar diffusion method as described in the Aida modified method **(Aida *et al.*, 2001)**. The microbial cultures were adjusted to 0.5 McFarland turbidity standards; and inoculated on Mueller hinton agar plate of diameter 9 cm. The plate was flooded with each of the standardized test organism (1 mL), and then swirled. A sterile cork borer was used to make a 6 mm diameter wells on the agar plates. Aliquots of the nanoparticle dilutions were mixed with 50% DMSO at concentrations of 200μg/L, 300μg/L, 400μg/L and 500μg/L and applied on each of the well in the culture plates previously inoculated with the test organisms. Augmentin standard drug was used as the positive control for the bacterial studies. These were then left on the bench for 1 hour for proper diffusion of the nanoparticles. Thereafter, the plates were incubated at 37°C for 24 hours. Antimicrobial activity was determined by measuring the zone of inhibition around each well (taking the average of the length and breadth including that of the well) for each nanoparticles obtained from the plant extract.

2.7 Statistical Analysis

All data were analyzed using Microsoft Excel 2007.

3. Results and Discussion

3.1 Formation of the Ag-Co bimetallic nanoparticles

The formation of the bimetallic nanoparticles was first noticed by color change after addition of cobalt solution, Figure 1**A**, silver solution Figure 1**B** and Aqueous root extract of Palmyra palm, Figure 1**C** from milky, Figure 1**D** to light brown, Figure 1**E** within 15 minutes as a result of the surface Plasmon absorption. The Surface Plasmon absorption in the metal nanoparticles was due to the collective oscillation of the free conduction band electrons which is excited by the incident electromagnetic radiation. The supernatant was decanted and the solid nanoparticles settled at the bottom and further evaporated. Both the supernatant liquid and the liquid were used for further analysis. The change in color is shown in Figure 1 and 2 for the metal precursor and the synthesized bimetallic nanoparticles respectively.

3.2 Optical measurement using UV-Visible Spectrophotometer of Silver-Cobalt Bimetallic nanoparticles

The UV-Visible spectrum of the synthesized Ag-Co NPs is shown in Figure 2 below. The synthesis of bimetallic nanoparticles was followed by UV-Vis spectroscopy. The maximum absorption peak was shown at 420nm. The maximum wavelength is similar to that obtained by **Akinsiku *et al.*, (2018)**. The maximum absorption wavelength was greater than that observed for Cobalt nanoparticles (300nm) by **Igwe and Ekebo (2018)** and as well greater than that observed by **Chikkanna and Neelagund (2018)** for the synthesis of Silver nanoparticles. This suggests that bimetallic nanoparticles have higher wavelength compared to monometallic counterparts.

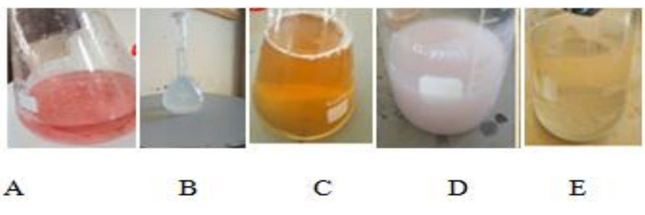

Figure 1. 0.01M $CoCl_2$ (**A**), 0.01M Ag (NO_3) (**B**), Aqueous root extract Palmyra palm(**C**), Mixture of Ag-Co immediately after addition(**D**), and Ag-Co BMNPs formation(**E**)

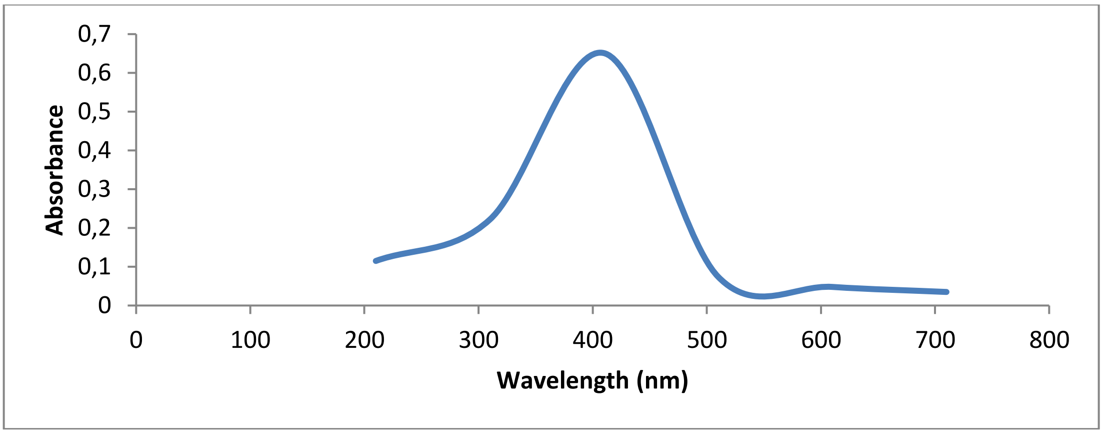

Figure 2. UV-Visible Spectrum for Ag-Co BMNPs.

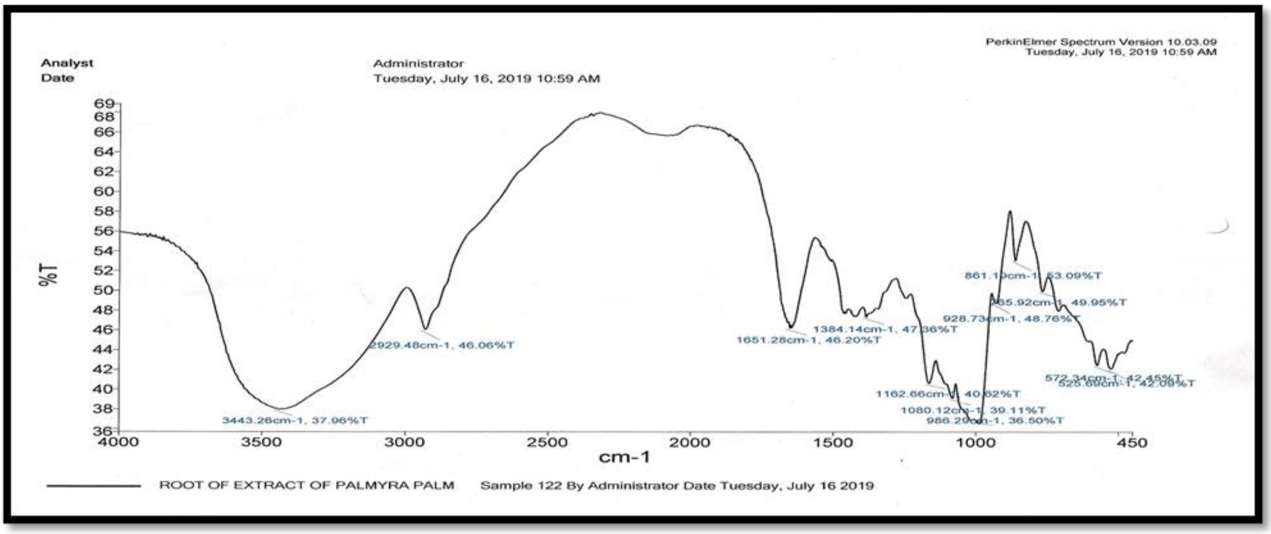

Figure 3. FT-IR Spectrum for root extract of *Borassus aethiopum*

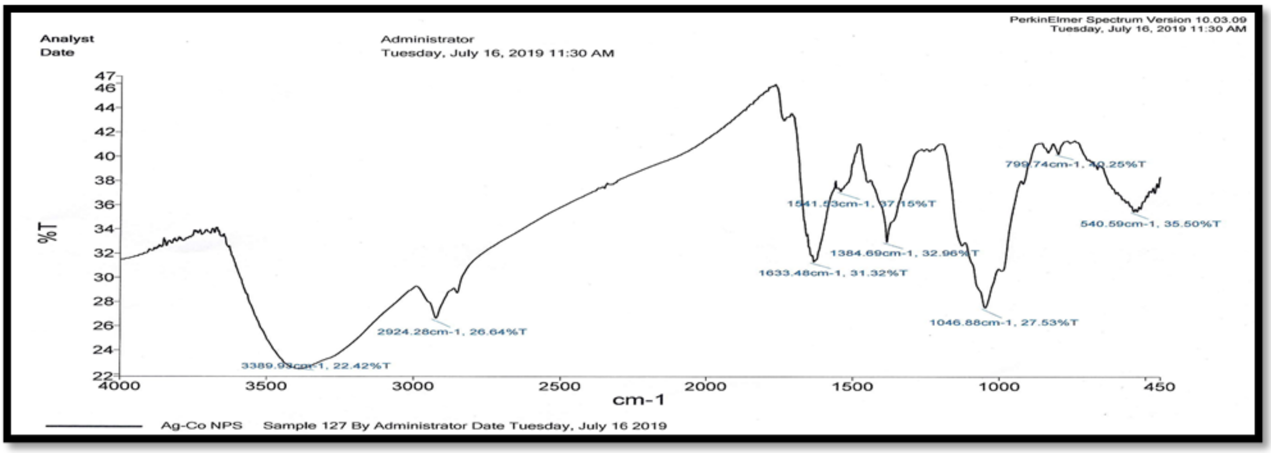

Figure 4. FT-IR Spectrum for Ag-Co BMNPs

3.3 FT-IR Result for Ag-Co BMNPs

FT-IR spectroscopy was applied to investigate the interactions between the aqueous root extract of *Borassus aethiopum* and the aqueous solution of the silver-Cobalt salt. The FT-IR spectra of the root extract and that of the biosynthesized Ag-Co BNPs are shown in Figures 3 and 4 respectively. Various functional groups present in the BNPS were identified. It displayed bands due to O-H stretching vibration mode at 3389.93 cm^{-1} which overlapped with the N-H stretching in terpenoid found within this region. Also observed, were a medium sharp peak for C-H absorption at 2924.28 cm^{-1}, C=C stretching at 1633.48 cm^{-1}, C=N stretching and the C-O deformation at 1541.53 cm^{-1} and 1046.88 cm^{-1} bands respectively. This is fairly the same with the result obtained by **Akinsiku *et al.* (2018)**. These have replaced those observed in the spectrum of the root extract that include peaks at 3443.26cm^{-1}, 2929.48 cm^{-1}, 1651.28 cm^{-1}, and 1080.12 cm^{-1}. Most notably is the appearance of a prominent peak at 1541.53cm^{-1} due to C=N stretching and the disappearance of the peaks at 1162.66 cm^{-1}, 986.29 cm^{-1}, 861.19 cm^{-1} and 525.69 cm^{-1}.

3.4 Antibacterial study results

Results of the antibacterial bioassay using agar diffusion test to identify the inhibitory activity of Ag-Co bimetallic nanoparticles against *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Bacillus subtilis* and *Salmonella typhi* at different nanoparticle precursor concentrations are presented in Table 1 and a chart relating the zone of inhibition with concentration for the five bacteria is depicted in Figure 5.
The test showed the activity of the biosynthesized Ag-Co bimetallic nanoparticles was based on the size of zones of inhibition in millimeter (mm). Agar diffusion test revealed that the nanoparticles possessed antibacterial property due to the zones of inhibition values at a reasonable level. Interestingly, screened nanoparticles exhibited a dose-dependent inhibitory activity on the organisms in agreement with **(Andrighetti-Frohner *et al.*, 2009)**. It was also observed that the Ag-Co BMNPs displayed high activity on all the organisms considered even at low concentration of 200μg/L as compared with the standard drug Augmetin at concentration of 200μg/L, Table 1 and Figure 8. This showed that Ag-Co BMNPs have strong potency against the investigated pathogens. It is interesting to note that for all concentrations of Ag-Co BMNPs as well as standard Augmentin drug, equal zone of inhibitions was observed for *S. typhi* and *E. coli*, Table 1. Thus, Ag-Co BMNPs could be used in the treatment of these bacterial strain diseases. Previously, $CaO@SiO_2$ nanocomposite was synthesis by our research group and its antibacterial activity was tested on various pathogens **(Lamayi *et al.*, 2019)**. At 500 μg/L of $CaO@SiO_2$ nanocomposite, the inhibition zones were found to be 14, 16, 22 and 20 for *E.* coli, *B.subtilis* , *P.aeruginosa* ,and *K.pneumonia* respectively. These results are far lower than in the current study. However, at 500 μg/L of $CaO@SiO_2$ nanocomposite, the inhibition zone was found to be 26 mm for S. *typhi*. Therefore, for *E.* coli, *B.subtilis* , *P.aeruginosa* ,and *K.pneumonia*, Ag-Co BMNPs proved to be a promising antibacterial agent than *S. typhi* as compared to $CaO@SiO_2$ nanocomposite.

Table 1. Result for Antibacterial Studies of Silver-Cobalt Nanoparticles against 5-bacteria Pathogens

Zone of inhibition for Ag-Co BMNPs (mm)

Concentration of Ag-Co BMNPs (μg/L)	*E. coli* (mm)	*B.subtilis* (mm)	*P.aeruginosa* (mm)	*K.pneumonia* (mm)	*S. typhi* (mm)
200	13	15	17	9	13
300	15	20	18	14	15
400	21	17	25	15	21
500	22	30	23	28	22
Augmentin 300μg/L	11	8	23	8	11

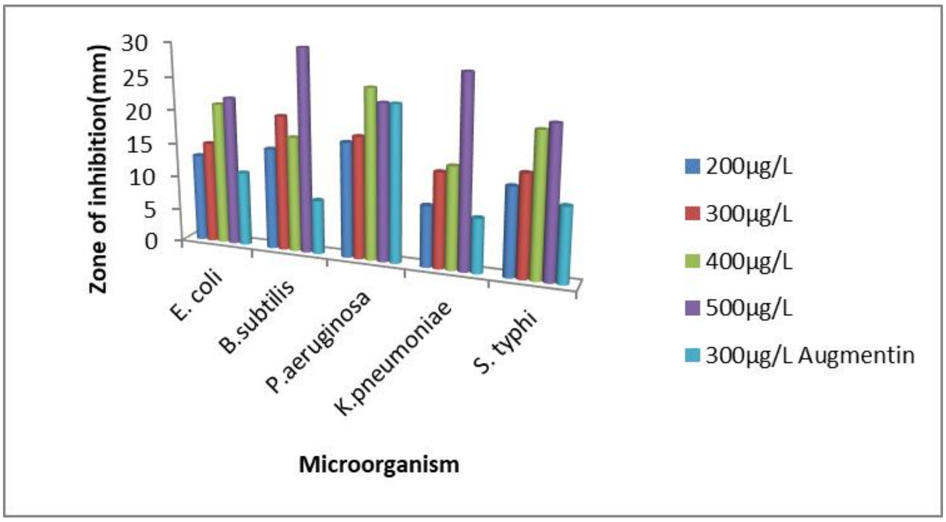

Figure 5. Chart for the antibacterial result for Ag-Co BMNPs

Conclusion

Motivated by the fact that little is known on the in vitro bacterial potency of Ag-Co bimetallic nanoparticles and other wider applications, these bimetallic nanoparticles were successfully synthesized from the locally available root extract of *Borassus aethiopum* and characterized using FT-IR and UV-Visible Spectrophotometers used frequently for identification of nanoparticles. Bacterial assay showed its effective efficacy as potential biocontrol for the treatment of bacterial diseases.

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Declaration of interest

Authors declare that there is no conflict of interest.

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