Bima Journal of Science and Technology, Vol. 7 (3) Sept, 2023 ISSN: 2536-6041

DOI: 10.56892/bima.v7i3.484

Green Synthesis and Characterization of Silver – Cadmium (Ag-Cd Bimetallic) Nanoparticles from *Ocimum Gratissimum* Leaves Extract and Evaluation of its Antimicrobial Activities

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ABSTRACT

Suitable and effective antimicrobial agent bimetallic silver-cadmium nanoparticles was prepared via reduction process using phytochemicals present in Ocimum gratissimum leaves extract as eco-friendly solvents, non-toxic reducing reagents, biodegradable capping and stabilizing agents; characterized by UV-Vis., FTIR, XRD and SEM techniques, and antimicrobial activities were tested on *H-pylori, K-pneumonia, S-Typhi, S-Aureus; Aspergillus niger, and Candida albican*. The UV-visible spectroscopic technique confirmed the formation of synthesized nanoparticles with plasma resonance peaks at 600nm for Ag-Cd bimetallic nanoparticles. SEM analysis revealed that Ag-Cd bimetallic nanoparticles, are merely spherical in form; FTIR analysis confirmed the presence of carbonyl, alcoholic groups, unsaturation due to both aliphatic and cyclic (ring) aromatic, metal linkage, aromatic amine and alkyl halides. The XRD analysis indicated Ag-Cd, bimetallic nanoparticles are Face Centered Cubic (FCC) structure with average crystalline size of 53.82nm. The antimicrobial evaluation results showed an increase in the bacterial and fungal growth inhibition with increase in concentration of the bimetallic nanoparticles with highest inhibition at 500ug/ml and 150ug/ml respectively. It could be concluded that the synthesized Ag – Cd could serve as an effective microbial inhibitor.

Keywords: Bimetallic, Silver, cadmium, antimicrobial, nanoparticle.

INTRODUCTION

The recent development and implementation of new technologies have led to new era with an increased interest in environmental issues which however the nano-revolution revealed the roles of nano-base materials on the environmental remediation (Kavitha *et al.,* 2013). This therefore, unfolds the need for efficient, low-cost and environmentally sustainable method of synthesizing nanoparticles. Although nanoparticles can be synthesized through array of conventional methods, biological route of synthesizing nanoparticles, seem to be efficient and effective approaches in contrast to physical, chemical and other techniques to overcome the limitation of the other conventional methods where plants and microorganisms are majorly exploited (Muhammad *et al.,* 2020).

Recently, bimetallic nanoparticles (NPs) have captured attention of many researchers due to their excellent optical, magnetic, electrical, and catalytic properties compared to their individual analogues. The, structure of bimetallic NPs is composed of two different elements that are either present in a core-shell arrangement or exist as randomly dispersed alloys. As such, bimetallic NPs do not only possess the properties of the combining metals but also produce emerging properties due to the synergistic effect between two metals (Nurafaliana *et al.,* 2018).

Bimetallic nanoparticles can occur naturally, be created as the by-products of combustion reactions, by biogenic synthesis or can be produced purposefully through engineering to perform a specialized function due to the ability to generate nano-materials in a particular way to play a specific role. The use of nano-materials spans across a wide variety of industries, from healthcare and cosmetics to environmental preservation, anticancer, antimicrobial and air purification. However, the biogenic fabrication of bimetallic nanoparticles from plants and microbes has captured the attention of many researchers because of it's economical, sustainable and eco-friendly (Fahmy *et al.,* 2021). Such that plants and their parts are known to have various kinds of primary and secondary metabolites (phytochemicals) which reduce the metal salts to metal nanoparticles (Xu *et al.,* 2011). The shape, size and stability of the nanoparticles are influenced by pH, temperature, incubation time and concentrations of plant extract and that of the metal salt. Thus, three main conditions of preparation of bimetallic nanoparticles are: the choice of environment-friendly solvent medium, reducing agent and a nontoxic material for their stabilization (Shalaka *et al.,* 2018). Thus, biological methods that use biogenic materials as a reducing and capping or a dispersing agent are emerging as a new green procedure for the production of bimetallic nanoparticles in an aqueous medium (Piermatti, 2021). The reduction of metal ions occurs by the proteins, amines, amino acids, phenols, sugars, ketones, aldehydes and carboxylic acids present in the plants and or microbes to obtain a nanoparticle of particular bimetallic of interest. (Khwaja *et al.,* 2016).

Ocimum gratissimum extracts have phytochemical components content such as polyphenol, flavonoids, terpenoids, polyols, glycosides, vitamins, amino acids and carboxylic that are therapeutics in nature. It is used in eye maintenance as it a source of vitamin A which promote good eyesight; improve heart function and lowers blood sugar in the body; it is use as insects and mosquito repellents, treatment of fungal infection and diarrhea. It also aids in digestion, and has anti-inflammatory property (Chun *et al.,* 2011).

However, several studies have provided direct evidence that the wide spread use of antibiotics has led to the emergence of multidrug-resistant bacterial strains, this raises the hope that nanoparticles would be less prone to promoting resistance in bacteria than antibiotics(Fatimah *et al*., 2017) as most of the antibiotic resistance mechanisms are irrelevant to that of nanoparticles because the mode of action of nanoparticles is direct contact with the bacterial cell wall, without the need to penetrate the cell. Therefore, attention has been drawn to an efficient and effective way of preparing nanoparticles-based materials with improved activity (Fahmy *et al.,* 2021).

This research is aimed at synthesizing Ag-Cd bimetallic nanoparticles via reduction process using phytochemicals present in *Ocimum gratissimum* leaves extract, and evaluation of the applications as antimicrobial agent.

MATERIALS AND METHODS

Preparation of *Ocimum gratissimum* Leaves Extract

The method used by Ahmad *et al.,* (2022) was adopted and slightly modified. The freshly collected *Ocimum gratissimum* leaves sample was washed several times with running tap water and then rinsed with distilled water to remove any adhered dirts and then air dried and ground using a wooden mortar and pestle. It was then sieved. A 30g of the powdered sample of *Ocimum gratissimum* was weight and dispersed into 200ml of distilled water in 500ml glass beaker and boiled at 80^0C for 30 minutes and then allowed to cool. After cooling, it was filtered using Whatmann filter paper and the filtrate was used immediately for the synthesis of the nanoparticles.

Synthesis of Ag-Cd Bimetallic Nanoparticles

The method adopted was that of Muhammad *et al.* (2022) with slight

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modification. A1mM aqueous solutions of silver nitrate and cadmium chloride were prepared in distilled water in a separate container. These two solutions *were* equally mixed to form a coffee brown color of Ag-Cd salt solution Muhammad *et al*., (2018 and 2022) approach was adopted and slightly modified. The prepared $AgNO_3$ and $CdCl_2.2.5H_2O$ solutions was added into the plant extract in the ratio of 1:5 that is 5ml extract and 25ml metal precursor with constant stirring at 80^0C for 60 minutes using magnetic stirrer. Within the first 15 minutes. The color changed from coffee brown to light brown as shown Figure 1 indicated the formation of Ag-Cd NPS nanoparticles. It was aged and allowed to settle for 24 hours after which it was decanted and dried at 100^oC for 2hours. It was then ground into powder and kept for further analysis.

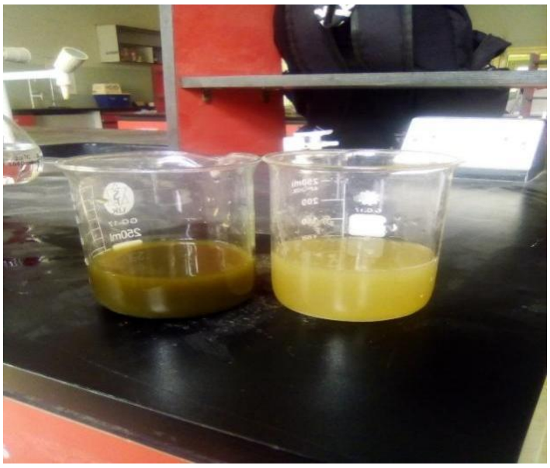

Figure 1: Formation of Ag-Cd NPS nanoparticles

Antibacterial Activity of Biosynthesized Ag-Cd NPs

The antibacterial activity of the biosynthesized Ag-Cd NPs against *H-pylori, K-pneumonia, S-Typhi, S-Aureus* bacteria species and two fungi: *Aspergillus niger*, and *Candida albican* were done by the Disk Diffusion method (Arunkumar *et al*., 2021; and Poirel *et al.,* 2017) slightly modified. Bacterial strains were spread on the Petri dishes, which contained autoclaved (Luria-Bertani LB) medium containing agar. Then the disks were soaked in distilled water as a control. The plant extract, and biosynthesized Ag-Cd NPs were separately placed on the Petri dishes containing LB media. Petri dishes were incubated at 37^0C. The inhibition zone of each disk was measured by a ruler after 18h.

Characterization

The UV visible spectrophotometric measurements were performed on the Elico spectrophotometer to ascertain the formation of the Ag-Cd nanoparticles (Subbaiya *et al*., 2014) The structures of the prepared Ag-Cd nanoparticles were analyzed by X-ray diffraction using X' Pert Pro X-ray diffractometer with Cu Kα as a source of radiation in a θ-2θ configuration (Yelil Arasi *et al*., 2012), whereby the bonding was studied by Perkin–Elmer 1600 spectrophotometer in KBr medium tools. JEOL JSM-6380 LA Scanning electron microscope with energy dispersive microanalysis of X-Ray (EDAX) was used to study particle morphology with metal confirmation of the sample (Arunkumar *et al*., 2021).

RESULTS AND DISCUSSIONS

The silver-cadmium bimetallic nanoparticles (Ag-Cd NPS) exhibited a surface plasmon resonance (SPR) band around 600nm which is due to the surface Plasmon vibration and excitation of the bio reduction and capping agent present in the leaves extract. The color of the solution changed from coffee brown to light brown after the addition of $AgNO_3$ and $CdCl_2.2.5H_2O$ solutions, indicating the deposition of Ag layer onto the surface of Cd NPs (Nurafalian *et al.,* 2018). This also corresponds with the literature of Kiranmai *et al*., (2017) which showed the highest absorbance peaks at 300nm and 650nm and also suggest that, this variation depends on the reducing agent and the type of metal salt used as a precursor

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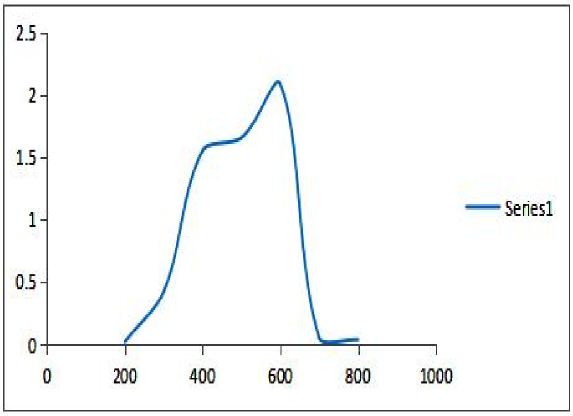

Figure: 2 Absorption Spectrum for Ag-Cd Bimetallic Nanoparticles

X- Ray Diffraction (XRD) Result for Ag-Cd NPS

The XRD pattern of the synthesized silver-cadmium bimetallic nanoparticles was shown in Figure 3 above. The XRD was done to determine the average crystalline size of the silver-cadmium bimetallic nanoparticles and the resultant peaks were observed at 2Θ = 15.04^0, 27.11^0, 33.63, and 49.76^0, with respect to the plane of (101), (210), (211) and (311). It shows Face Centered Cubic (FCC) structure and the average crystalline size of 53.82nm. This is also nearly in agreement with the work done by Flora *et al.* (2018).

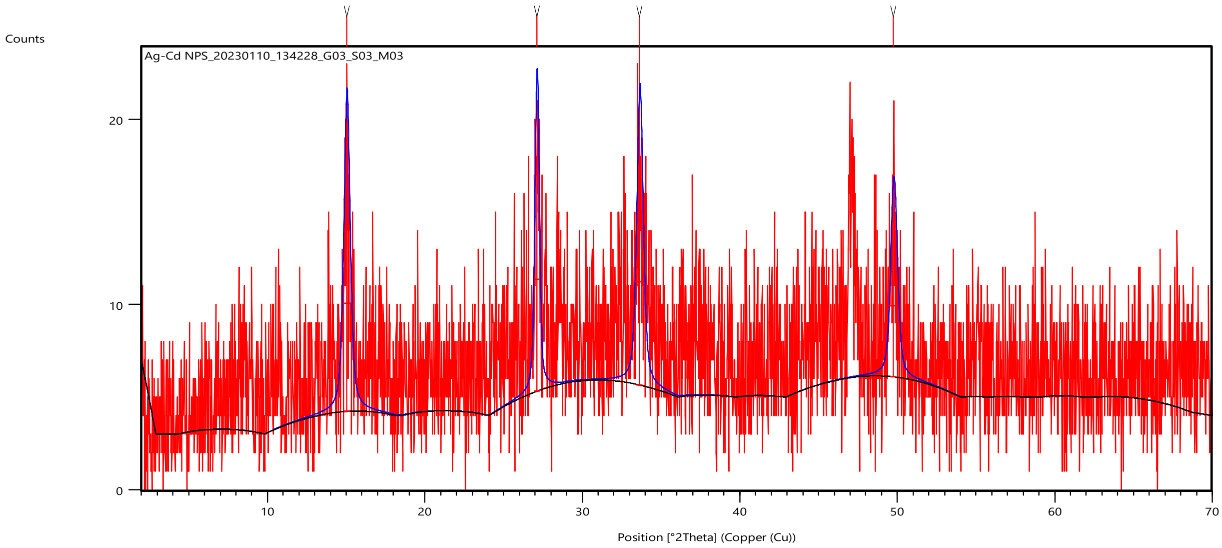

Figure 3: XRD band for Ag-Cd NPS

FTIR Results for Ag-Cd Bimetallic Nanoparticles

The FTIR spectra of the Ag-Cd bimetallic NPS in Figure 4 show several peaks. Where peak at 3402.54cm^{-1}, represents O-H stretching H-bonded due to alcohol/phenols; 2924.18cm,$^{-1}$ peak represent C-C triple bond stretching due to the alkynes; while peak at 2360.95 cm^{-1}, represent C-C triple bond stretching due to the alkynes and peak at 1635.69cm^{-1}, was due –C=C stretching indicated alkenes aromatic and the metal bond or linkage, 1404.22 cm^{-1}; peak obtained at 1149.61 cm^{-1} shows –C-C bonding in aromatic ring; peaks appeared at 671.25cm^{-1}; 609.53cm^{-1} and 478.36cm^{-1} indicates C-O stretch alcohol finger print; also peaks at 3402.54cm^{-1}, 2924.18cm^{-1}, 2360.95cm^{-1}, 1635.69cm^{-1}, 1404.22cm^{-1}, 1149.61cm^{-1}, 671.25cm^{-1}, 609.53cm^{-1} and 478.36cm^{-1}. 3410.26cm^{-1} represent O-H stretching H-bonded due to alcohol/phenols. 2357.09cm^{-1} represent C-C triple bond stretching due to the alkynes, 1639.55cm^{-1} indicate –C=C stretching due to alkenes aromatic and the metal bond or linkage, 1408.08cm^{-1} –C-C (in ring) aromatic, 1330.98cm^{-1} C-N stretching aromatic amine, 1203.63 cm^{-1} and 1161.19 cm^{-1} indicates C-

H (wag) alkyl halides(-CH_2X). 999.16 cm^{-1} and 964.44 cm^{-1} represents -C=C-H and =C-H bends alkenes while 663.34 cm^{-1} and 605.67 cm^{-1} are responsible for –C triple bond C, C-Br alkyl halides respectively. The FTIR analysis therefore, confirmed the presence of carbonyl, alcoholic groups; unsaturation due to both aliphatic and cyclic (ring) aromatic (benzene, alkene, and alkyne aromatic); metal linkage; aromatic amine and alkyl halides. These proved the possible formation of the bimetallic nanoparticles.

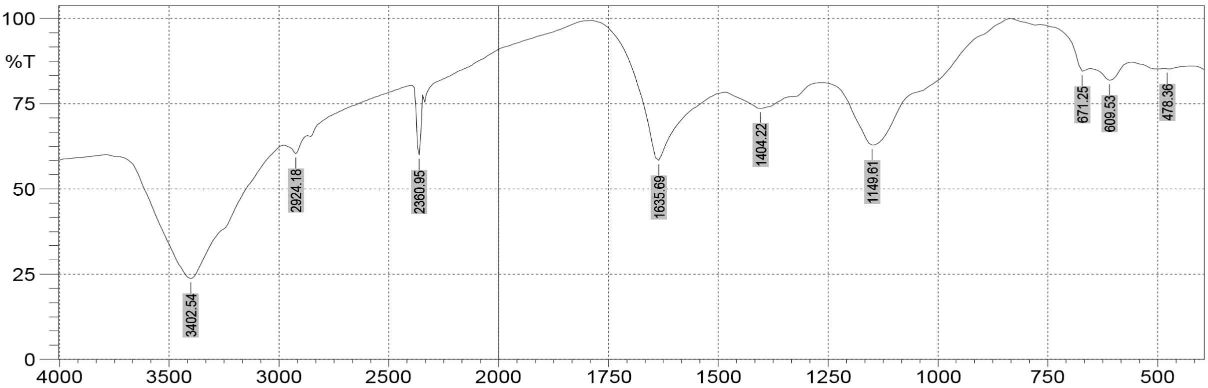

Figure 4: FTIR spectra of Ag-Cd NPs

Scanning electron microscopy (SEM)

The result shows that, the particles are partially spherical in form. Similar to the work done on *Papaver sonmiferum* by Wali *et al.,* (2017) which showed the morphology of the nanoparticles to be crystalline and partially spherical in nature.

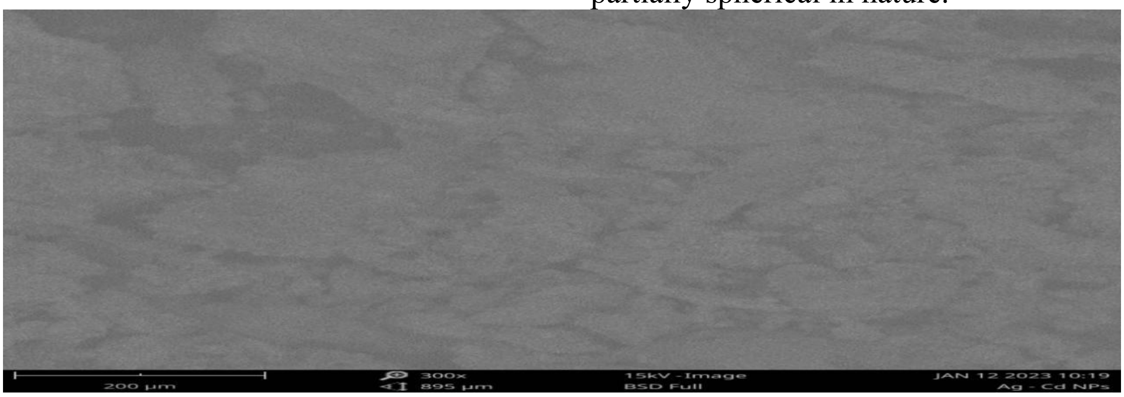

Figure 5: SEM image obtained for Ag-Cd NPs

Antibacterial Activity of Ag-Cd NPs

The zones of inhibition measured are summarized in Table 1 and 2 from the tables; it is evident that the synthesized nanoparticles are good candidates for their usage in antibacterial drugs (Arena *et al*., 2015). This sample shows remarkable activity against *H-pylori, K-pneumonia, S-Typhi, S-Aureus; Aspergillus Niger, and Candida albican*.

As shown in Table 1 there are increase in the bacteria growth inhibition with increase in concentration of the Ag-Cd nanoparticles synthesized. The inhibition was high at 150ug/ml.

Also, from Table 2 below, the synthesized silver-cadmium bimetallic nanoparticles show an increase in fungal growth inhibition with increase in concentration of the synthesized drug (Ag-Cd NPs), it shows highest inhibition at 150ug/ml with 19mm for *Candida albican* while that of the *A.*

niger, also shows an increase in fungal growth inhibition with increase in concentration of the nanoparticles. It shows 25mm fungal growth inhibition which is closed to that of the control drug (Fulcin which shows 31mm inhibition performance.

Table 1 Antibacterial activity test result for silver-cadmium bimetallic nanoparticles.

S/ N	Organism	Concentrations				Control
		150ug/ml	100ug/ml	50ug/ml	25ug/ml	Aug 30mg
1	S. Typhi	25mm	13mm	10mm	09mm	28mm
2	S. Aureus	24mm	21mm	17mm	14mm	32mm
3	H. Pylori	29mm	13mm	16mm	08mm	31mm
4	K. Pneumonia	26mm	29mm	22mm	12mm	22mm

Table 2 Anti-fungal activity Test Result of Ag-Cr NPS on Selected Fungal Pathogen

S/N	Organism	Concentrations				Control
		150ug/ml	100ug/ml	50ug/ml	25ug/ml	Fulcin30mg
1	Candida	19mm	11mm	13mm	09mm	27mm
2	A. Niger	25mm	21mm	17mm	13mm	31mm

CONCLUSION

The green synthesis of Ag-Cd nanoparticles using *Ocimum gratissimum* leaves extract as a simple, efficient, eco- friendly method was successfully achieved and the reduction of silver and cadmium ions takes place simultaneously. It physical and chemical properties was analyzed by SEM, XRD, UV-Visible and FTIR techniques. The antimicrobial activity of the synthesized bimetallic nanoparticles was tested on *H-pylori, K-pneumonia, S-Typhi, S-Aureus; Aspergillus Niger, and Candida albican* and the results showed that, the synthesized nanoparticles is a good candidate for their usage in antibacterial drugs. This method is one of the economic viability methods for the synthesis of nanoparticles. Further detailed characterization, properties, and applications of Ag-Cd nanoparticles is the future direction of this work.

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