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GREEN SYNTHESIS, CHARACTERISATION AND ANTIBACTERIAL ACTIVITY EVALUATION OF SILVER SULFIDE NANOPARTICLES OF *SENNA OCCIDENTALIS* (L.)

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ABSTRACT: The study focuses on the green synthesis of silver sulfide nanoparticles using aqueous Senna occidentalis (L.) extract as reducing, capping, and stabilizing agents. Fourier Transform Infra-red Spectroscopy (FTIR) and Scanning electron microscopy (SEM) characterized the synthesized silver sulfide nanoparticles. FT-IR spectrum of *Senna occidentalis* leaves aqueous extract as shown in **Fig. 3** below shows a number of peaks, thus reflecting its complex nature. The bands at 3175cm^{-1} is characteristic of the alcohol/phenol –OH stretching vibration, 2361.44cm^{-1} an attribute O=C= Stretching representing carbon dioxide. The weak peak at 1594cm^{-1} is assigned for C=O bending, indicating carboxylic acid, and the band at 995cm-1 represents the C=C bending signifying alkene. **Fig. 4** below shows the FTIR result of the synthesized silver sulfide nanoparticle at different bands. The bands at 3785 and 3703 cm^{-1} correspond to O-H stretching vibration, indicating the presence of alcohol/phenol; the peak at 3406 cm^{-1} reflects N-H stretching of alphatic primary amine. Bands at 2919 and 2855.82cm^{-1} correspond to the C-H stretching of aromatic compounds. The band at 1713.88cm-1 indicates C=N stretching attributed to amine, the band at 1601cm^{-1} is assigned to C=C, a characteristic of conjugated alkene. The shape of the silver sulfide nanoparticles is spherical with few exceptional as ellipsoidal. *Senna occidentalis* Ag_2S nanoparticles were observed to possess antimicrobial activity as it inhibited some of the tested microorganisms (bacteria and fungi), giving clear inhibition zones that were the same or higher in some cases to those of the standard antibiotic or antifungal used. The nanoparticles antifungal inhibition activity was seen to be better than griseofulvin (the standard antifungal used) in the case of *Aspergillus fumigatis*.

INTRODUCTION: Metallic nanomaterials have been used in a wide-ranging applications in various fields. Specifically, shapes, size, and composition are significantly linked to their physical, chemical, and optical properties.

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Technologies based on nanoscale materials have been exploited in various fields, from chemistry to medicine [1-3]. Silver sulfide (Ag_2S) nanomaterial is an important material used in various areas of studies, including antimicrobial activity [4, 5].

The synthesis of silver sulfide nanoparticles (Ag_2SNPs) using several different methods had been reported, such as template-based method at room temperature and ambient pressure [6], water-in-CO_2 micro emulsions [7], a microwave-assisted template-free method [8], modified homogeneous

precipitation route [9], sonochemical synthesis [10], hydrothermal method [11], by multi-solvent thermal decomposition method [12], *via* a one-pot method in ethylene glycol with 3-mercaptopropionic acid [13], modified chemical bath deposition technique [14], synthesis of silver sulfide nanoparticles capped with either chitosan, green tea, *Combtretum molle* or black wattle extracts [15], chemical method [16], multi-solvent thermal decomposition method [17]. All the methods and routes by which silver sulfide nanoparticles are synthesized are highly disadvantageous due to toxic chemicals used and waste products, which create environmental problems, high energy consumption, the difficulty of large-scale processes, and wasteful purifications [18-21]. In recent studies, the green or benign approach in synthesizing nanomaterials has attracted significant attention to protect the environment from hazardous wastes [22]. *Senna occidentalis* (L.), a small shrub about 3 ft. high belonging to the Leguminosae family [23], have been extensively investigated because of their rich medicinal (anti-inflammatory, anti-carcinogenic, anti-mutagenic, anti-plasmodial, anti-rheumatic and hepatoprotective) and economic uses [24–28]. It is known to have been used locally in treating eczema and some other skin infections by people of Oju local area of Benue state [29]. However, in an attempt to continuously search for and to bring a solution to man's health challenges, especially microbial infections causing morbidity and mortality due to the development of resistant strains of the virus, bacteria, pathogenic fungi, and protozoa through the use of natural endowment in a more enhanced form that is eco-friendly, the objective of the present research work was to synthesize silver sulfide nanoparticles by green route using *Senna occidentalis* (L.) leaves aqueous extract at room temperature and study it's antibacterial activity

MATERIALS AND METHODS:

Collection and Identification of Plant Sample: *Senna occidentalis* leaves were collected from Mararaba in Nasarawa states, Nigeria; the leaves were identified and authenticated at the Department of Biological Sciences, Benue State University Makurdi by a botanist Mr. Waya.

Plant Extraction: *Senna occidentalis* leaves were rinsed with distilled water to remove foreign matters and air dried in the chemistry laboratory Benue State University Makurdi, at room temperature for 15 days. The dried leaves were crushed and further pounded into powder using a porcelain mortar and pestle. 20 g of the powdered sample was weighed using an electronic weighing balance and transferred into an 800 mL conical flask. 200 mL of distilled water was measured using a measuring cylinder and was poured into a conical flask containing the crushed sample. The mixture was boiled for 10 minutes using a heating mantle/hot plate, after which the solution was filtered with the aid of cotton wool and a glass funnel, into a 250mL conical flask to obtain an aqueous extract kept for further analysis.

FIG. 1: *SENNA OCCIDENTALIS* LEAVES AND THEIR AQUEOUS EXTRACT

Green Synthesis of Silver Sulfide (Ag_2SNPs): Silver sulfide nanoparticles were synthesized by mixing silver nitrate with *Senna occidentalis* aqueous extract and sodium sulfide at ambient temperature. In this synthesis, 1g of silver nitrate was dissolved in 100 ml of *Senna occidentalis* aqueous extract under magnetic stirring at 27°C (room temperature) for 10 min.

After that, a sodium sulfide solution was prepared by dissolving 1g of Sodium Sulfide in 100 mL of distilled water and gently swirling for homogeneity. The sodium sulfide solution was then added dropwise to the solution of $AgNO_3$, and *Senna occidentalis* extract under continuous

magnetic stirring until the colour of the solution changed to a suspended gray-black color indicating the formation of silver sulfide nanoparticles is shown below [30]. The mixture was centrifuged at 8000rpm for 4 hours, the supernatant was taken out, and the residue was washed twice with 10mL of distilled water. The obtained nanoparticles were further transferred to a sample bottle for further analysis.

FIG. 2: AQUEOUS EXTRACT OF *SENNA OCCIDENTALIS* AND SYNTHESIZED AG2SNPS (LEFT TO RIGHT)

Characterization of Silver Sulfide Nanoparticles:

Fourier Transform Infrared Spectroscopy (FT-IR): Fourier transforms infrared spectroscopy (FT-IR) analysis was performed in all samples isolated to have a prompt result regarding the biomineral. A few crystals were mixed with KBr (Merck for spectroscopy) and pulverized in an agate mortar to form a homogenous powder from which the appropriate pellet was prepared under a pressure of 7 tons. All spectra were recorded from 4000 to 400 cm^1 using the Pelkin Elmer 3000 MX spectrometer. Scans were 32 per spectrum with a resolution of 4 cm^1. The IR spectra were analyzed using the spectroscopic software Win-IR Pro Version 3.0 with a peak sensitivity of $2cm^1$.

Scanning Electron Microscopy: Samples were coated with a platinum coating of electrically conducting material, deposited on the sample either by low-vacuum sputter coating or by high-vacuum evaporation. To examine the morphology of the synthesized Ag_2S Nanoparticles.

Antimicrobial Assay:

Source of Microorganisms: Pure isolates of bacteria: *Staphylococcus aureus, Pseudomonas aeruginosa*, *Klebseilla* spp, *Escherichia coli*; fungi: *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigates*, *Candida* albicans, maintained in the microbiological laboratory of Benue State University, Makurdi were received and used for the assay.

Culture Media Preparation, Inoculation and Sensitivity Testing: Nutrient agar (Titan Biotech Ltd TMG 341) and Potato dextrose agar (HKM HCM050) were prepared according to manufacturer's instructions following the general procedure for culture media preparation by [31]. Antibiotic (chloramphenicol capsule) was added to potato dextrose agar (PDA) to inhibit bacteria growth and allow only the seeded fungal growth.

All prepared media was aseptically poured into sterile plastic petri dishes and allowed to set. The plates were subjected to a sterility test for 24 hours; only sterile plates were used for the analysis. The plates were properly labelled with codes representing the various organisms in duplicate. The bacteria were individually streaked with a wire loop uniformly on the nutrient agar plates. A suspension of the fungi spores was prepared by washing the surfaces of each fungi plate with sterile

water into a sterile sample bottle. The spores in suspension were individually streaked uniformly on the potato dextrose agar plates using a sterile wire loop. The inoculated plates were incubated for 2 hours. Paper disc of 5 mm each was prepared by punching Whatman No 1 filter paper. The paper disc was separated into labelled containers for samples and control and then sterilized in an oven (UNISCOPE LAB OVEN SM 9053) at 170 °C for 30 min. They were saturated with synthesized *Senna occidentalis* Ag_2S nanoparticles, standard antibiotic (Ciprofloxacin 500 mg) or antifungal (Greosefulvin); respectively. The disc 3 per plate was placed at equidistance on the surfaces of the labelled inoculated and pre - incubated plates following the method of [32]. The plates were incubated for 24 hours and zones of inhibition where present were measured diameter-wise with the use of a transparent plastic ruler.

RESULTS AND DISCUSSION:

Fourier Transform Infrared Spectroscopy (FT-IR): FT-IR characterization was done to identify the types of chemical bonds (functional groups) present in aqueous extract of *Senna occidentalis* leaves and synthesized silver sulfide nanoparticles. FT-IR spectrum of *Senna occidentalis* leaves aqueous extract highlighted in **Fig. 3** below shows a number of peaks, thus reflecting its complex nature.

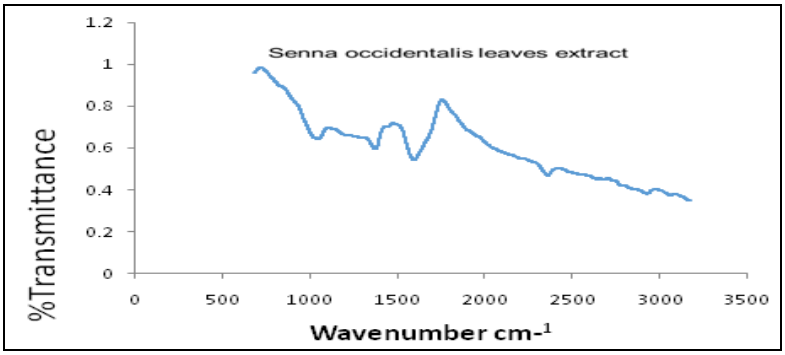

FIG. 3: FT-IR SPECTRUM OF AQUEOUS *SENNA OCCIDENTALIS*

The bands at 3175cm^{-1} is characteristic of the alcohol/phenol –OH stretching vibration, 2361.44cm^{-1} an attribute of O=C= stretching, representing carbon dioxide. The weak peak at 1594cm^{-1} is assigned for C=O bending, indicating carboxylic acid and the band at 995cm-1 represents the C=C bending signifying alkene [33]. **Fig. 4** below shows the functional groups present in the synthesized silver sulfide nanoparticle. The bands at 3785 and 3703 cm^{-1} correspond to O-H stretching vibration, indicating the presence of alcohol/phenol, the peak at 3406 cm^{-1} reflects N-H stretching of aliphatic primary amine. Bands at 2919 and 2855.82cm^{-1} correspond to the C-H stretching of aromatic compounds. The band at 1713.88cm^{-1} indicates C=N stretching attributed to amine, the band at 1601cm^{-1} is assigned to C=C a characteristic of conjugated alkene. These functional groups play an important role as stabilizing / capping agents in synthesizing silver sulfide nanoparticles. Also, for the fingerprint, bands at 1372 and 1038 cm^{-1} signify S=O stretch indicating sulfonamide and sulfoxide, respectively while the band at 1280 indicates C-O stretching representing aromatic esters [34].

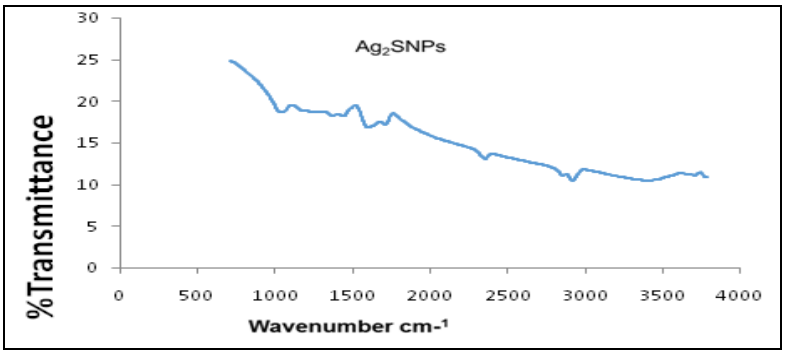

FIG. 4: FT-IR SPECTRUM OF SYNTHESIZED AG_2SNPs

Scanning Electron Microscopy (SEM): The shape of the silver sulfide nanoparticles is spherical with few exceptional as ellipsoidal **Fig. 5** and **6.** The larger silver sulfide particles may be due to the aggregation of the smaller ones due to the SEM measurements.

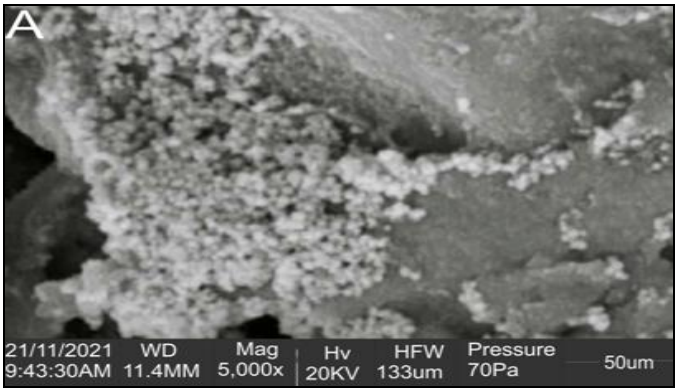

FIG. 5: SCANNING ELECTRON MICROSCOPY (SEM) OF SYNTHESIZED AG_2SNPs OF 50UM AT A MAGNIFICATION OF 5000X

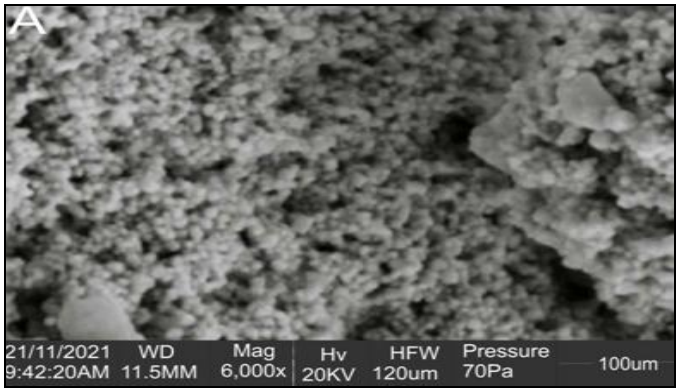

FIG. 6: SCANNING ELECTRON MICROSCOPY (SEM) OF SYNTHESIZED AG_2SNPS OF 100UM AT A MAGNIFICATION OF 6000X

TABLE 1: ANTIMICROBIAL ACTIVITIES OF SENNA OCCIDENTALIS EXTRACT AG_2S NANO PARTICLES ON SOME MICROORGANISMS (MM)

Miicroorganism	Disc number	Test exreact	Control
Staphylococcus aureus	1	8.0	37.5
	2	8.5	38.0
	3	7.5	35.0
	Average	8.0	36.8
Pseudomonas aeruginosa	1	-	14.0
	2	-	13.5
	3	-	13.0
	Average	-	13.5
Klebseilla spp	1	-	30.0
	2	-	32.5
	3	-	32.5
	Average	-	31.7
Escherichia coli	1	8.0	10
	2	8.0	9.5
	3	8.0	12.5
	Average	8.0	10.7
Aspergillus niger	1	11.0	9.0
	2	12.0	10.0
	3	10.5	9.0
	Average	11.2	9.3
Aspergillus flavus	1	9.5	10
	2	9.0	10
	3	9.0	10
	Average	9.2	10
Aspergillus fumigatis	1	9.5	-
	2	9.5	-
	3	9.5	-
	Average	9.5	-
Candida albicans	1	8.5	8.0
	2	8.0	9.0
	3	9.0	10.0
	Average	8.5	9.0

Antimicrobial Studies: Antimicrobial inhibition test was conducted using the synthesized Ag_2S nanoparticles and it was observed to have inhibition activity against *Staphylococcus aureus* and *Escherichia coli* bacteria; however, *Pseudomonas aeruginosa* and *Klebseilla* spp were

not inhibited; fungi *Aspergillus niger*, *A. flavus*, *A. fumigatus* and *Candida albicans* were inhibited giving clear zones of inhibition as shown in Table1. The sensitivity of staphylococcus aureus to standard antibiotic ciprofloxacin was higher than that recorded by the other test bacteria and the finding agrees with work done by [35]. Fungi *Aspergillus fumigates* was inhibited by the *Senna occidentalis* Ag_2S nanoparticles but showed no inhibition by the standard antifungal drug used as control; this agrees to the fact that antimicrobial are met with resistance from various microorganisms, this is in agreement with the findings of [36].

CONCLUSION: In recent studies, green approach to synthesizing nano materials has attracted significant attention to protect the environment from hazardous wastes**.** However, the importance in the use of a benign approach in the synthesis and development of nanoparticles through the fast, eco-friendly, and convenient method cannot be overemphasized. In this study, a simple, fast, eco-friendly and convenient method was used to prepare silver sulfide nanoparticles for antimicrobial activity evaluation using *Senna occidentalis* leaves aqueous extract. These particles are monodispersed and spherical, with few exceptionally ellipsoidal. The method enables the bioprocess with the advantage of being environmentally friendly as no chemical reagent was used. The color change occurs as a result of the reaction with the plant moiety leading to the formation of silver sulfide nanoparticles, as confirmed by SEM and FTIR results. Antimicrobial inhibition test was conducted using the synthesized Ag_2S nanoparticles, and it was observed to have inhibition activity against *Staphylococcus aureus* and *Escherichia coli* bacteria; however, *Pseudomonas aeruginosa* and *Klebseilla* spp were not inhibited; fungi *Aspergillus niger*, *A.flavus*, *A. fumigatus* and *Candida albicans* were inhibited giving clear zones of inhibition as shown in Table1. The sensitivity of staphylococcus aureus to standard antibiotic ciprofloxacin was higher than that recorded by the other test bacteria and the finding agrees with work done by [35]. Fungi *Aspergillus fumigates* were inhibited by the *Senna occidentalis* Ag_2S nanoparticles but showed no inhibition by the standard antifungal drug used as control; this agrees that antimicrobial is met with resistance from various microorganisms; this is in agreement with the findings of [36]. *Senna occidentalis* Ag_2S nanoparticles was observed to possess antimicrobial activity as they inhibited some of the test microorganisms (bacteria and fungi), giving clear inhibition zones that were the same or higher in some cases than those of the standard antibiotic or antifungal used. The inhibition activity on bacteria was selective as it did not inhibit some of the test organisms (*Pseudomonas aeruginosa* and *Klebseilla* spp). The nano particles' antifungal inhibition activity was seen to be better than griseofulvin (the standard antifungal used) in the case of *Aspergillus fumigatis*.

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CONFLICT OF INTEREST: The authors declare no conflict of interest.

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