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SYNTHESIS OF SILVER NANOPARTICLES (AgNPs) FROM BARK AND ROOT OF AFRICAN MAHOGANY (*Khaya senegalensis*) AND THE COMPARATIVE STUDIES OF THEIR ANTIMICROBIAL PROPERTIES

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. Authors MEA, OBA, OOO and ABB designed the study, wrote the protocol and interpreted the data. Authors MEA, OSO and ABB anchored the field study, gathered the initial data and performed preliminary data analysis. Authors MEA, OBA, OOO and AS managed the literature searches and produced the initial draft. All authors read and approved the final manuscript.

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ABSTRACT

Stem bark and root of *Khaya senegalensis* were extracted with ethyl acetate and n-Hexane and extracts screened for metabolites, antibacterial activity. There were also used to reduce $AgNO_3$ to synthesize AgNPs and their antibacterial effects studied. A number of metabolite example flavonoids, steroids, tannins etc were discovered. The stem bark extract show higher activities on all the four bacterial isolates compared to the root extract: on *K. pneumoniae* (28.1±0.42), *B. subtilis* (20.1±1.21).while the root extract show lower activity on the test organisms at 26.3± 0.12 on *K. pneumoniae* and 22.1±0.20 on *S. epidermidis*. The activities of the nanoparticles are higher than those of the crude extracts and standard antibiotics used as control. The zones of inhibition around the bacterial colonies by the particles are far wider around each organism challenged. AgNPs bark extract on *B. subtilis* produced 31.2±0.11zone of inhibition of 24. 24.3±1.21 and 28.2±2.12 by Streptomycin and Tetracyclines. Strikingly, both the ethyl acetate and n-Hexane bark extracts of the plant demonstrated higher activities on the test isolates compared to the root extracts but the nanoparticles of the root had more activity compared to the stem bark. The activity of the metabolites in the root may have been enhanced when they were carried by the nanoparticles. The potency of *Khaya senegalensis* on microbial pathogens may be broadened when processed into its nanoparticles.

Keywords: Synthesis; silver nanoparticles (AgNPs); stem bark; African mahogany; comparative studies.

1. INTRODUCTION

Khaya senegalensis also known as African Mahogany, Benin Mahogany, Dry Zone Mahogany, Senegal Mahogany, is believed to grow in Benin, Chad, Gambia, Cameroon, Ghana, Guinea, Guinea-Bissau, Mali, Niger, Nigeria, Senegal, Sierra Leone, South Sudan, Sudan, Togo, and Uganda. According to

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report, it is a mahogany species, widespread in high-rainfall savannah woodland. This is a tree that can grow up to 15-30 meter in height and 1 m in diameter. The colour of the bark may be dark grey or grey brown but the heartwood is usually brown and having a pink-red pigment made up of coarse interlocking grains. The leaves are arranged in a spiral formation which clustered at the end of branches. The flowers are white and have sweet smell, the fruits turn black as they ripen [1]. Natural products from plants have been exploited over the years for the production of drugs for human healthcare delivery. Many shrubs as well as higher plants are ready source of active principles used for curative purposes all over the world. While shrubs, grasses and higher plants are known sources of drugs, antibiotics, on the other hand, are produced by microorganisms such as bacteria and fungi although many semi-synthetic and full synthetic antibiotics are now available together with naturally occurring ones. Many research works had and are been conducted with the sole aim of finding novel material from plants and microorganisms that could help ameliorate the sufferings of mankind at the hand of sicknesses and diseases. Many research works have also focused on bioeffects of plants extracts or metabolic product on control of insects and pests. [2] reported that the leaf of *Helianthus annuus* has Antihyperglycemic and Antioxidant Properties. [3] reported the acaricide activity of aqueous extracts of caatinga plants on the cassava green mite. According to [4], stem bark *Mahonia aquifolium* may be active against Malsseizia species. Studies on 45 Indian medicinal plants against multi-drug resistant *Stapylococcus aureus* and ascertained the medicinal values of the plants [5-12] all claimed (through their various research efforts) to have detected antimicrobial activities with various plants and their products. Good as these may seem, the menace of development of resistance to drugs makes researchers not to relent in their search for solutions against pathogens. Available evidences have shown that microbial pathogens are developing resistance to existing drugs and antibiotics steadily and this call for grave concerns. [13], threat report indicates increasing antibiotic/antimicrobial resistance and [14] gave insight into Methicillin-resistant *Staphylococcus aureus* and also [15,16]. Many plants' extracts have demonstrated activities in-vitro against resistant strains of microorganisms [5,17,18], however, more potent ways of dealing with the menace of microbial resistance to drugs and antibiotics are needed. It is against this backdrop that scientist started looking at drug delivery and substances that can enhance drug delivery via deeper penetration into the cell. Nanoparticles of different sizes have been accessed for drug delivery. It is believed that nanoparticles because of the millionth minute sizes have the capacity to penetrate deeper into cells to cause damage to unwanted cells.

Compounds such as silver, gold, copper have been used in green synthesis and utilized for antimicrobial investigations [19,20,21].

The present research was conducted to synthesize nanoparticles in green synthesis from root and bark extracts of African mahogany (*Khaya senegalensis*) which have previously demonstrated antimicrobial activity against some known microbial pathogens.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

Plant materials were collected from Bida, Nigeria. Bida is the traditional headquarters of the Nupe tribe and it is on latitude 9006 N and longitude 6001 E on the Nupe sand stone formation. The geographical coordinates are 9° 5 0" North, 6° 1 0" East. It is located 19 kms North of River Kaduna, along Mokwa Bida road and 86 kms South East of Minna Niger State. The bark and root of the plant were collected with help of the locals and was identified and authenticated by a Botanist and Taxonomist. The samples were dried in the drier at low temperatures (25°C±1) for about two week. When well dried, they were pulverized and blended into fine powder.

2.2 Extraction of Plant Materials

Extraction of plant parts was done using the method of [22]. 100 g of the powdered samples were soaked in 500 ml of ethanol and allowed to stand for about 72 hours for extraction. The extract was then filtered. The filtered extract in solution were sterilized by passing through Millipore filter and then evaporated to dryness and kept for further use.

2.3 Phytochemical Screening

The extracts from n-Hexane (bark and root) were investigated for the presence of plant secondary metabolites such as carbohydrates, alkaloids, saponins, proteins, amino acids, phenol, diterpenes, tannins and phytosterols by following standard biochemical methods as described by [23] and [24].

a. Alkaloids- 1 ml of 1% HCl was added to 3 ml of the extract in a test tube. The mixture was then heated for 20 min, cooled and filtered about 2 drops of Mayer's reagent to1 ml of the extract. A creamy precipitate was an indication of the presence of alkaloids.

b. Tannins- 1 ml of freshly prepared 10% KOH was added to 1 ml of the extract. A dirty white precipitate showed the presence of tannins.
c. Glycosides- 10 ml of 50% H_2SO_4 was added to 1 ml of the extract and the mixture heated in boiling water for about 15 min. 10ml of Fehling's solution was then added and the mixture boiled. A brick-red precipitate was confirmatory for the presence of glycosides.
d. Saponins- Frothing test: 2 ml of the extract was vigorously shaken in the test tube for 2 min. No frothing was observed.
e. Flavonoids- 1 ml of 10% NaOH was added to 3 ml of the extract. There was no yellow colouration which is indicative of the absence of flavonoids.
f. Steroids- Salkowski test: 5 drops of concentrated H_2SO_4 was added to 1 ml of the extract in a test tube. Red colouration was observed which is indicative for the presence of steroids.
g. Phlobatanins- 1 ml of the extract was added to 1% HCl. No red precipitate observed which means negative result.
h. Triterpenes- 1 ml of the extract was added to 5 drops of Acetic anhydride and a drop of concentrated H_2SO_4 added. The mixture was then steamed for 1 h and neutralized with NaOH followed by the addition of chloroform. Absence of blue-green colour indicates the absence of triterpenes.

2.4 Microbial Cultures for Antibacterial Assays

Staphylococcus epidermidis, Bacillus subtilis, Escherichia coli and *Klebsiella pneumoniae* were the isolates used for these experiments. They are both clinical and environmental specimens. Organisms were isolated using standard methods and maintained on agar slants and refrigerated for further use.

2.5 Synthesis of Ag Nanoparticles

Analytical grade of silver nitrate was used for the silver nanoparticles synthesis. In a typical reaction procedure, 1ml crude extract of each plant part was diluted to 300 ml using triply distilled de-ionized water to make it 3% and 20 ml of this extract solution was mixed with 20 ml 5×10−3M aqueous silver nitrate solution. The resulting mixture was then heated at 85°C with constant stirring for about 4 hours in oil bath which yielded silver nanoparticles. Hyaluronic acid was added as a stabilizing agent to avoid aggregation in green synthesis of these plant parts.

2.6 Characterization of the Nanoparticles

Morphology and size of silver nanoparticles were investigated using transmission electron microscopy (TEM) images. TEM images were obtained on a Ziess Leo 910 transmission electron microscope using the method described by [20]. In this experiment, 10 µL of each sample was placed on the carbon coated copper grid and excess of the sample was removed by a blotting paper. The grid was dried under an infrared lamp. The accelerating voltage was 40–120 kV and images were taken by 0.4 nm resolution and a Gatan SC1000 camera.

2.7 Antibacterial Activity of Crude Plant Extracts and $AgNO_3$ on the Four Pathogens

Bioactivities of the crude extracts and the silver nitrate solution were determined by using the disc diffusion method described by [25]. Sterile paper discs (6 mm) impregnated with the plant bark and root extracts were suspended in sterile distilled water and were left to dry at 37°C for 24 hours in a sterile condition. The test organisms were prepared in saline solution from nutrient agar plate, the agar plates were grown for 18 hours. A loop full of the test isolates were inoculated into 5ml of nutrient broth and incubated for 24 hours. 0.2 ml from the overnight culture of the organisms were dispensed into 19ml of sterile nutrient broth and incubated for 3-5hours by using McFarland turbidity standard using the spectrophotometer of 600 nm to standardize the culture to 10^6cfu/ml. Muller Hinton was inoculated the organisms using swab and the discs into which the synthesized AgNPs have been soaked were placed on the surface and then incubated for 24 hours at 37°C. The zones of inhibition were measured after incubation and recorded. $AgNO_3$ was evaluated the same way to determine its bioactivity. Disc diffusion method as described and adopted by [26] was used.

2.8 Antibacterial Activity of Silver Nanoparticles on the Four Pathogens

The antimicrobial assay was conducted according to the method of [27] and [28]. The sterile paper discs (6 mm) impregnated with silver nanoparticles derived from the plant bark and root extracts were suspended in sterile distilled water and were left to dry at 37°C for 24 hours in a sterile condition. Cultures for inoculation were prepared from colonies on nutrient agar plate, the agar plates were grown for 18 hours. Procedure for inoculation was as stated above and plates were incubated for 24 hours. 0.2 ml from the overnight culture of the organisms were dispensed into 19 ml of sterile nutrient broth and incubated for

3-5 hrs using McFarland turbidity standard using the spectrophotometer of 600 nm to standardize the culture to 10^6cfu/ml. The surface of Muller Hinton Agar was completely inoculated using a sterile swab. The discs impregnated with silver nanoparticles were then placed on the inoculated agar and incubated at 37°C for 24 hours and zones of inhibition measured. Chloramphenicol (30 µg) and Streptomycin (30 µg) were used as the positive standards control.

3. RESULTS AND DISCUSSION

Search for active drugs to fight emerging and reemerging diseases is ongoing with snail speed successes. The slow rate of success has prompted nano-research that aim at drug delivery accounting for enhanced delivery of active components of drugs to target organs. Plants are known to produce certain chemical substances which they either use for defense purposes or they simply accumulate them as waste materials. The former are usually accumulated at the bark of the plant and remain part of the plant till it dies. These substances are fortunately used to treat diseases since they have antimicrobial effects. From Table 1 plant secondary metabolites such as Triterpenes, Flavonoids, Saponins, Tannins, Alkaloids, Glycosides and Steroids were identified through phytochemical screening of the bark and root extracts. The chemicals identified in this plant bark and root may be effective against a number of bacteria, protozoa and fungi [29,30]. Antibacterial activity of extracts obtained from ethyl acetate and n-Hexane extracts of bark and root of *K. senegalensis* against *S. epidermidis, B. subtilis, E. coli* and *K. pneumoniae* brought to light differences in their antibacterial activities. The bark extract show higher activities against all the four bacterial isolates compared to the root extract. Taking a critical look at Table 2 it is clear that the most profound activity was against *K. pneumoniae* (28.1±0.42) while the least activity was against *B. subtilis* which produced the zone of inhibition of 20.8±1.21. The root extract show lower activity against the test organisms with the highest being 26.3±0.12 on *K. pneumoniae* and the lowest as 22.2±0.20 on *S. epidermidis*. The n-Hexane extracts of the bark as well as the root of the plant had lower activities on the pathogens with activity on *B. subtilis* by the n-Hexane bark extract (21.1±0.00 and *K. pneumoniae* by the n-Hexane root extract (18.1±1.20). Certainly, the bark extract has shown to be more active against the bacterial isolates. Rarely do researchers focus on parts of these plants, most researchers studied stem bark, root, leaves separately. [31] studied only the stem bark. Also [29] studied the stem bark extract against plasmodia. The ethyl acetate of both root and bark extracts were used to synthesize silver nanoparticles (AgNPs) in a controlled green synthesis in the laboratory using appropriate technology. The progression of the AgNPs synthesis is as shown in Fig. 3. The evolving nanoparticles peaked at the wavelength of between 450-600 nm on the spectrophotometer (Figs. 1 and 2). The scanning electron microscope and transmission electron microscope imagery of the minute nanoparticles are shown in Fig. 5. It is expected that these particles are impregnated with the active compounds of the extracts like "ballistic missiles" carry nuclear warheads. It is also expected that these particles deliver these active materials to target parts on the organisms that could eventually inhibit the growth of

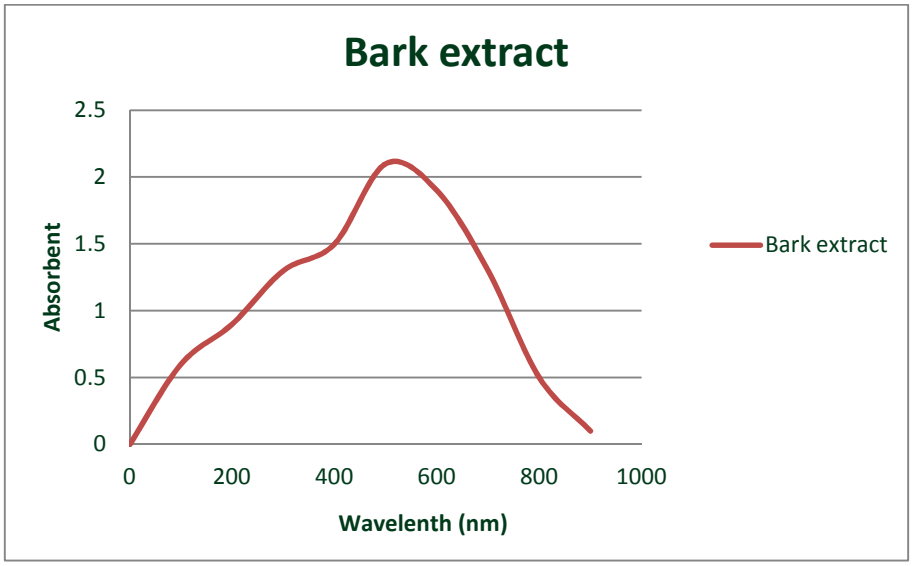

Fig. 1. Green synthesis of AgNPs from bark extract of *K. senegalensis*

the organisms or lyse them altogether attacking the cell wall synthesis, cell membrane functions, protein synthesis or amino acid synthesis. The activities of the nanoparticles are higher than those of the crude extracts. The zones of inhibition created by the particles are far wider around each organism challenged. The following results, as contained in Table 3, validate this claim: AgNPs bark extract against *B. subtilis* produced 31.2±0.11 zone of inhibition as against 24.2±1.21 and 28.2±2.12 by Streptomycin and Tetracyclines respectively. We have stumble on something that arouse our curiosity in the course of this work. Both the ethyl acetate and n-Hexane bark extracts of the plant demonstrated higher activities on the test isolates compared to the root extracts (Table 2). One would have expected that the nanoparticles of the stem bark will have more activity on the isolates than the root nanoparticles. This was surprisingly, not the case here! The root nanoparticles have higher activities than the stem bark nanoparticles. What could have been responsible for these differences in activity? What could have enhanced the activity of the root above the stem bark? We feel may be, active compounds contained in the roots were properly and rightly delivered far into the organisms to cause enough damage when in nano form. The nanoparticles have the ability and capability of penetrating far deeper into microbial cells in order to elicit cidal effects. The potency of *Khaya senegalensis* against microbial pathogens can be broadened if it can be processed into its nanoparticles.

Table 1. Presence of secondary metabolites in bark and root ethyl acetate extracts of plant

Metabolite	Stem bark	Root
Triterpenes	+	-
Flavonoids	-	+
Saponins	+	+
Tannins	+	+
Alkaloids	+	+
Phlobatannins	-	-
Glycosides	+	+
Steroids	+	+

Key:+=Present
-= Absent

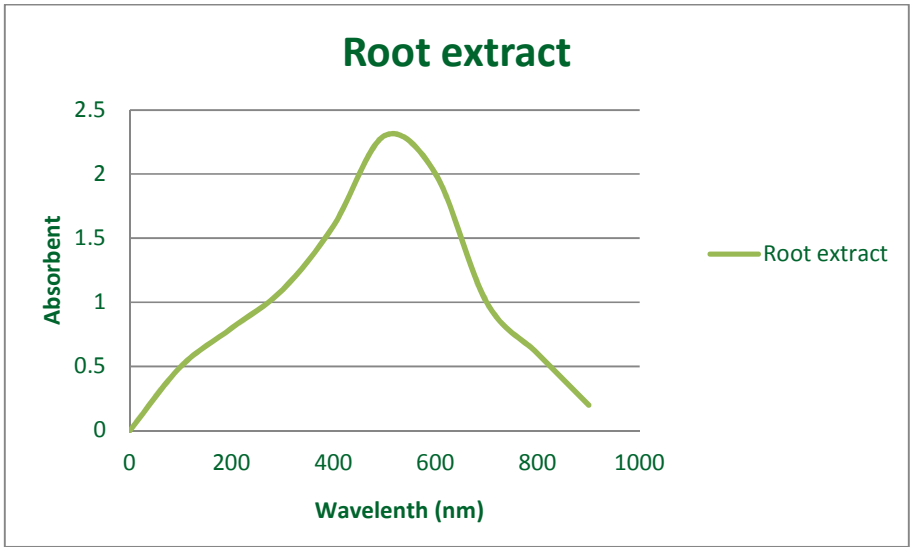

Fig. 2. Green synthesis of AgNPs from root extract of *K. senegalensis*

Table 2. Zones of inhibition (mm) of the ethyl acetate extracts of stem bark and root of *K. senegalensis* and control against the test bacterial strains

Extracts	Clinical bacterial isolates			
	S. epidermidis	***B. subtilis***	***E. coli***	***K. pneumoniae***
Ethyl acetate bark extract	24.1 ± 1.20	20.8 ± 1.21	25.3 ± 0.21	28.1 ± 0.42
Ethyl acetate root extract	22.2 ± 0.20	25.0 ± 0.01	23.1 ± 3.01	26.3 ± 0.12
n-Hexane bark extract	18.0 ± 1.01	21.1 ± 0.00	18.3 ± 1.14	16.6 ± 2.10
n-Hexane root extract	15.3 ± 1.31	17.1 ± 1.12	14.3 ± 0.24	18.1 ± 1.20

**(Mean ± S.E.M)*

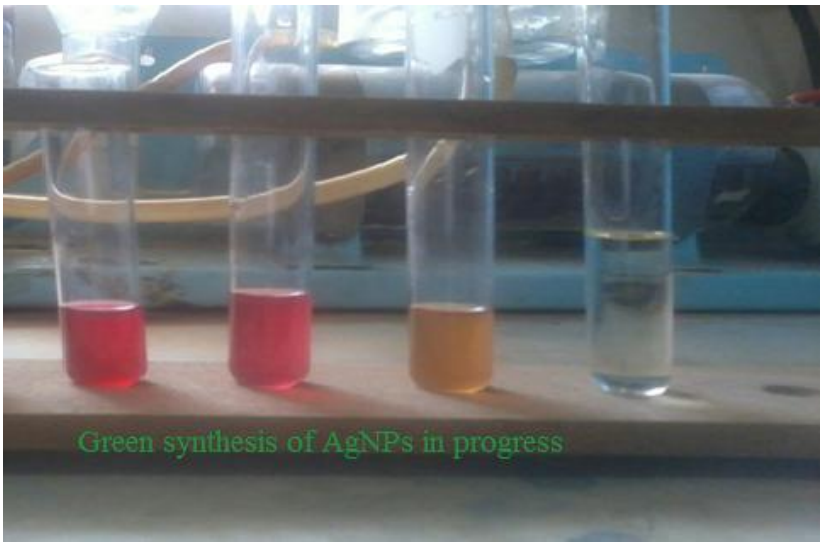

Fig. 3. Synthesis of silver nanoparticles in test tubes

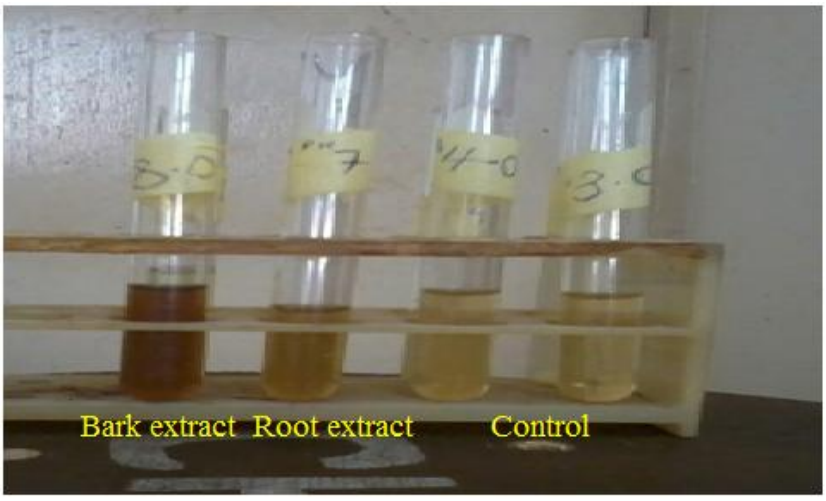

Fig. 4. Extracts from stem bark and roots of *Khaya senegalensis* with control

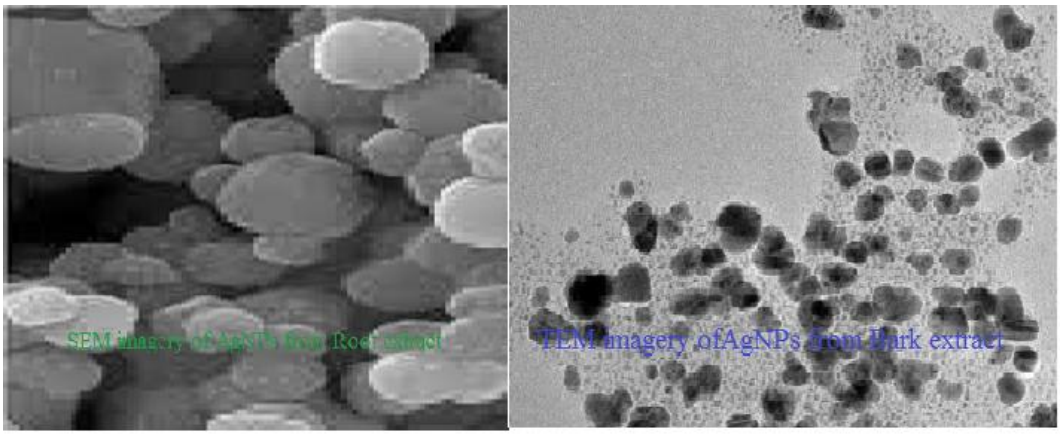

Fig. 5. SEM and TEM imagery of AgNPs from *K. senegalensis*

Table 3. Zones of inhibition (mm) of the n-Hexane extracts of stem bark and root of *K. senegalensis* and control against the test bacterial strains

Extracts	Clinical bacterial isolates			
	S. epidermidis	***B. subtilis***	***E. coli***	***K. pneumoniae***
AgNPs bark extract	28.3 ± 0.32	31.2 ± 0.11	28.1 ± 0.00	27.2 ± 1.01
AgNPS root extract	33.1 ± 2.12	34.1 ± 1.14	29.2 ± 0.40	36.1 ± 0.21
Streptomycin	22.3 ± 0.12	24.3 ± 1.21	23.2 ± 2.04	26.2 ± 1.04
Tetracyclines	24.1 ± 2.22	28.2 ± 2.12	26.0 ± 0.10	24.5 ± 2.01

**(Mean ± S.E.M)*

4. CONCLUSION

Extracts of *K. senegalensis* have considerable activities against these pathogenic bacteria. The silver nanoparticles of the stem bark and root however, have higher activities on the organisms. The silver nanoparticles synthesized using the root extract to reduce $AgNO_3$ to AgNPs have more elaborate activities against the organisms compared to those from the stem bark extract. This is unexpected as the stem bark extract had higher activity in the crude form. It is suspected that the root extract contained active components which have far reaching antibacterial effects on the organisms but were only aided when used in nanoparticles. Nanoparticles enhance the delivery of active antimicrobial compounds to their target sites.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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