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Green Synthesis, Characterization and Antimicrobial Studies of Ag/Ni Bimetallic Nanoparticles from *Tamarindus indica* Aqueous Stem Extract

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Abstract
In this study, the mixture of Ag^{+}/Ni^{2+} was reduced to Ag/Ni BMNPs using Tamarindus indica Aqueous Stem Extract as a green reducing agent. The formation of Ag/Ni bimetallic nanoparticles was first observed based on the visual change in color of the reaction mixture at room temperature from light brown to deep brown within 20 min. UV analysis of the sample revealed that the maximum absorption peak was at absorbance of 0.8 with corresponding wavelength (λ_{max}) at 500 nm. The FT-IR studies detected the presence of C-H, C-O, N-H, O-H and C=C functional groups as revealed by different absorption bands. Similarly, the surface morphology of the nanoparticles as revealed by SEM result is spherical. Furthermore, the synthesized Ag/Ni BMNPs were examined for antimicrobial efficacy against S. aureus, P. aeruginosa, C. albicans, and A. niger with augmentin being used as control throughout the studies. Although the inhibition zone increases with increase in concentrations of Ag/Ni BMNP of all the pathogens, P. aeruginosa, demonstrated higher zone of inhibition as compared to other pathogens, hence, Ag/Ni BMNPs may be a potential antimicrobial agent for the posterity.

Keywords: Green Synthesis, Characterization, Antimicrobial Studies, Ag/Ni Bimetallic Nanoparticles, *Tamarindus indica*, Aqueous Stem Extract

1. Introduction
Nanoparticles of transition metals have received special attention due to their wide range of properties and potential applications in the fields of physics, chemistry, materials science, biology and catalysis [1]. Nanoparticle research is presently an area of strong scientific interest due to a wide variety of Potential applications in biomedical, optical and electronic fields. Many plant parts have been used in green synthesis of silver nanoparticles including grape skin power, *Moringa oliefera* root [2, 3]. Bimetallic nanoparticles specifically offer a feasible alternative for the potentiation of the metallic properties of the nanoparticles. This is because these hybrids consist of two combined nanoparticles with different functionalities with a common interface. Synthesis of bimetallic nanoparticles has employed many applications especially as larvicidal agents, and these bimetallic nanoparticles can be produced via a cost-effective and eco-friendly route [4, 5]. Recently, researchers have focused on the selective fabrication of new bimetallic nanoparticles in various forms, such as a core-shell, alloys, and a contact aggregate, as they may have unique electronic, optical and catalytic properties, absent from corresponding monometallic nanoparticles. Furthermore, Bimetallic nanomaterials have raised more and more significant concern from worldwide researchers in recent years due to their new physical and chemical properties derived from synergistic effects between the two metals. These hybrid species are highly desirable for specific technological applications, especially for antimicrobial study [6, 7]. It is now no news that plant mediated nanoparticles have been investigated to possess many applications in many fields such as pharmaceuticals, therapeutics and other commercial products [8, 9]. In a separate study, Silver-Cobalt Bimetallic Nanoparticles were reported as a potent nanotechnological method for control of *Culex quinquefasciatus*-borne diseases. These entities have been found to have a lot of applications ranging from biological to catalytic applications [10, 11, 12].

2. Materials and methods
2.1 Collection of plant materials
Fresh stems of Tamarind tree were collected from Banganje, Billiri Local Government Area of Gombe State. The stem sample was identified and authenticated at the Taxonomy Section in Federal University of Kashere.

2.2 Preparation of Aqueous Plant Extract
The stem sample was, under running tap water, thoroughly washed and rinsed severally with distilled water followed by sun drying to expunge residual moisture. The dried materials were cut into fine pieces and 30g of it was weighed and dispersed in 600 ml of sterile distilled water in a 1000 ml glass beaker and boiled at 100^0C for 30min and was allowed to cool. The solution was filtered through Whatman No.1 filter paper and kept for further use.

2.3 Green Synthesis of Ag/Ni Bimetallic Nanoparticles
One hundred milliliters of the filtrate obtained was measured in a measuring cylinder and transferred into a 1000 ml beaker. A 250 ml each of 10^{-2}M $AgNO_3$ and 10^{-2}M $Ni(NO_3)_2{\cdot}6H_2O$ were mixed in a standard Erlenmeyer flask and added gradually to the boiling stem extract with frequent stirring using a glass rod for 30 minutes. Nanoparticles were allowed to settle, decanted and dried in an oven.

2.4 UV-visible Spectroscopic Analysis
Here, the bio reduction process of Ag/Ni bimetallic nanoparticles in aqueous solution was measured by sampling of 1 ml aliquot compared with 1 ml of distilled water used as blank and subsequently measuring the UV-visible spectrum of the solution. UV-visible spectrum was monitored on Cary Series UV-Vis spectrophotometer Agilent Technology, operated within 200 to 800 nm wavelength range.

2.5 Infra-Red Spectral Analysis
Here, a very minute quantity of the dried Ag/Ni bimetallic nanoparticles were taken and made into a pellet using pelletizer and hydraulic presser then placed into FT-IR sample holder and introduced into the infrared spectrophotometer for analysis**.** The FT-IR spectrum was recorded in the range of 450-4000cm^{-1} using Perkin Elmer Spectrum version 10.03.09.

2.6 Antibacterial activity of the Ag-Ni NPs
The biosynthesized AgNPs were checked for their antibacterial activity using well diffusion method [13] against *Staphylococcus aureus (S. aureus), a Gram-positive bacterium, Pseudomonas aeruginosa (P. aeruginosa), a Gram-negative bacterium, Candida albicans (C. albicans), and Aspergillus niger (A. niger).* 24h old culture suspensions were inoculated into molten Mueller Hinton agar butt, and poured into sterile petri plates. Wells were made in each plate using the sterile cork borer of 6mm diameter and the solutions were added to the plate. The plates were then incubated at 37°C for 24h. After 24h, the zones of inhibition were measured and interpreted.

3. Results and discussion
3.1 UV-Visible Spectroscopic Analysis of Cu/Ni BMNPs
The formation of Ag/Ni bimetallic nanoparticles was first recorded based on the visual alteration in color of the reaction mixture at room temperature from light brown to deep brown within 20 min which signaled formation of nanoparticles. This is as a result of electronic transitions within the structure of Ag/Ni nanoclusters as they interacted with light. The electronic transitions within the structure of metallic nanoparticles resulted in emergence of surface plasmon resonance (SPR) which increased in peak intensity and confirmed Ag/Ni NPs formation. This phenomenon corresponds to the investigation carried out by the earlier researcher [14]. The UV–Vis absorption spectrum of the synthesized Ag/Ni BMNPs is depicted in Fig 1. It was observed that, the maximum absorption peak was at absorbance of 0.8 with corresponding wavelength (λ_{max}) at 500 nm. Less information is given on the characterization of these NPs using this equipment, however, the λ_{max} is in concord with 500nm reported by a researcher in a separate study on Cu/Ni BMNPs [15].

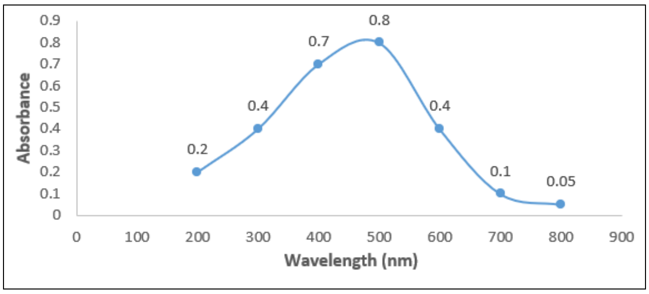

Fig 1: UV-visible spectrum of Ag/Ni bimetallic nanoparticles

3.2 Fourier-Transform Infrared Spectroscopy
Here, an FT-IR analysis was carried out (fig 2) so as to identify the biomolecules contained in the tamarind stem extract, as these are mostly responsible for the reduction of Ag^+ and N^{2+} ions in the synthesis of bimetallic nanoparticles. The FTIR spectrum of the Ag/Ni bimetallic nanoparticles showed absorptions at 1090.80 cm^{-1}, 1384.50 cm^{-1},1638.75 cm^{-1}, 3436.44 cm^{-1}, and 3787. 71 cm^{-1}. Investigation reveals a medium sharp peak absorption at 1090.80 cm^{-1} which may be attributed to the stretching of aliphatic hydrocarbon (C–H). A peak at 1384.50 cm^{-1} corresponds to C-O stretching while the absorption bands at 1638.75 cm^{-1} and 3436.44 cm^{-1} may be assigned to N-H and O-H stretching vibration modes respectively. Similarly, a peak was seen at 3787.71 cm^{-1} corresponding to C=C. The result of the FT-IR analysis investigated in this study, not only agrees with the UV-visible result, but also corresponds to the result of other researchers [16] which then confirmed the bio fabrication of Ag/Ni bimetallic nanoparticles.

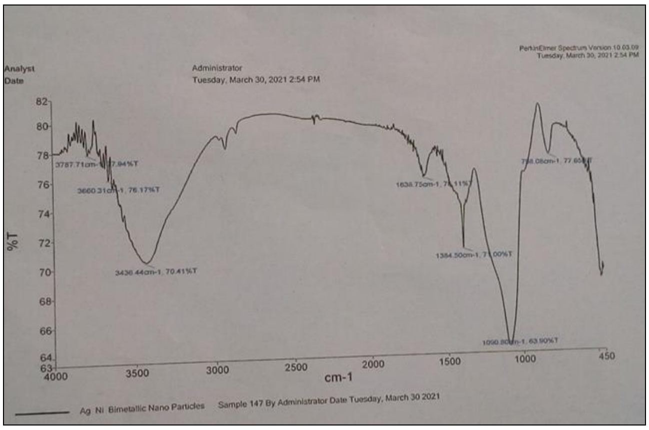

Fig 2: FT-IR Spectrum of Ag/Ni BMNPs

3.3 Scanning Electron Microscopy
The electron microscopic study observed shows that the structure seen in the SEM image (fig 3) is spherical in shape. It is observed that the Ag/Ni nanoparticles are scattered over the surface and no aggregates are noticed

under SEM, the difference in size is possibly due to the fact that bimetallic nanoparticles were formed at different times. It is however, worthy of note that, very limited information was reported regarding the characterization of Ag/Ni bimetallic nanoparticles using this technique in particular.

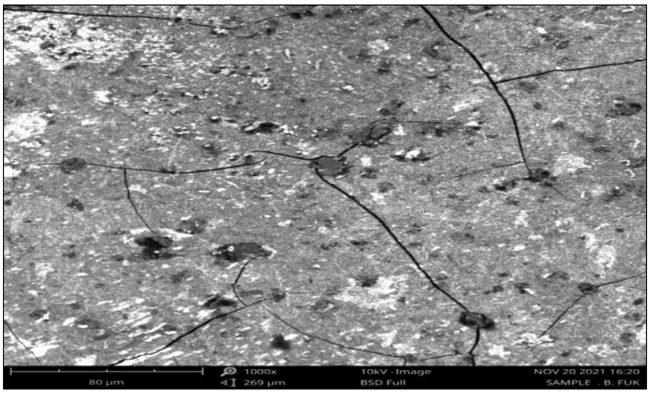

Fig 3: SEM Spectrum of Ag/Ni Nanoparticles

3.4 Antimicrobial activity.

In search of a new therapeutic alternative, here, the antimicrobial potency of Ag–Ni bimetallic NPs against selected Pathogens was determined. The result of microbial studies of Ag/Ni BMNPs against *S. aureus* (a Gram-positive bacterium), *P. aeruginosa* (a Gram-negative bacterium), *C. albicans*, and *A. niger* is presented on table 1. Augmentin was used as control throughout the studies at concentration of 300µg/L. Different concentrations of 100, 200, 300, 400 and 500µg/L of Ag/Ni BMNP was tested against each microbe. The inhibition zone increases with increase in concentrations of Ag/Ni BMNP of all the pathogens. At higher concentration of 500µg/L, the zones of inhibition were in the following order: 24.44mm, 19.52mm, 17.66mm, and 16.99mm for *P. aeruginosa, S. aureus, C. albicans* and *A. niger* respectively. For each concentration investigated, *P. aeruginosa*, demonstrated higher zone of inhibition as compared to other pathogens, an indication that Ag/Ni BMNPs is potent against *P. aeruginosa* than any other pathogen under investigation for this research.

Table 1: Antimicrobial Activity of Ag/Ni BMNPs

AgNPs	TEST Organism	Concentration (mm)					
		100µg/L	**200**µg/L	**300**µg/L	**400**µg/L	**500**µg/L	**Control (Augmentin) 300**µg/L
	S. aureus.	13.50mm	13.55mm	14.50mm	16.00mm	19.52mm	20.00mm
	P.aeruginosa	14.33mm	15.22mm	17.55mm	20.00mm	24.44mm	26.22mm
	C. albican	13.11mm	13.55mm	14.55mm	16.66mm	17.66mm	21.55mm
	Aspergillus Niger	11.44mm	13.00mm	15.66mm	16.10mm	16.99mm	18.00mm

4. Conclusion

Tamarindus indica stem extract was used as reducing agent in the fabrications of Ag/Ni BMNPs via green route which is considered to be environmentally friendly as it does not require use of toxic chemicals. The synthesized nanoparticles were characterized using UV-visible, FT-IR and SEM to ascertain their formation. The antimicrobial studies revealed that Ag/Ni BMNPs might be potent against the selected pathogenic microbes.

5. Funding

This study receives no specific financial assistance.

6. Conflicts of Interest

The authors declare that there are no conflicts of interest.

7. Acknowledgement

All authors contributed equally to the conception and design of the study.

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