



## Synthesis of silver nanoparticles using coffee senna (*Senna occidentalis*) stem extract and its antimicrobial evaluation

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### Abstract

This research was carried out to synthesize silver nanoparticles (AgNPs) using the stem extract of *Senna occidentalis* Linn (Coffee senna) and testing its antimicrobial activity. In this study, the crushed stem of *Senna occidentalis* Linn was subjected to sequential extraction by increasing polarity index of solvents (hexane, ethyl acetate, methanol and water). The water extract gave the highest percentage yield of extract in the sequential extraction. The AgNPs were characterized and tested for their antimicrobial activity using the methanol and water extracts. UV-visible spectrophotometer and Fourier Transform Infrared (FTIR) Spectrometer were used to analyse the AgNPs. Colour changes were observed for each extract indicating AgNPs formation. The UV-VIS spectra showed peaks at 440nm, 440nm, 620nm, 620nm for the hexane, ethyl acetate, methanol and water extracts of the synthesized AgNPs respectively. The FTIR analysis indicated the presence of active functional groups for the methanol and water extracts. The synthesized AgNPs for the methanol and water extracts showed antimicrobial activity against all the test organisms used (*Klebsiellapneumoniae*, *Shigellaspp*, *E. Coli*, *Salmonella typhi*, and *S. Aureus*), however, hexane and ethyl acetate crude extracts prove positive for only *E.coli*, while water crude extract showed negative for all the test organisms. The crude extracts and the synthesized AgNPs showed medium zone of inhibition, with only the methanol AgNPs showing a higher zone of inhibition than the control for *S. aureus*. The synthesized nanoparticles via biological method is safe and can be used to modify and improve the efficacy of drugs through drug discovery.

**Keywords:** anticancer, bioactive, free radicals, silver nanoparticles, *Senna occidentalis*

### 1. Introduction

Researchers are now focused on the use of sustainable ideas that uses green chemistry for synthesis in all scientific fields; as this will eliminate complex synthetic routes and the hazards that comes with these synthetic routes (Akinsiku et al., 2015) <sup>[1]</sup>.

Over the past few years, the chemistry Community has been mobilized to develop new chemistries that are less hazardous to human health and the environment. This new approach has received extensive attention and goes by many names including Green chemistry, clean chemistry and environmental chemistry. Simply stated, green chemistry involves the use of chemistry techniques and methodologies that reduces or eliminates the use of products, by-products, solvents, reagents that are hazardous to human health or the environment. It involves the approach to the synthesis, processing and use of chemicals that reduces risk to humans and the environment (Anastas, and Williamson, 1996) <sup>[3]</sup>.

Nanotechnology is a new and emerging field of science that is bound to have tremendous impact on mankind by helping solve major challenges facing humanity in health and energy. Nanoparticles are particles that have a size of 1nm-100nm in at least one dimension and possess unique physical and chemical properties due to their large surface area to volume ratio and smaller size (Jeremy, 2011) <sup>[9]</sup>. Metal nanoparticles have wide range of applications in various areas of physics, chemistry, material science and biological science. Intrinsic chemical optical, electronic, sensing and catalytic properties of metal nanoparticles largely depends on their shapes and sizes (Zhou and

Wang, 2012) <sup>[17]</sup>. One of such important member of the noble metal nanoparticles are silver nanoparticles (AgNPs). These are broadly applied in shampoos, soaps, detergents, cosmetics, toothpaste, medical and pharmaceutical products and are hence directly encountered by human systems (Banerjee et al., 2014) <sup>[4]</sup>. Synthesis of these nanoparticles has different methods of which the common ones were found to involve much expenses, hazardous chemicals and time consuming. However, biosynthesis of the silver nanoparticles using plants and biological systems were found to be environmentally friendly (Vijayakumar and Sinthiya, 2018).

*Senna occidentalis* (Linn) is a weed of the leguminosae family, and it is distributed throughout the tropical and subtropical regions of the world. It can be found in open pastures, fertile soils, along road margins, as well as a contaminant in fields cultivated with cereals such as soya bean, corn, sorghum and others (Schmitz and Denton 1977; Colvin et al., 1986; Flory et al., 1982) <sup>[15, 6, 8]</sup>; thus during harvest, it is almost impossible to prevent this plant from mixing with the cultivated crops. The plant is a shrub that grows between 5 to 8m in height, elliptical pointed dark green leaves, bright golden yellow flowers (Barros et al., 1993) <sup>[5]</sup>, and is commonly found in the tropics (Muhammad et al., 2017). The plant is widely distributed in the coast of Asia, America and Africa. *Senna occidentalis* has a pantropical distribution and probably originated in tropical America, however in recent time, the plant is reported to be available in many parts of the world of which Nigeria is inclusive (Akinsiku et al., 2015) <sup>[1]</sup>.

*Senna occidentalis* is commonly known as "Esdafwan" by the mhisip tribe of Chip district, Pankshin Local Government Area of Plateau State, Nigeria, the name describes the plant's seed comparably with Rabbit's faeces. The plant is also commonly called "Khinkho'om" by the Jipal tribe of Pankshin LGA of Plateau State, Nigeria. This name also describes the plant's leaves comparably with that of groundnut plant leaves (Personal interview, 2018).

According to Lombardo (2014)<sup>[11]</sup>, *Senna occidentalis* has toxic properties; the plant is incriminated as the cause of poisoning in some animals because of its toxicological properties. This legume is used as a daily food by the poor classes of Ceylon and India, the pods are cooked and have a pleasant taste similar to beans (Lombardo, 2014)<sup>[11]</sup>. *Senna occidentalis* is widely used to treat liver diseases, combating malaria, constipation, measles, smallpox, eczema and many fungal infections. *Senna occidentalis* has a high therapeutic value for many clinical conditions from throat inflammation and gastritis to hemorrhage and cancer.

Muhammad et al., (2017) also pointed out that the leaves of the plant are used in the treatment of yaws, scabies, itches and ringworm. It is also used as an effective treatment for hepatitis among the rural dwellers in northern part of Nigeria, and also in the treatment of snake bite.

## 2. Materials and Methods

### A. Sample collection and identification

The plant *Senna occidentalis* Linn (Coffee senna) was collected from Jenta Adamu in Jos North Local Government Area of Plateau State, Nigeria. The plant was then identified in the department of Plant Science and Biotechnology, University of Jos.

### B. Plant pre-treatment

The plant samples collected were freed from twigs and extraneous matter. The plant sample was thoroughly washed under tap water and rinsed with distilled water. The stem was detached and was dried in the shade at room temperature for 14 days. The plant sample was pulverized to lower surface area using pestle and mortar and stored in polythene bags for further analysis.

### C. Plant Extraction

500g of the plant sample was weighed and transferred into a conical flask (1000ml), the plant sample was extracted sequentially using cold maceration. The plant sample was sequentially extracted using the four solvent systems (n-hexane, ethyl acetate, methanol and water) for 72 hours each based on increasing polarity. Each mixture was filtered using Whatman No 42 filter paper. The extracts were concentrated using a rotary evaporator, however the water extract was concentrated using the water bath. The crude extracts were weighed and transferred into labeled and pre-weighed sample bottles. The percentage yield of the extracts was then obtained using the relation below as described by Anokwuru et al., (2011) with modifications.

$$\%Yield = \frac{\text{Weight of concentrated crude extract}}{\text{Weight of the dried crushed plant sample used}} \times 100$$

### D. Silver Nanoparticles Synthesis using *Senna occidentalis* (Linn) Stem Extract

2g of concentrated crude extract was dissolved in 20mL of its solvent in a 2:20 ratio (at room temperature). 100ml of 0.001M silver nitrate solution was measured into a beaker and placed on a magnetic stirrer hot plate, 10mL of the extract solution was transferred into the beaker and left to stir for one hour. The extract solution was transferred and mixed into aqueous solution of the silver ion complex colour changes is observed in respect to phenomena surface plasmon resonance (SPR). This was done separately with each type of plant extract (Deshi et al., 2016).

### E. UV-Vis and FTIR Spectra Analysis

The synthesized silver nanoparticles for all the samples were monitored using UV-visible spectrophotometer to ascertain the completion of bio reduction of  $Ag^+$  to  $Ag^0$ . The suspension was analyzed between wavelengths of 200nm to 1020nm in a UV-visible spectrophotometer. As the extracts were added to the aqueous  $AgNO_3$  solution, colour changes were observed in the solution; brown hexane extract turned light yellow, dark green ethylacetate extract turned green, red methanol extract turned brown while the brown water extract turned light brown indicating formation of AgNPs. Similar changes in colour have also been observed in previous studies (Zhou and Wang, 2012 and Suriati et al., 2014)<sup>[17]</sup>.

Fourier transform infrared spectral analysis of the filtered dried silver nanoparticles of the methanol and water was carried out to check the active functional groups present (Banerjee et al., 2014)<sup>[14]</sup>.

### F. Antimicrobial analysis of the crude extract

#### 1. Preparation of culture media

Nutrient ager powder was dissolved into distilled water. The dissolved nutrient ager was then homogenized by heating. This was further sterilized by autoclaving at 121 °C 15l for 15 min. It was then allowed to cool at 47°C and then dispensed into sterile Petri dishes which was used for culturing and sensitivity test of the organisms as described by Shagal et al., (2012)<sup>[14]</sup> with slight modifications.

#### 2. Determination of antimicrobial activities.

Five different organisms *S.aureus*, *Salmonella typhi*, *Shigella sp*, *E.coli*, and *Klebsiella sp* were inoculated into the culture media, each extract was dissolved into its respective solvent, and the nanoparticles were also dissolved into normal saline. Small piece of sterilized filter paper was impregnated with the extract and placed on the culture media for sterility test. The already impregnated filter paper discs were placed on the culture plates containing the different inoculum and culture plates were inverted and incubated at 37°C for 24 hours in an incubator. After incubation, the inoculated plates were observed for zones of inhibition (in mm diameter). The results obtained were observed as zones of inhibition of the organisms by the test extract as described by Shagal et al., (2012)<sup>[14]</sup> with slight modification.

### 3. Results

**Table 1:** Weight and Percentage Yield of Extract.

Extract	Colour of Extract	Weight of Sample (g)	Weight of Extract (g)	Percentage Yield (%)
Hexane	Brown	500	11.20	2.24
Ethylacetate	Dark green	500	14.60	2.92
Methanol	Reddish brown	500	18.40	3.68
Aqueous	Dark brown	500	20.80	4.16
Total			65.00	13.00

**Table 2:** UV-VIS result showing highest absorbance and wavelength

	Silver nanoparticles (AgNPs) Extracts			
	HE	EE	ME	WE
Absorbance	0.882	1.452	0.850	0.600
Wavelength	440nm	440nm	620nm	620nm

**Key:** HE= Hexane Extract, EE= Ethylacetate Extract, ME= Methanol Extracts, WE= Water Extracts

**Table 3:** FTIR results showing functional group

	Wavelength (cm <sup>-1</sup> )	Functional group
AgNPs methanol extract	3749	OH (alcohol)
	3272	OH (carboxylic acid)
	2922	C-H (alkane)
	2851	C-H (alkane)
	2098	SCN (isothiocyanate)
	1703	C=O (conjugated aldehyde)
	1595	N-H (amine)
AgNPs water extract	1509	N-O (nitro compound)
	3749	O-H (alcohol)
	3276	O-H (carboxylic acid)
	2922	C-H (alkane)
	2161	SCN (Isothiocyanate)
	1950	NCS (thiocyanate)
	1595	N-H (amine)

**Table 4:** Showing zone of inhibitions found in bacteria cultures

Test organisms	Zone of inhibition (in mm)						Control (Gentamicin)
	HCE	ECE	MCE	WCE	MAGNPs	WAGNPs	
<i>S. aureus</i>	-	-	14	-	25	16	24
<i>S.typhi</i>	-	-	-	-	12	14	27
<i>E. coli</i>	13	12	14	-	15	17	25
<i>Shigella sp</i>	-	-	12	-	14	14	22
<i>Klepsiella sp</i>	-	-	-	-	15	15	23

Cultures were treated with crude extracts and AgNPs.

**Key:** sp= specie

AgNPs= silver nanoparticles,

HCE= hexane crude extract,

ECE= ethyl acetate crude extract,

MCE= methanol crude extract,

MAGNPs= Methanol silver nanoparticles,

WCE= Water Crude Extracts,

WAGNPs= Water silver nanoparticles

PSAgNPs= Pelargonium sidoides silver nanoparticles

### 4. Discussion

#### A. Percentage Yield

Percentage yield depicts the ability of a given solvent to extract the phytochemicals or bioactive compounds present in a plant. Sase et al (2019)<sup>[13]</sup>, pointed out that the percentage yield of plant extraction is dependent on the solvent used in the extraction. According to their work, polar solvent usually has the highest extraction yield compared to non-polar solvents, this is because

the Polar compounds are highly extracted in polar solvents compared to non-polar solvents.

From this research, the water extract was found to have the highest percentage yield with a percentage yield of 4.16%, followed by the methanol extract (3.68%), ethyl acetate extract (2.92%), and the hexane extract (2.24%). This is indicative that the plant possesses more polar compounds as these compounds will be dissolved in similar polarity of solvents, having the

percentage yield increase with increasing polarity of the solvents used in the extraction.

### B. UV-vis analysis

UV-Visible spectroscopy can be used as a simple and reliable method for monitoring the stability of nanoparticle solutions. Plasmons are the oscillations of free electrons that are the consequence of the formation of a dipole in the material due to electromagnetic waves. In metal, coupled state arises between a plasmon and a photon which is known as Plasmon polariton. Surface Plasmon Resonance (SPR) is the coherent excitation of all the free electrons within the conduction band, leading to an in-phase oscillation. The resonance falls into the visible region for

SNPs and hence SNPs have characteristic optical absorption spectrums (Vijayakumar et al., 2018)

In the present study, the absorbance of the reaction mixture was measured for the AgNPs synthesized by the different extracts. The highest SPR absorption was observed at 440nm for the AgNP synthesized from the hexane and ethyl acetate extract, while the methanol and water extracts showed absorption peak at 620nm, with little peaks as shown in the figures below. Previous research by Vijayakumar et al., (2018) reported that the particles in SPR region of around 410-450nm could be attributed to spherical nanoparticles. The observed absorption peaks confirmed the synthesis of silver nanoparticles (AgNPs).

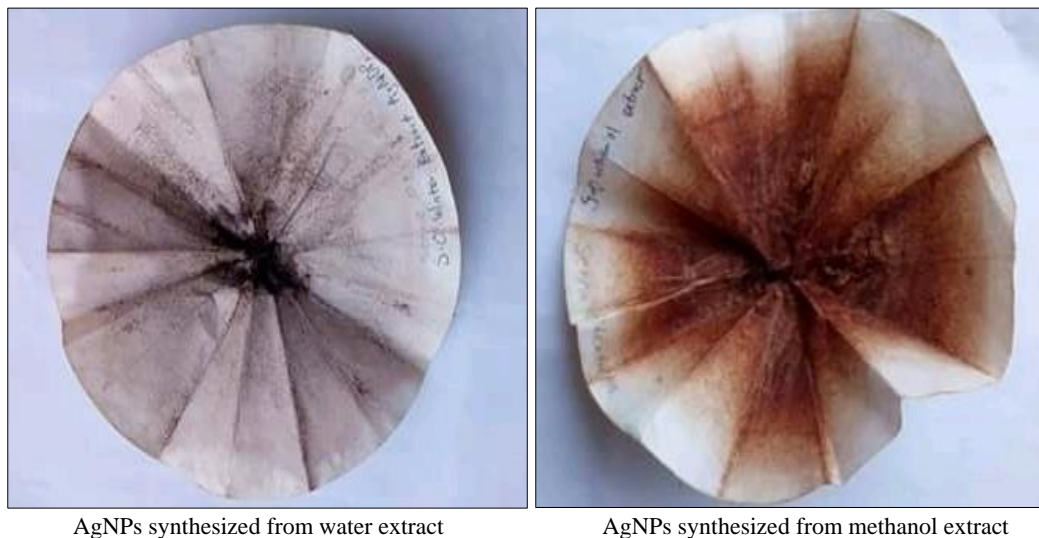


Fig 1: Nanoparticles synthesized from water and methanol extracts

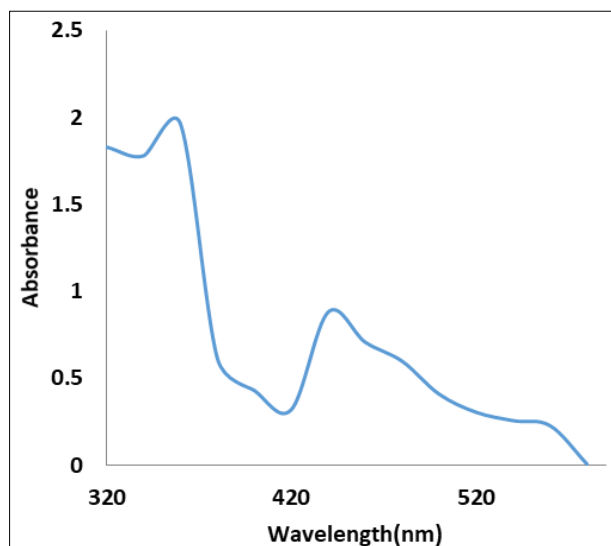


Fig 2: UV-VIS spectra of silver nanoparticle hexane extract.

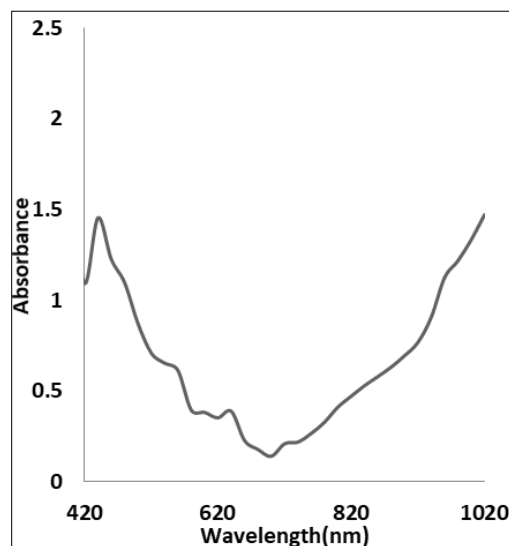


Fig 3: UV-VIS spectra of silver nanoparticles ethyl acetate extract

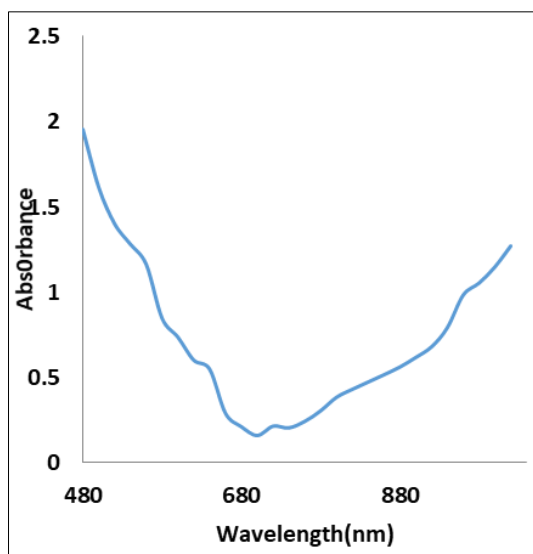


Fig 4: UV-VIS spectra of silver nanoparticles by *Senna occidentalis* water extract.

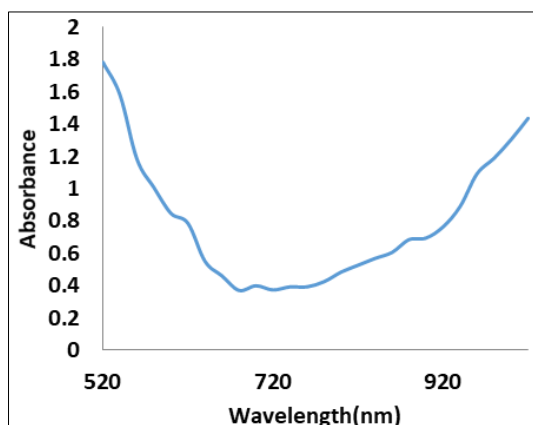


Fig 5: UV-VIS spectra analysis of Silver nanoparticles by *Senna occidentalis* methanol extract.

### C.FTIR Analysis

FTIR analysis was carried out to identify the possible functional groups responsible for capping and efficient stabilization. FTIR analysis was performed for the AgNPs synthesized from the methanolic and ethyl acetate extract. The AgNPs synthesized from the methanol extract showed 8 prominent peaks at the functional group region, with peaks at 3749, 3272, 2922, 2851, 2098, 1703, 1509 and 1595  $\text{cm}^{-1}$  which are representative of  $\text{-OH}$  (alcohol),  $\text{-OH}$  (carboxylic acid),  $\text{N-H}$  (amine),  $\text{C-H}$  (alkane),  $\text{SCN}$  (isothiocyanate),  $\text{C=O}$  (conjugated aldehyde), and  $\text{N-O}$  (nitro compounds). The other peaks at the fingerprint region were indicative of the presence of phenol, aromatic amine, tertiary alcohol, alkenes, halocompounds, ether and esters. The AgNPs synthesized from the water extract had 6 prominent peaks of 3749, 3276, 2922, 2161, 1950, and 1595  $\text{cm}^{-1}$  which are indicative of  $\text{-OH}$  (alcohol, carboxylic acid),  $\text{N-H}$  (amine),  $\text{SCN}$ ,  $\text{NCS}$ ,  $\text{C=O}$  (carbonyl) functional groups. The peaks at the fingerprint region revealed the presence of phenol, Alkene, esters, Ester's and halo compounds.

The observed peaks in the FTIR spectrum of the AgNPs showed

that the absorption of phytoconstituents on the surface of the nanoparticles are responsible for capping and efficient stabilization of the silver nanoparticles (Vijayakumar et al., 2018). Hence the phytochemicals present in the plant are responsible for the bio-reduction of the metal nanoparticles, as the presence of active functional groups in the stem extract of *Senna occidentalis* results in the reduction of silver ions to silver nanoparticles.

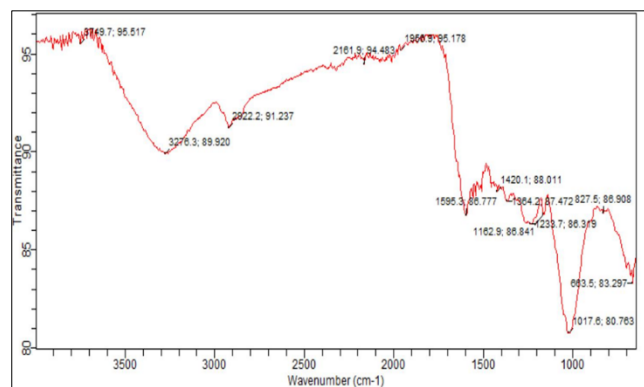


Fig 6: The absorption spectra of AgNPs of *Senna occidentalis* methanol extract

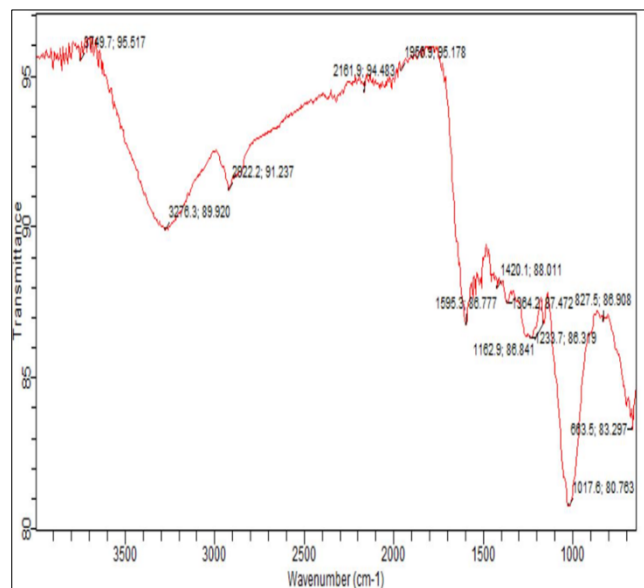
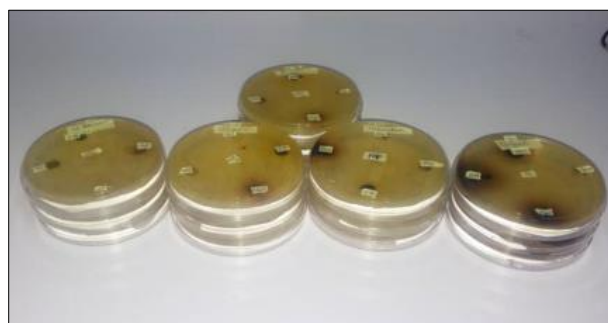
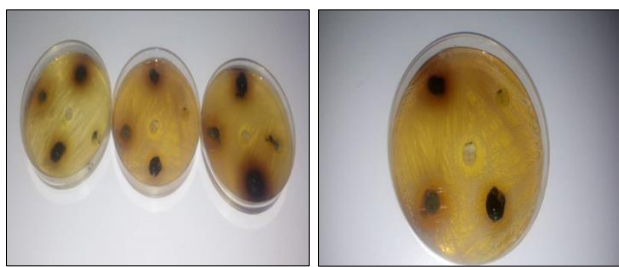


Fig 7: IR Graph of silver nano particles from water extract of *Senna occidentalis*





**Fig 8:** Petri dishes showing zone of inhibition

#### D. Antimicrobial

The research work used the crude extracts of the plant *Senna occidentalis* and the synthesized silver nanoparticles to test on microorganisms that are pathogenic. The test microorganisms used were *Klebsiella pneumoniae*, *Shigella sp.*, *E. coli*, *Salmonella typhi*, and *S. aureus*. The hexane and ethyl acetate crude extracts showed inhibition zone only on *E. coli*, this is in agreement with Alaribe et al., (2011) [2] who reported the effectiveness of crude hexane extract of stem bark of *Ficus congensis* on *E. coli* and other microbes. The present study showed the zone of inhibition of 13mm using the crude hexane extract of the stem of plant used on *E. coli* which is in agreement with reports of Musa et al., (2015) [4] showing 6mm zone of inhibition of the hexane crude extract of the leaf *Anisopus manni* on *E. coli*. The higher zone of inhibition in the present study indicates that the bioactive compound in the stem of *Senna occidentalis* has high antimicrobial activity

crude extract gotten from distilled water as solvent showed no inhibition for all the test organisms while the methanolic crude extract showed activity on *S. Aureus*, *E. coli* and *Shigella sp.*, with inhibition on *Salmonella typhi* and *Klebsiella pneumoniae*. The synthesized nanoparticles from methanol and water extracts showed inhibition zones for all the microorganisms used, which shows their efficacy against the test microorganisms as compared with the positive control gentamicine. There was a higher inhibition for the nanoparticles synthesized from water extract.

This disparity in the results showed that the silver nanoparticles have more antimicrobial property than the crude extracts. Only the methanol AgNPs showed a higher zone of inhibition than the control for *S. aureus*. The silver nanoparticles have more antimicrobial activity than the plant extracts when used in crude form, effective antimicrobial activity was also reported for PSAgNPs compared to the crude extract of *Pelargonium sidoides* DC (Kgatshe et al., 2019) [10], hence silver nanoparticles can be used as a better and improved technology to synthesize more effective antibacterial drugs.

#### 5. Conclusion

From the results obtained in this study, the synthesis of AgNPs was successfully carried out with the stem bark extract of *Senna occidentalis* through green chemistry. The success of synthesizing the AgNPs showed an easy, low cost and less time consuming way of synthesizing silver nanoparticles without using harmful chemicals. The antimicrobial results showed the advantage of using nanotechnology in drug production to combat bacteria efficiently. Activity wasn't uniform for the crude extracts but it was however more efficient for the silver nanoparticles on the microorganisms. This indicates that there can be a way of improving already produced antibacterial drugs. The extracts

used initiated nucleation and growth of the nanoparticles. The phytochemicals present such as alkaloids, flavonoids among others in the plant extract have been considered to be responsible for the bioreduction process. The research validated the concept of like dissolves like through calculating the percentage yield and finding an increase in the yield with increase in polarity.

#### 6. Recommendations

From the results obtained through this research work, the following recommendations are enlisted;

- That FTIR analysis should further be done for silver nanoparticles synthesized from hexane and ethyl acetate extracts.
- That further characterization should be performed such as XRD, and SEM/TEM on all extracts.
- Other metal nanoparticles should be used such as gold and copper nanoparticles in the synthesis.
- The leaves, seeds, and roots of *Senna occidentalis* should be used in the synthesis of the nanoparticles.
- Further isolation of the compounds using column chromatography should be performed on the crude extracts
- Further test on different species of bacteria should be carried out using synthesized silver nanoparticles.

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