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Synthesis and Bioactivity of Silver Nanoparticles Against Bacteria (*E. coli* and *Enterococcus sp.*) Isolated from Kalamu River, Kinshasa City, Democratic Republic of the Congo

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Abstract: The emergence of new infectious agents is a potential risk associated with genetic manipulation and field cultivation of genetically modified organisms and constitutes a new challenge in molecular epidemiology. The main objective of the current study was to synthesize silver nanoparticles and evaluate the antibacterial activity of these nanoparticles. *E. coli* and *Enterococcus sp.* were isolated from wastewater samples collected from Kalamu River. The preliminary characterization of silver nanoparticles was carried out using UV-visible spectrophotometer. Noble metals, such as silver nanoparticles, exhibit unique and adjustable optical properties due to their external plasmon resonance. The reduction of silver ions was monitored by measuring the UV-visible spectrum of the solutions after dilution of a small aliquot (0.2 mL) of the aqueous component. The antibiotic susceptibility test results confirmed the inactivity of these antibiotics tested against the wild strain of *Enteroccocus sp.* The synthesized silver nanoparticles displayed a good antibacterial activity against *Enterococcus sp.* The synthesis of silver nanoparticles is designed precisely to alleviate this situation; and these results provide a strong evidence that silver nanoparticles can be used to fight antibiotic-resistant bacteria.

Keywords: Silver Nanoparticles, Antibacterial Activity, Antibiotic-Resistant Bacteria, Kalamu River, *Annona senegalensis*

1. Introduction

The emergence of new infectious agents is a potential risk associated with genetic manipulation and field cultivation of genetically modified organisms (GMOs) and constitutes a new challenge in molecular epidemiology. In fact, it was reported that transgenic plants grown on surface are likely to release their DNA and this DNA can go through different

environmental compartments and end up in the groundwater and then reaches the gastrointestinal tract via water consumption [1]. It is well established and known that in plant transgenesis, the gene of interest is merged with an antibiotic resistance gene in order to facilitate the selection of transgenic explants leading to the uncertainty of using genetic modified organisms (GMOs). Therefore, bacteria have developed different mechanisms to render ineffective the antibiotics used against them. The genes encoding these defense mechanisms are located on the bacterial chromosome or on extrachromosomal plasmids, and are transmitted to the next generation (vertical gene transfer). Genetic elements, such as plasmids, can also be exchanged among bacteria of different taxonomic affiliation (horizontal gene transfer) [2].

In the particular case of transplatomic plants (i.e. modified at cp DNA), the dead leaves can release plant cells into the soil of the transgenic DNA by lysis. In the soil, the transgenic DNA can be protected from the nucleases by adsorption on the clay particles [3]. The high degree of homology between chloroplast DNA and bacterial genome as well as the diversity of naturally competent telluric bacteria constitute potential risks related to the environmental release of recombinant DNA both in the biogeochemical cycle as well as in the trophic chain [4].

Since times immemorial, among various antimicrobial agents, silver has been most extensively studied and used to fight against infections and prevent spoilage [5]. Silver nanoparticles are among the most widely commercialized engineered nanomaterials, because of their antimicrobial properties. They are already commonly used in medical devices, household products and industry [5]. The use of nanoparticles for therapeutic purposes was envisaged some 20 years ago and continues to inspire active research in this field, particularly in the controlled release of drugs [6] or the improvement medical imaging techniques [7] [8]. Silver nanoparticles are used in particular for their biocidal property, so they are found in antibacterial and anti-odor textiles, as well as in antibacterial packaging and plastics [9]. In recent years, Nanotechnology has attracted considerable attention to scientists due to its various applications. The impact of the nanostructured materials can bring improvement on the quality of life and preservation of the environment, and also represents a promising field for generating new types of nanomaterials with biomedical and environmental applications [5].

The main objective of the current study was to synthesize silver nanoparticles and evaluate the antibacterial activity of these nanoparticles. Specific objectives were: (1) to isolate the bacteria indicative of faecal pollution (*E. coli* and *Enteroccocus sp.*); (2) to run an antibiotic susceptibility test of isolated bacteria in order to assess the chemo-resistance; (3) to perform the biogenic synthesis of silver nanoparticles (AgNPs) using green chemistry; (4) to characterize silver nanoparticles using UV-Visible spectrophotometer and (5) to assess the antibacterial activity of these nanoparticles by determining the Minimum Inhibitory Concentration (MIC). The significance of nanoparticles continues relentlessly because of their usefulness in several scientific fields such as pharmacy where they are used for their broad spectrum of activity towards bacterial strains.

The hypothesis of the current study was the accumulation of heavy metals such as Cd, Cu, Hg, and Zn in tropical ecosystems (pollution) would promote bacteria transformation by producing antibiotic resistance genes. The acquisition of this drug resistance may constitute a major public health problem and the dissemination of antibiotic resistance genes in the environment would be via hydro-dispersive transport of DNA via soil and/or groundwater. This DNA would be biologically active and capable of transforming wild competent bacteria. It would come from transgenic plants or from the gastrointestinal tract (fecal pollution) to the environment.

2. Material and Methods

2.1. Study Area

The current study was carried out in Kalamu River where samples were collected and transported to the laboratory for further analyses. The geographic coordinates of inspected sites and the image of the site collection are presented in table 1 and figure 1 below. These coordinates were taken by a GPS (GARMIN brand).

Figure 1. *Kalamu river – Site of sample collection (border of Lemba and Ngaba townships).*

Table 1. *Geographic coordinates of different sites of collection.*

SITES	Geographic coordinates	Altitude (m)
Upstream 1	S: 04°22.717 E:015°19.841	289
Upstream 2	S: 04°22.733 E: 015°19.822	290
Landmark 1	S: 04°22.704 E: 015°19.861	292
Landmark 2	S: 04°22.704 E: 015°19.861	292
Downstream 1	S: 04°22.672 E: 015°19.902	293
Downstream 2	S: 04°22.672 E: 015°19.908	288

2.2. Material

2.2.1. Plant Material

As plant material, the root bark of *Annona senegalensis* (*A. senegalensis*) was used. It was collected in September 2016 at Matadi-kibala district, Mont-Ngafula Township, Kinshasa city. The identification of this plant was performed in the

Herbarium in the department of Biology, Faculty of Science, University of Kinshasa.

2.2.2. Bacterial Strains

In the current study, two bacterial strains were used namely *Escherichia coli* (*E. coli*) and *Enterococcus sp*. They were isolated from the wastewater collected from Kalamu river and these water samples were analyzed in Molecular Biology Laboratory, Faculty of Sciences, University of Kinshasa.

2.3. Methods

2.3.1. Conditioning of Plant Material

Plant sample was dried for two weeks in room temperature (about 27°C) under shade in the Molecular Bio-prospection Laboratory at the Department of Biology, University of Kinshasa. Having dried, the plant material was crushed in a mill and screened with one mm diameter sieve to obtain fine powder. Wastewater samples were collected into plastic bottles in September 2016 and were kept in a cool box at 4°C and were directly carried to the laboratory to avoid any disturbance of parameters taken *in situ*.

2.3.2. Physico-Chemical Analyses

Physico-chemical analyses were carried out *in situ* using a multi-parameter probe (HQ40d brand). The parameters used to assess the properties of this water were pH, temperature, conductivity, dissolved oxygen and total dissolved solids (TDS). All these analyses were carried out according to the standard methods as previously described [10]. For each parameter, 25 mL of wastewater were placed into a beaker and the electrode of the probe was inserted in and the result was read in the device for pH, temperature, conductivity, dissolved oxygen and TDS respectively.

2.3.3. Bacteriological Analysis

The bacteriological analysis for the search and enumeration of *E. coli* and *Enterococcus sp.* was performed as described by Luboya and Kilunga [11] [23]. The culture of these bacterial strains was carried out each in its selective medium; TBX for *E. coli* and SBA for *Enterococcus sp.* respectively. A stock solution was prepared from the wastewater samples out of which several dilutions were performed. Then, at the smallest dilution the bacterial suspension (diluted wastewater) was cultured in different media contained in the petri dish. After culture, they were incubated in the oven at 44°C pending 24 hours for *E. coli* and 48 hours for *Enterococcus sp.* In TBX medium, the presence of *E. coli* was confirmed by the appearance of blue colonies while in SBA medium the presence of the Enterococci was confirmed by the appearance of red-like tending to pink colonies.

2.3.4. Phytochemical Screening of A. senegalensis Root Bark

The phytochemical screening is a chemical screening that includes a number of qualitative analysis that allows the identification of secondary metabolites present in a certain sample. The detection of these chemical groups is performed through color and precipitation reactions occurring with the addition of specific reagents [12-14]. This phytochemical screening was carried out according to the standard protocol as previously described by Ngbolua *et al.* [12] and it can be performed in aqueous as well as in organic phases [15].

i. Preparation of Aqueous and Organic Extracts

Ten grams of the powder were weighed and placed in an Erlenmeyer where 100 mL of distilled water and methanol were added respectively. The mixture was incubated for 48 hours and then filtered using Whatmann's n°1 filter paper to obtain the aqueous and organic extracts respectively.

ii. Search for Steroids and Triterpenoids

In five mL of organic extract evaporated to dryness, one mL of acetic anhydride and 0.05 mL of concentrated H_2SO_4 (Leibermann reagent) were added in the test tube. A purple coloration indicates the presence of triterpenoids and steroids when mixed while terpenes give a complex purple and steroids display a green coloration.

2.3.5. Synthesis of Silver Nanoparticles

i. Preparation of Aqueous Extract and Silver Nitrate Solution

Ten grams of *A. senegalensis* powder were introduced into an Erlenmeyer where 100 mL of distilled water were added and incubated for 24 hours then filtered. Meanwhile the solution of silver nitrate (0.001 M) was prepared by weighing 170 mg of silver nitrate ($AgNO_3$) which was introduced into a beaker and a liter of distilled water was added.

ii. Synthesis of Silver Nanoparticles

In a test tube, five mL of the aqueous extract was introduced to which 95 mL of silver nitrate solution was added. Then, this mixture was heated for 10 min at 90°C. Having heated, the mixture was centrifuged at 10 000 rpm for 10 min at 4°C, afterwards a washing was performed with water (the residue is diluted with water). At last, UV-visible spectrophotometer was used to read the results (wavelength between 200 and 700 nm).

2.3.6. Characterization of Silver Nanoparticles

The preliminary characterization of silver nanoparticles was carried out by UV-visible spectroscopy, using a spectrophotometer (HITACHI U-3900H brand). Noble metals, such as silver nanoparticles, exhibit unique and adjustable optical properties due to their external plasmon resonance, depending on the shape, size and distribution of nanoparticle sizes. The reduction of silver ions was monitored by measuring the UV-EIDENT spectra of the solutions after dilution of a small aliquot (0.2 mL) of the aqueous component.

2.3.7. Chemical Characterization of Water Samples

The analysis of chemical parameters involved the determination of Copper ions (Cu^{2+}, Cu^{3+}), Cadmium (Cd^{2+}), Mercury (Hg^{2+}) and Zinc (Zn^{2+}) using a computer-assisted

spectrometer (Xepos III ED-XRF brand). Different chemical parameter analyses were carried out using the X-ray fluorescence spectrometer (EDP-XRF, XEPOS III brand). Therefore, samples were measured on the above mentioned device using four secondary targets notably Molybdenum (39.76 KV of voltage and 0.88 mA of current), Aluminum oxide (49.15 KV of voltage and 0.7 mA of current), Cobalt (35.79KV of voltage and 1mA of current) and last HOPG Bragg Crystal (17.4KV voltage and 1.99 mA current) of the anode in palladium.

In general, the sample (pellet) to be analyzed is placed under a beam of X-rays and under the effect of these rays, the sample resonates and re-emits X-rays which are its own and they are fluorescent. If we have a look at the energy spectrum of fluorescent X-rays, we can perceive characteristic peaks of different elements present in the sample. Therefore, it helps to know what elements are present and the height of these peaks helps to determine in what quantity are these elements. The Kα1 peak (3.313 Kév) of the K was used for the calculation; Bragg's HOPG Crystal target (17.4KV voltage and 1.99 mA of current) gave surfaces that were normalized compared to the peak from coherent and incoherent diffusion.

2.3.8. Antibiotic Susceptibility Test

The antibiotic susceptibility test was assessed using the diffusion method with discs of antibiotics for a sensitivity test.

i. Antibacterial Activity

The antibacterial activity was evaluated using the micro-dilution method in liquid medium as previously reported by Ngbolua *et al.*, [16]. The extract to be tested (10 mg) was dissolved in 250 µL of DMSO and the final volume was adjusted to five mL in Mueller Hinton culture medium (final concentration of DMSO 5%). In two mL of saline solution, bacterial suspension is prepared by introducing two colonies isolated from the strains to be tested by incubating for 24 hours in order to obtain 0.5 Mc Farland (10^8 cells/mL) Then, the bacterial suspension was diluted in order to obtain 10^6 cells/mL (1/100).

The micro-dilution test was carried out in a 96-well sterile polystyrene microplate. Briefly, 100 µL of culture were placed inside wells (A_2 to A_8, then in the 11^{th} and 12^{th} columns as controls). Using a micropipette, 200 µL of the extract to be tested (1000 µg/mL) is placed inside well A_1 (*A. senegalensis* extract), 100 µL of the extract stock solution is then sampled to carry out serial dilutions of 2 by 2 up to the eighth column and the last 100 µL (column 8) were removed.

Then five µL of the inoculum (10^8 CFU/mL) were removed aseptically using a micropipette and transferred to all wells along the microplate except for wells of the 11^{th} column which serve as control for the bacterial growth (inoculum and culture medium). Wells of the 12^{th} column are used as control of sterility of culture medium. The microplate was incubated in an oven at 37 °C for 24 hours. After the incubation, five µL of Resazurin dye 1% (7-Hydroxy-3H-phenoxazin-3-one 10-oxide) were added to each well and the microplate was then incubated again for five hours. The minimum inhibitory concentration (MIC) (first well showing no bacterial growth) was determined after 24, 48 and 72 hours respectively.

ii. Determination of the Minimum Inhibitory Concentration (MIC)

The MIC was read after addition of five µL of Resazurin dye 1% (7-Hydroxy-3H-phenoxazin-3-one 10-oxide) to each well having a concentration of 0.1 $mg.mL^{-1}$, a blue dye which is less fluorescent. The test was valid only if acceptable growth was observed in these control wells. If the growth is insufficient in these wells, the microplate is reincubated and the MIC was read after 48 hours. It should be noted that when the MIC is 250 $\mu g.mL^{-1}$, the drug was considered active on the bacterial strains [17].

3. Results and Discussion

3.1. Physico-Chemical Parameters

Physico-chemical parameters of wastewater samples of Kalamu river are presented in table 2 below.

Table 2. *Physical and chemical parameters of Kalamu river.*

	Units	Standards of WHO	Sites						Mean ± SD
			Upstream 1	Upstream 2	Landmark 1	Landmark 2	Downstream 1	Downstream 2	
Parameters									
pH	-	6.5-8.5	6.28	6.19	6.26	6.26	6.21	6.21	6.235±0.036
Temperature	°C	12-25	26	25.3	26.2	26.1	25.4	25.4	25.73±0.408
Conductivity	($\mu S.\ Cm^{-1}$)	400-1250	428	434	421	421	405	395	417.3±14.624
Dissolved oxygen	$mg.L^{-1}$	5	2.89	2.53	2.38	2.35	3.63	3.68	2.91 ±0.608
TDS	Ppm	500-1500	228	230	228	228	215	223	225.3±5.574

Legend: SD: Standard deviation, TDS: Total dissolved solids

It emerges from the above table that the pH of Kalamu river ranges between 6.21 and 6.28 having an average of 6.2 ± 0.036 which is below WHO standards (6.5-8.5). There is no pH threshold based on health, although the range between 6.5 and 8.5 is often recommended for the fact that aquatic life is negatively affected at pHs below 6.0 [18]

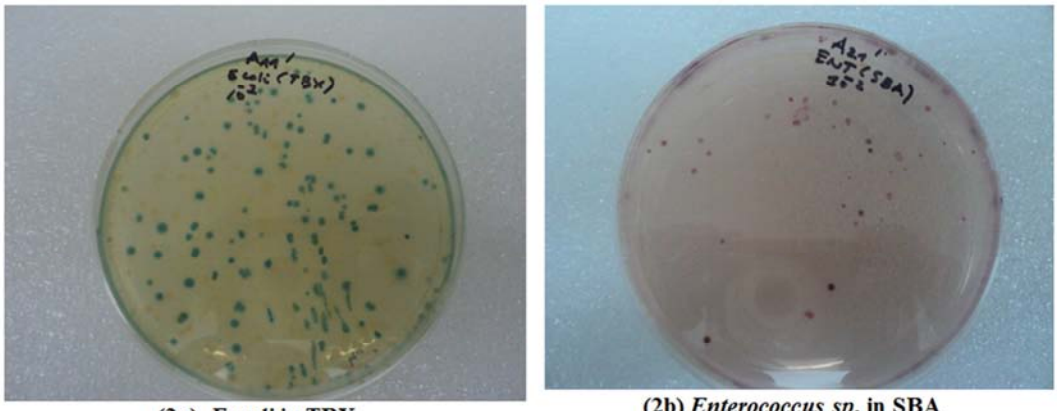
Conductivity in water is due to the degree of mineralization of this water and it depends on the solubility of dissolved compounds and dissociated from ion mobility as well as the temperature of water. The values found in this

study ranged between 395 and 434 µS.cm^{-1} with an average of 417.3 ± 14.624 which is close to WHO standards for chemical substances (50 to 400 µS.cm^{-1}); this could be due to the high number of dissolved ions carried by rainwater. TDS as conductivity is an indication of organic as well as inorganic solids totally dissolved in water. WHO recommends 1200 mg.mL^{-1} for TDS and the Environmental Protection Agency (EPA) recommends at 500 mg.mL^{-1}. All our calculated values range between 215 and 230 ppm with an average value of 225.3 ± 5.574. These values are below the standards established by WHO. This may be due to the dilution of ions in rainwater and their transport by rain. This parameter is important for it indicates the degree of pollution caused by chemical fertilizers and other agricultural products used by riparian for their crops along the bank of Kalamu river as reported in other countries [19]. The value of water temperature in this study ranged between 25.4 and 26.2 with an average of 25.7 ± 0.4. These values are within the standards established by WHO (25°C to 29°C). This could be justified considering the season by which samples were collected. Dissolved oxygen is an important indicator of aquatic ecosystem health because it expresses the amount of oxygen present in water at a given temperature. Our findings provide values ranging between 2.35 mg.L^{-1} and 3.68 mg.L^{-1} with an average 2.91 ± 0.6. This low value could result in a high temperature, since dissolved oxygen is always proportional to water temperature [20]; and the abundance of aquatic microorganisms which metabolic activities require dissolved oxygen consumption in water [20]. However, there are several problems with the discharge of sewage (wastewater) into watercourses. First, polluted water by wastewater discharges is a threat to public health because they carry several pathogens. Second, sewage generates two serious environmental problems: the enrichment of water in organic matter and the decrease in dissolved oxygen content of watercourses [21].

3.2. Bacteriological Analysis

3.2.1. Bacterial Isolation

Figure 2 shows the results of the isolation of wild strains of *E. coli* and *Enteroccocus sp.*



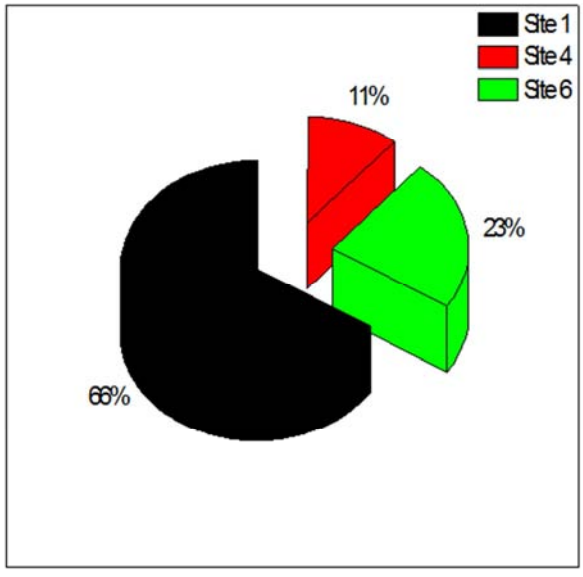
(2a) ***E. coli* in TBX** (2b) ***Enterococcus sp.* in SBA**

Figure 2. *Isolation of wild bacteria strains from Kalamu Wastewater River.*

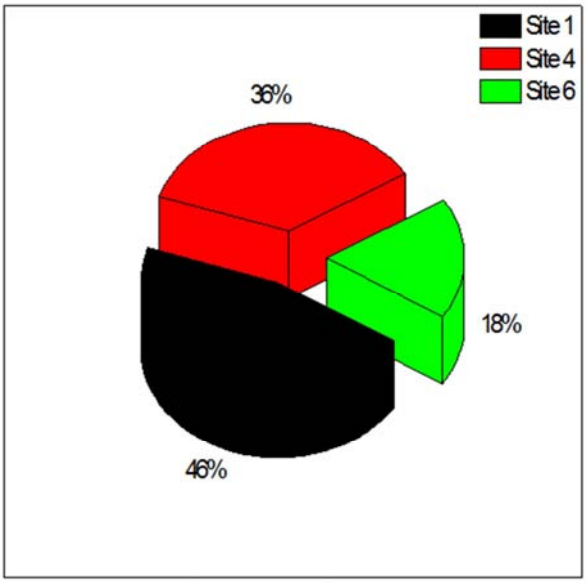
Considering specific characteristics and colors as indicated by the manufacturer, the figure above illustrates the bacterial strains of *E. coli* isolated in the TBX medium (figure 2a), and the strains of *Enterococcus sp.* isolated in the SBA medium (figure 2b). These media being specific for each of these two species, *E. coli* growth in TBX medium is observed by the presence of blue colonies while *Enterococcus sp.* is present by displaying a red color which tends to pink in SBA medium.

3.2.2. Enumeration Test

Figure 3 gives the results of the bacterial load in the studied sites



(3a) *E. coli*



(3b) *Enterococcus sp.*
(Legend: Site 1: upstream; Site 4: landmark; Site 6: downstream).

Figure 3. *Bacterial load of different sites.*

From the above figure, it can be observed that water from Kalamu River is more polluted upstream than downstream from the reference point (landmark). Faecal contamination of rivers in Kinshasa is certain. Indeed, all water samples collected from Kalamu River revealed the presence of faecal pollution indicators (*E. coli* and *Enteroccocus sp.*). The bacteriological analysis gives a high concentration of *E. coli* upstream than downstream as well as for *Enteroccocus sp.* Several studies reported that *E. coli* is a bacterial species indicative of recent pollution, and its presence in an aquatic ecosystem is an indicator of other pathogenic microorganisms [22]. These findings really show that Kalamu River is polluted every time while riparian put all canalization of their septic tanks in the river. The high concentration of *Enterococci* observed upstream and downstream would result in their adaptation to water environmental conditions, particularly with respect to physico-chemical parameters and the distribution of dissolved heavy metals in water. The EPA [22] reported that the presence of *Enterococci* in an ecosystem is indicative of recent and old pollution; and their presence in an aquatic ecosystem is an indication of the presence of other pathogenic microorganisms having the same characteristics than them. Kilunga *et al.* [23] reported that the discharge of untreated wastewaters and excreta into the urban environment of Kinshasa leads to the faecal contamination of the rivers thus increasing the potential risks of human infections by direct uptake (drinking water), which constitutes a possible source of bacterial contamination in raw vegetables or contamination during recreational activities.

3.3. Phytochemical Screening of A. senegalensis Pers. Root Bark

The phytochemical screening carried out in aqueous and organic phases of *A. senegalensis* root bark extract is presented in table 3 below.

Table 3. *Phytochemical screening of A. senegalensis root bark extract.*

Chemical groups	Results
Aqueous Phase	
A. Polyphenols	+
Anthocyanins	+
Leucoanthocyanins	+
Bound Quinones	+
Tannins	+
Flavonoids	+
B. Alkaloids	+
C. Saponins	+
Organic Phase	
Terpenoids	+
Free Quinones	+

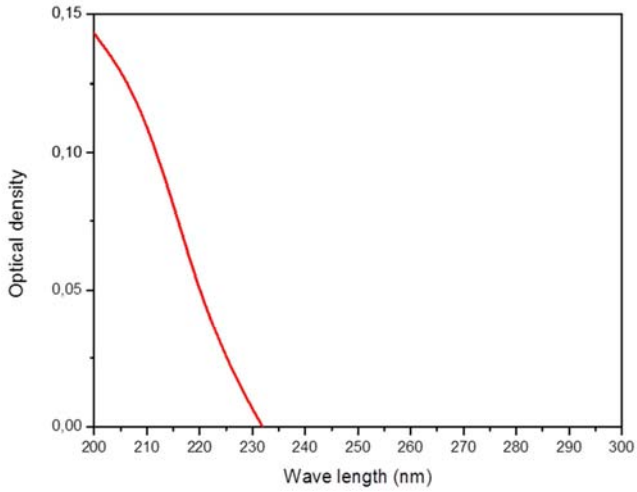
Legend: +: presence, -: absence.

From the above table, it is observed that *A. senegalensis* root bark is rich in secondary metabolites notably polyphenols, flavonoids, anthocyanins, leucoanthocyanins, tannins, bound quinones, free quinones alkaloids, saponins and terpenoids. These results corroborate with the ones of Okoli, *et al.* [24] and Ngbolua *et al.* [25]. Moreover, the presence of quinones both in the organic and aqueous phases suggests that these metabolites are in their free and bound forms in the form of heterosides [26]. All these secondary metabolites are endowed with remarkable pharmacological properties trying to justify the partial use of these plants in African traditional medicine against various infections. In fact, the presence of secondary metabolites such as flavonoids, anthocyanins and tannins could be justified by the physiological roles they provide in the plant including protection against sunlight and predators and the coloring of plants [26].

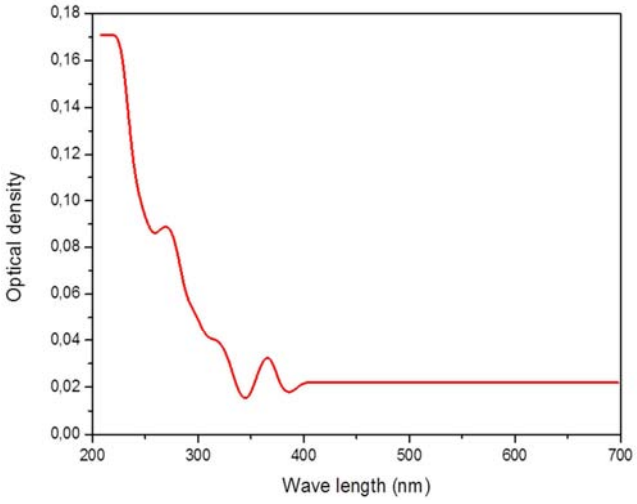
3.4. Characterization of Silver Nanoparticles

Figures below illustrate the pellets containing the silver nanoparticles, the spectrum of the aqueous extract of *A. senegalensis*, the spectrum of AgNPs and the compared spectra of AgNPs and aqueous extract respectively.

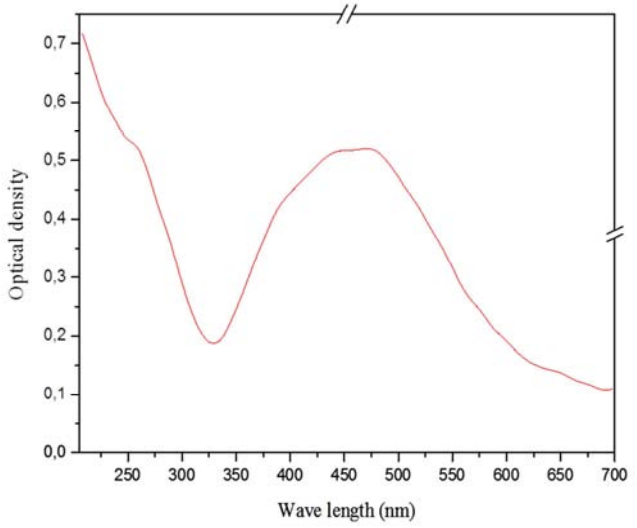
Figure 4. *Residue of AgNPs.*



(5a). Spectrum of $AgNO_3$ aqueous solution (0.15 M)



(5b). Aqueous extract of *A. senegalensis* (10%)



(5c). Silver Nanoparticles (AgNPs)

Figure 5. *UV-visible spectra of $AgNO_3$ solution, Plant extract and AgNPs.*

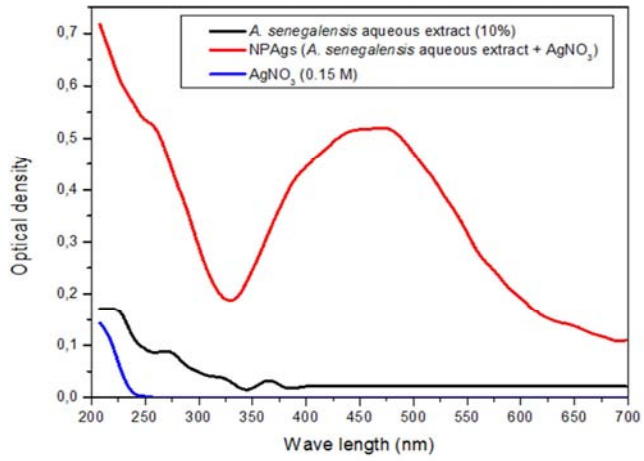


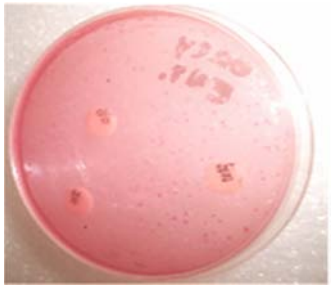
Figure 6. *Comparative UV-visible spectra of aqueous solution of $AgNO_3$, aqueous extract of A. senegalensis and silver nanoparticles.*

Figures 5a, 5b, 5c and 6 describe the spectra of the $AgNO_3$ solution, aqueous extract, silver nanoparticles and the compared spectra of $AgNO_3$, aqueous extract and silver nanoparticles respectively. In view of the above, figure 6 provides sufficient evidence that silver nanoparticles are really present in our residue because this spectrum coincides with data in the literature, according to which the UV-visible spectrum shows a peak between 400 and 500 nm corresponding to the Plasmon absorbance of the AgNPs (surface plasmon resonance peak) [27] [28].

3.5. Antibiotic Susceptibility Test

Figure 7 illustrates the antibiotic disks on our different culture media inoculated with the isolated bacterial strains.

(7a) *E. coli* in TBX medium



(7b) *Enterococcus* sp., in SBA medium

Figure 7. *Antibiotic disks on different culture media inoculated with the isolated bacterial strains.*

These figures reveal the sensitivity of *E. coli* strain to some used antibiotics (figure 7a) and the insensitivity of *Enterococcus sp.* to all antibiotics available used in the current study (figure 7b). The inhibition average obtained at the concentration of 1000 µg.mL^{-1} of the antibiotic discs on the wild isolates of *E. coli* and *Enterococcus sp.*, are presented in the table below.

Table 4. *Antibiotic susceptibility test of bacteria isolated from water samples.*

Antibiotics (µg)	*E. coli*	*Enterococcus sp.*
GN(10)	+	-
NOR(10)	-	-
CIP(30)	-	-
VA(30)	+	-
NA	-	-

Legend: GN (10): Gentamycine loaded at 10 µg; NOR (10): Norfloxacine loaded at 10 µg; CIP (30): Ciprofloxacine loaded at 30 µg; VA (30): Vancomycin loaded at 30 µg; (NA) Nalidixic acid, -: insensitive strain to antibiotics

In view of the above table, the wild strain of *E. coli* showed resistance to three antibiotics notably Norfloxacin (10 µg), Ciprofloxacin (30 µg) and Nalidixic acid while it was sensitive to the remaining antibiotics namely Vancomycin (inhibition diameter 20 mm) and Gentamycin (inhibition diameter 18 mm). Norfloxacin is an antibiotic with a spectrum of activity on enterobacteria; it inhibits the synthesis of bacterial DNA by preventing the synthesis of gyrase DNA and topoisomerase, being naturally insensitive to Gram positive bacteria.

Our results corroborate with those of Bryskier [29]. Gentamycin has an effect on Gram^{+} bacteria, but also on Gram^{-} bacteria and it acts on 30S subunit of ribosome which induces an error in reading the genetic code during protein translation [30]; our findings show that the isolated strains of *Enterococcus sp.* are resistant to gentamicin. Vancomycin is an antibiotic having a spectrum of activity on Gram+ bacteria mainly *Enterococci* [31]; our results show that *Enterococci* strains are resistant to vancomycin; and that its inactivity against *E. coli* is proven because this antibiotic does not have a spectrum of activity against *E. coli*.

3.6. The Micro-dilution Test in a Liquid Environment

The values of the minimal inhibitory concentrations (MIC) on the strains of *Enterococcus sp.* are presented shown in the table below.

Table 5. *MIC values of silver nanoparticles.*

Residue	Concentrations (µg/mL)									MIC (µg.mL^{-1})
	1000	500	250	125	62.5	31.25	15.625	7.813	3.906	
Enterococcus sp. AgNPs	-	-	-	+	+	+	+	+	+	**250**

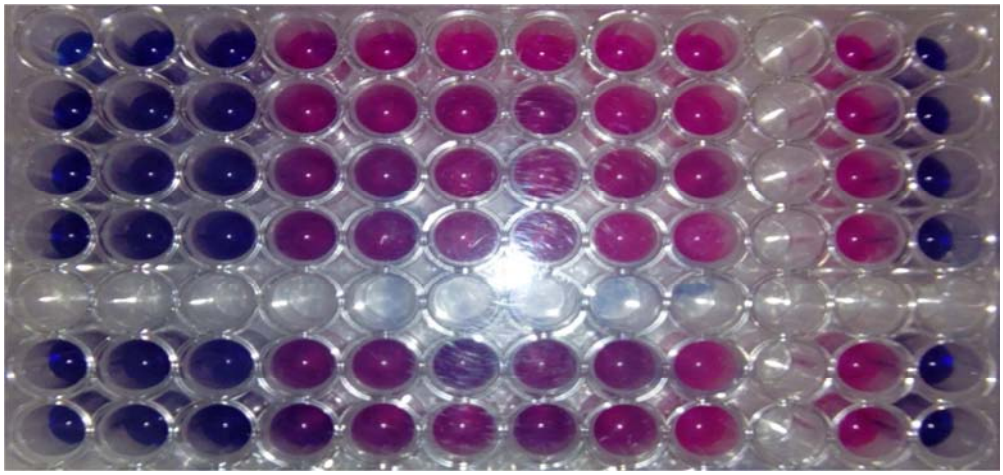


Figure 8. *Legend: +: bacterial growth (pink color); -: growth inhibition (blue color), MIC: minimal inhibitory.*

The antibiotic discs used were chosen according to the spectrum of activity that each antibiotic has towards each group of bacteria. The antibiotic susceptibility test results confirmed the inactivity of these antibiotics tested against the wild strain of *Enterococcus sp.* i.e. this strain is resistant. The emergence of bacteria resistant to antibiotics is common in areas where antibiotics are used, but antibiotic-resistant bacteria also increasingly occur in aquatic environments [2]. The synthesis of silver nanoparticles is designed precisely to alleviate this situation; and these results provide ample evidence that silver nanoparticles can be used to fight antibiotic-resistant bacteria.

The nanoparticles are considered as the most promising as they contain remarkable antibacterial properties due to their large surface area, which is of interest for researchers due to the growing microbial resistance against metal ions, antibiotics and the development of resistant strains [32]. Silver nanoparticles are an arch product from the field of nanotechnology which has gained boundless interests because of different properties that they display namely antibacterial, anti-viral, antifungal, anti-inflammatory, antiplasmodial, anti-cancer and antioxidant activities and

help in the fumigation of medical devices and home appliances to water treatment [33-35]. For biomedical applications; being added to wound dressings, topical creams, antiseptic sprays and fabrics, silver nanoparticles functions' as an antiseptic and displays a broad biocidal effect against microorganisms through the disruption of their unicellular membrane thus disturbing their enzymatic activities [34]. This can be explained by the fact that bacteria are sensitive towards AgNPs because of the variation in thickness and the molecular composition of their membrane structures [35]. As well, AgNPs also react with sulphur and phosphorus-rich biomaterials like DNA or proteins (membrane proteins) which affect the respiration, division and ultimately the cell survival. Once inside the bacterial cell wall, AgNPs can enter into cells, leading to the aggregation of damaged DNA and exert effect on protein synthesis [36]. The synthesis of silver nanoparticles is of much interest to the scientific community because of their wide range of applications and the main focus in the current research was the assessment of antibacterial activity.

All these applications are due to the advancement of the green synthesis (green chemistry) over chemical and physical methods, which is: environment friendly, simple, dependable, cost effective, pollution-free, biocompatible and easily scaled up for large scale syntheses of nanoparticles, furthermore there is no need to use high temperature, pressure, energy and toxic chemicals [34] [37]. This green chemistry approach uses microorganisms such as bacteria (*E. coli*, *Lactobacillus* strains, *Pseudomonas aeruginosa*), fungi (*Fusarium oxysporum*) and plant extracts (*Allophylus cobbe*, *Artemisia princeps*) as well as several biomolecules such as biopolymers, starch, fibrinolytic enzyme as well as amino acids and these materials used are always available [37]. But understanding the mechanism by which these biomolecules of these organisms are involved in the synthesis is not fully known [38]. The increasing use of AgNPs in day to day life will increase their release to the environment and would require the assessment of environmental risks associated with these particles [37] [38].

3.7. Heavy Metal Composition of Wastewater

Table 6 presents the composition in heavy metals of our wastewater samples collected from Kalamu river.

Table 6. *Determination of some heavy metals.*

	Elements/Concentrations											
Samples	**Aluminium**	**Silicium**	**Phosphorus**	**Sulphur**	**Chlorine**	**Potassium**	**Calcium**	**Titanium**	**Chromium**	**Manganese**	**Iron**	**Cobalt**
FSB1740	541	127.1	180.3	153.0	<2.0	69.2	237.6	9.9	1.9	3.0	44.5	< 3.0
FSB1741	<20	761	183.6	103.8	< 2.0	56.4	177.5	10.1	2.6	1.8	34.2	< 3.0
FSB1742	< 20	677	183.7	99.4	< 2.0	55.4	194.2	8.5	0.8	1.9	41.2	< 3.0
FSB1743	419	131.4	202.8	156.2	< 2.0	68.1	199.5	12.2	3.3	2.2	59.7	< 3.0
FSB1744	381	128.7	203.4	112.9	< 2.0	63.7	227.5	13.4	1.4	2.1	47.2	< 1.8
FSB1745	99.1	119.5	192.5	127.9	<2.0	56.9	160.2	9.2	0.9	1.7	40.9	< 3.0
FSB1746	< 20	690	166.3	78.6	< 2. 0	47.3	140.6	7.3	1.3	3.3	35.5	< 0.5
FSB1747	323	115.8	209.9	133.7	< 2.0	72.1	329.0	16.5	5.8	3.5	83.8	< 3.0
FSB1748	146.6	114.3	187.2	120.5	< 2.0	69.7	353.6	12.4	1.5	2.1	47.9	< 3.0

Table 6. *Continue.*

	Elements/Concentrations												
Samples	**Nickel**	**Copper**	**Zinc**	**Arsenic**	**Selenium**	**Silver**	**Cadmium**	**Tin**	**Cesium**	**Cerium**	**Mercury**	**Lead**	**Uranium**
FSB1740	2.2	2.5	3.2	< 0.5	< 0.5	< 2.0	<2.0	< 3.0	< 2.0	< 1.0	< 1.0	1.1	< 1.1
FSB1741	2.6	41	3.1	< 0.5	< 0.5	< 2.0	<2.0	< 3.0	< 4.0	< 2.0	< 0.2	1.0	< 1.0
FSB1742	2.7	2.8	2.3	< 0.5	< 0.5	1.3	1.3	< 3.0	< 2.0	1.6	< 1.0	< 0.3	< 1.0
FSB1743	3.4	3.0	3.8	< 0.5	< 0.5	2.5	2.5	< 3.0	< 2.0	< 1.0	< 1.0	< 0.3	< 1.0
FSB1744	2.2	15	4.0	< 0.5	< 0.2	< 2.0	<2.0	< 3.0	< 2.0	< 0.5	< 1.0	0.7	< 1.0
FSB1745	3.5	2.1	3.8	< 0.5	< 0.5	< 2.0	<2.0	< 3.0	< 2.0	< 1.0	< 0.4	0.6	< 1.0
FSB1746	2.5	2.4	3.3	< 0.5	< 0.5	2.0	2.0	< 3.0	< 2.0	17.1	< 0.2	< 1.0	< 1.0
FSB1747	4.1	2.5	5.0	< 0.5	< 0.5	1.2	1.2	< 3.0	< 2.0	2.4	< 1.0	< 0.5	< 1.0
FSB1748	2.5	2.3	3.8	< 0.5	< 0.5	< 0.2	<2.0	< 3.0	< 2.0	< 0.5	< 0.2	< 0.4	< 1.0

Legend: FSB1740: Upstream 1; FSB1741: Upstream: 2; FSB1742: Upstream 3; FSB1743: Landmark 1; FSB1744: Landmark 2; FSB1745: Landmark 3; FSB1746: Downstream 1; FSB1747: Downstream 2; FSB1748: Downstream 3.

Of the whole list of chemical elements measured following the hypothesis of the current study, only four chemical elements were reported as being heavy metals notably Cu, Cd, Zn and Hg of environmental importance as involved in the resistance of microorganisms as previously described [39].

In some natural environments with microbial communities, combined contaminations of heavy metals and antibiotics contribute to the occurrence and spread of microbial antibiotic resistance; and sometimes multidrug resistance evolves [39]. Some instances are: the co-exposure to Zn and antibiotics such as oxytetracycline in activated sludge bioreactors appears to improve the resistance of the microbial community towards antibiotics. The amendment of Cu in agricultural soils selects for Cu resistance and further co-selects for resistance to ampicillin, chloramphenicol and tetracycline. Cd in combination with Ni increased the frequency of bacterial resistance in microcosms to

chemically unrelated antibiotics like ampicillin or chloramphenicol [39]. One possible explanation of such improvement of antibiotic resistance is that the presence of heavy metals enhanced the enrichment and growth of indigenous bacteria in the microbial community, which are already bearing antibiotic resistance genes; another possibility is that the resistance in bacteria which is sensitive to antibiotics could be induced due to the co-existence of heavy metals and antibiotics in the environment. Some investigations have demonstrated the positive correlation between the abundance of antibiotic resistance genes and the elevated concentrations of antibiotic and heavy metals in environments [39]. We have to note that the environment acts both as a reservoir of resistance traits and a bioreactor containing chemical stressors and opportunities for genetic exchange. The potential for these traits to disseminate to clinically relevant pathogens becomes a consequence [40].

Based on our findings, the analysis shows that heavy metals such as: Cd, Cu, Zn and Hg are present in this aquatic ecosystem at an abnormally high threshold compared to that set by WHO while Hg is in the range as indicated by WHO. The concentration of Cd is present in the sampling site with an average of 1.89 ± 0.398 $mg.kg^{-1}$; this threshold is higher than the one recommended by WHO (5 $\mu g.kg^{-1}$). This could be justified by the use of this metal as an additive in the production chain of industries. Since this metal is included in the list of heavy metals, its presence in a medium is independent of a threshold because it is not biodegradable. It is toxic in the ionic form Cd^{2+}, found in contaminated sources [41].

The average concentration of Cu (2.58 ± 0.712) present in water is at a higher threshold than that set by WHO (0.5 $mg.kg^{-1}$). This could be due not only to its use as an additive, but also to its use as raw material in the production of utensils from different production lines and effluent collectors. The average concentration of Hg recorded is 0.756 ± 0.371 $mg.kg^{-1}$, this threshold is far higher than that indicated by WHO (1 $\mu g.kg^{-1}$). It is toxic in the ionic form Hg^{2+}, but these are the organic forms (methyl-mercury and ethyl-mercury) that have the ability to pass the meningeal barrier and exert a nerve toxicity [41].

The average concentration recorded for Zn is 3.59 ± 0.74, this threshold is within the range recommended by WHO (1.5-5 $mg.kg^{-1}$); its presence is justified by leaching thru rainwater because this element enters the constitution of the earth's crust. Although the concentration of this metal is included in the range indicated by the WHO, being non-biodegradable, its accumulation in this ecosystem would lead to the phenomenon of bioaccumulation which is very dangerous for human health. The presence of heavy metals at abnormally high concentrations makes Kalamu River an opened bioreactor which offers all susceptible conditions for bacterial transformation by new infectious agents from transgenic plants (risks linked to environmental dissemination of GMOs). The findings of the current study show that in front of such bio-molecular catastrophe (dissemination of antibiotics resistant genes), the green chemistry offers the possibility of depolluting of contaminated water by the nanoparticles.

4. Conclusion

The main aim of the current study was to synthesize silver nanoparticles and evaluate their antibacterial activity. The waters of Kalamu River are heavily loaded with bacteria indicative of faecal pollution namely *E. coli* and *Enterococci* and these strains showed resistance to antibiotics used especially for *Enterococcus sp.*; the permanent danger of these bacteria in this aquatic ecosystem is the transfer of this character responsible for antibiotic resistance to other bacteria. *A. senegalensis* root bark contains groups of secondary metabolites such as anthocyanins, leucoanthocyanins, bound quinones, tannins, alkaloids, flavonoids, saponins, free and triterpenoid quinones which may confer not only biological interest, but also can play a reducing role towards an oxidant. The characterization of silver nanoparticles was confirmed as per literature data (415-417nm). The antibacterial activity of the nanoparticles gives a MIC of 250 $\mu g.mL^{-1}$, this proves that the drug thus synthesized is active vis-à-vis our bacterial strains (*Enterococcus sp.*). The analysis of heavy metal assay showed that their concentrations are above the standards as recommended by WHO. The threat of contamination and intoxication weighs on the life of the aquatic ecosystems of Kinshasa city. The harmful effects of heavy metals are not daunting because in the long run these metals are both toxic and bioaccumulative. While these metals reach critical concentrations, they can damage the exposed system and cause genetic disorders or death by intoxication. The release of liquid industrial effluents into the various rivers of Kinshasa remains a problem to be solved for the environmental protection. Further studies are required where there would be a need of setting a water purification plant which would help to treat both hospital waste and liquid industrial effluent waste prior to any spill into a watercourse. It is a long-term project that requires great resources to be affected.

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