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Green synthesis of silver nanoparticles from *Lantana camara and Morinda morindoides* leaf extracts and their antimicrobial and catalytic activities

Mwanze R. Sikumbili[1], Kalulu M. Taba[1], Justin B. Safari[2,3]and Rui W.M. Krause[2]

[1] Department of Chemistry, Faculty of Science, University of Kinshasa, B.P. 212, Kinshasa XI, Democratic Republic of Congo.

[2] Department of Chemistry, Faculty of Science, Rhodes University, PO Box 94, Makhana 6140, South Africa

[3] Department of Pharmacy, Faculty of Pharmaceutical Sciences and Public Health, Official University of Bukavu, Bukavu B.P. 570, Democratic Republic of the Congo

Keywords
Antimicrobial activity, catalytic activity, green synthesis, *Lantana camara,* leaf extract, *Morinda morindoides*, silver nanoparticles.

Abstract

Synthesis of metal nanoparticles using plant extracts is a flourishing field of research due to its ease of implementation, low cost and agreement with current trends of green chemistry which are considered advantages over chemical and physical methods. In this study, the green synthesis method was exploited to prepare silver nanoparticles (AgNPs) using aqueous leaf extracts of *Lantana camara* and *Morinda morindoides.* Synthetized AgNPs were characterized for their elementary composition, average size, and shape by energy dispersive X-rays spectroscopy (EDX), dynamic scattering light (DLS) and transmission electron microscopy (TEM) respectively. FTIR spectroscopy was used to study the nature of molecules on the surface of nanoparticles. Antibacterial and catalytic activities of silver nanoparticles were evaluated. The results of DLS and TEM revealed the presence of nanoscale particles with average sizes of 127 nm and 240 nm grouped into agglomerations. Silver nanoparticles presented a significant antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* strains with inhibition zones varying from 9 mm to 20 mm. A significant discoloration of methylene blue solutions was observed in the presence of silver nanoparticles suggesting their catalytic activity.

INTRODUCTION

Silver nanoparticles (AgNPs) have aroused a great interest in the scientific community working in the field of nanotechnology due to their exceptional physicochemical and biological properties. Among many properties, silver nanoparticles have thermic and electric conductivity, catalytical and optical properties, antimicrobial, anticancer, antiviral, larvicidal and anti-inflammatory activities which raised several applications in many fields **[1], [2], [3], [4].** Historically, silver nanoparticles were prepared using various physical and chemical methods. Vaporization/condensation, laser ablation, pulverization, thermal decomposition, chemical and electrochemical reduction are the most reported methods. However, these classical methods have turned out to be expensive and environmentally unfriendly. Thus, recently, biochemical methods using microorganisms, algae and plants extracts, generally considered green, were developed. These methods exploit biomolecules from plants and microorganisms as reducing and stabilizing agents for the synthesis of silver nanoparticles **[5], [6], [7].** Among these methods, the green synthesis of silver nanoparticles using plants extracts is the most popular due to its simplicity and consist mainly of mixing a silver salt aqueous solution with an aqueous plant extract as reducing

agent. Many plants have been used in the green synthesis of silver nanoparticles such as *Azadirachta indica* **[8],** ***Cassia occidentalis*** **[9],** *Cucumus prophetarum* **[10],** *Gmelina arborea* **[11],** *Piper chaba* **[12]**, *Salvia spinoza* **[13]**, *Emblica officinalis, Eucalyptus globulus*, *Ocinum sanctum*, *Piper nigrum* **[14]**, etc.

Lantana camara and *Morinda morindoides* are medicinal plants widely used in tropical Africa for the treatment of various diseases such as malaria, diabetes, asthma, hypertension, rheumatism, tetanus, varicella and diarrhea **[15], [16], [17], [18], [19], [20].** In this study, *Lantana camara* and *Morinda morindoides* leaf extracts were used in the green synthesis of silver nanoparticles and then assessed for their antibacterial and catalytic activities. To our knowledge, *Morinda morindoides* leaf extract has not been tested in the green synthesis of silver nanoparticles yet. However, previous studies have reported green synthesis of silver nanoparticles using *Lantana camara* leaf aqueous extract **[21], [22], [23].** Nevertheless, the operating conditions (silver salt solution concentration, time reaction, volume ratio of reagents) used in this study were significantly different from those previously reported. It is quite useful to study the behavior of a process in various conditions so as to deepen the knowledge around it.

MATERIALS AND METHODS

Material

Silver nitrate was for analytical grade and was purchased from *Merck*. Leaves of *Lantana camara* and *Morinda morindoides* were collected from the surroundings of the University of Kinshasa (D.R.C). *Escherichia coli* and *Staphylococcus aureus* bacterial strains identified respectively as ATCC (American Type Culture Collection) 2592 and ATCC 25923 were obtained from the Faculty of Pharmacy at the University of Kinshasa.

Preparation of *Lantana camara* and *Morinda morindoides* leaf extracts

Lantana camara and *Morinda morindoides* aqueous leaf extracts were prepared using the same procedure described by that of Gondwal and Pant 2018 with slight modifications [9]. Fresh leaves were collected early in the morning and washed several times with tap water and, subsequently with deionized water. They were dried in shade at room temperature for 3 weeks. Dried leaves were grounded to powder. Aqueous leaf extracts were prepared by boiling 15 g of powder in 150 mL of deionized water at 95°C for 15 minutes under magnetic stirring. Extracts were cooled, filtered with *Whatman* n°1 filter papers and stored at 4°C for further use.

Green synthesis of silver nanoparticles

For the green synthesis of silver nanoparticles, the same procedure inspired by the one reported by Gondwal and Plant 2018 was used for both plants [9]. Briefly, a volume of 100 mL of $AgNO_3$ 0.005 M was prepared in deionized water then, 10 mL of leaf extract (*L. camara* or *M. morindoides*) were added under magnetic stirring at room temperature. An hour later, the stirring was stopped and the mixture was kept in shade for 24 h. The silver nanoparticles suspension was centrifugated at 5 000 rpm for 15 min and the resulted precipitate was washed with deionized water. This operation was repeated 3 times before drying the product at 60 °C for 24 h. The dried product was then kept in a glass vial for analysis. In this study, the silver nanoparticles obtained for *L. camara* and *M. morindoides* leaf extracts will be named respectively as AgNPs-Lc and AgNPs-Mm.

Characterization

UV-Visible spectroscopy was used to detect silver nanoparticles by their characteristic peak using UV-Visible spectrometer JENWAY 7315 in the wavelength range of 300-500 nm. An appropriate amount of the sample was dispersed in deionized water then sonicated for a good dispersion; the resulting suspension was used for UV-Vis characterization. A Perkin Elmer Spectrum 100 FTIR Spectrophotometer (Massachusetts, USA) was used to perform, Fourier Transform Infrared (FTIR) spectroscopy analysis. The frequency ranged

from 4000 to 650 cm and for each sample 4 scans were performed. Silver nanoparticles average size was obtained by dynamic scattering light (DLS) using a zetameter ZEN 3600 MAL1043132. After dispersing AgNPs in deionized water, ordinary and capillary cuvettes were used for particles size and Zeta Potential measurements, respectively. The experiments were carried out at 25°C with a 173° scattering angle. Whereas the morphology was studied by TEM using a Zeiss Libra-120 kV transmission electron microscope. A drop of the suspension used for DLS studies was put on a copper grid the excess of water was absorbed by a filter paper and after 24h the AgNPs was observed using a TEM. EDX were carried out to determine the product elemental chemical composition and thus, its purity using an INCA PENTA FET coupled to VAGA TESCAM scanning electron microscope. The software Origin 2019 was used to process UV-vis and FTIR.

Evaluation of activities

Antibacterial activity

Antibacterial activity of silver nanoparticles was studied against *Staphylococcus aureus* and *Escherichia coli* strains using well diffusion method. Distilled water was used as negative control. A volume of 80 µL for different concentration of silver nanoparticles (150, 100, 75, 50 and 20 µg/mL) was placed in 6 mm-diameter wells dug in Muller-Hinton medium. Preparations were incubated for 18 h at 37°C and antibacterial activity was evaluated by measuring inhibition zones with a ruler.

Catalytic activity

Catalytic activity of silver nanoparticles was investigated on the reduction of methylene blue by plant extracts as reported by Edison and Sethurama 2012 with some modifications [24]. In this reaction, methylene blue (blue color) is supposed to be reduced in leucomethylene (colorless) by phytochemicals present in plant extracts under silver nanoparticles catalysis. The reaction was monitored by UV-visible spectroscopy, following the behavior of methylene blue characteristic peak at its maximum wavelength of 664 nm.

Three mixtures were prepared for each plant to study the catalytic activity and the same procedure was used for both plants. The first mixture (Reaction 1) contained plant extract and methylene blue. The second one (Reaction 2) was made of plant extract, methylene blue and silver nanoparticles. The last mixture (Reaction 3) was formed by methylene blue and silver nanoparticles only. A 100 µg/mL silver nanoparticles suspension and a 6 ppm methylene blue solution were used for all the tests. In a 4 mL cell, different mixtures were prepared according to the details reported in Table 1:

Table 1: Composition of the 3 mixtures used in the evaluation of catalytic activity

Reaction	Volume of Ag-nps suspension	Volume of methylene blue	Volume of plant extract	Volume of deionized water
1.	0 mL	2 mL	0.25 mL	0.75 mL
2.	0.75 mL	2 mL	0.25 mL	0 mL
3.	0.75 mL	2 mL	0 mL	0.25 mL

These volumes were calculated in order to obtain concentrations of 4 ppm for methylene blue and 25 µg/mL for silver nanoparticles in the mixtures.

RESULTS AND DISCUSSION

Characterization

UV-visible spectroscopy

Synthesis of AgNPs was first suggested by the change in color of the reaction mixture which indicated the reduction of the Ag^+ into

Ag^0. As illustrated in **Figure 1**, this suggestion was reinforced by characteristic peaks of silver nanoparticles in UV-vis that appeared at maximum wavelengths of 436 nm and 440 nm for AgNPs-Lc and AgNPs-Mm respectively. These results are in agreement with those previously reported by many other studies [8], [10], [13], [22], [25].

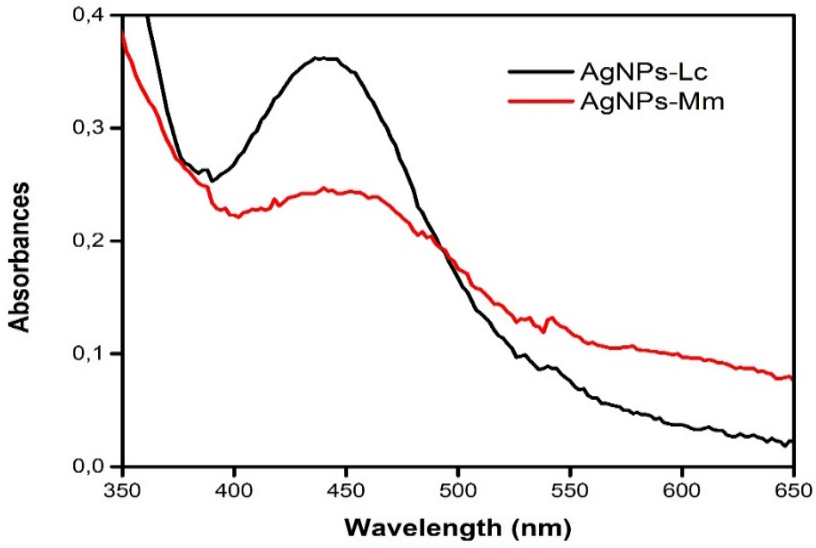

Figure 1: *UV-visible spectra of AgNPs-Lc and AgNPs-Mm*

Fourier Transform Infrared spectroscopy (FTIR)

FTIR spectroscopy analysis was performed to assess the chemical composition on the surface of synthetized silver nanoparticles. **Figure 2** present the FTIR spectra of AgNPs prepared using aqueous leaf extracts of *Lantana camara* and *Morinda Morindoides*. As depicted in the spectra mains vibration peaks of several organic function group can be noticed at different wavenumbers. The broad-band in the region of 3300–3260 cm^{-1} corresponds to -OH stretching vibration. Absorptions bands at around 2790-2925, 1590-1680 and 1025-1100 cm^{-1} may be attributed to C-H, C = O and C-O-C stretches, respectively suggesting the presence of phytochemical compounds containing alcohol and carbonyl groups that might be polyphenols. However, the nitro group (N-O) which appear around 1280 cm^{-1} was not observed in the spectra indicating that there are no noticeable traces of nitrates [26], [27].

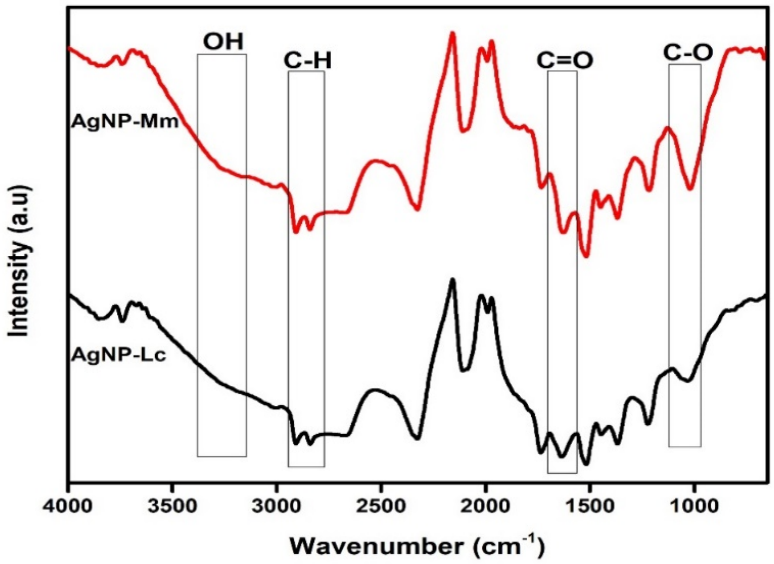

Figure 2: *FTIR spectra of green synthesized AgNPs.*

EDX

The yield of silver nanoparticles from *L. camara* leaf extract was superior to that of *M. morindoides*. EDX spectra of AgNPs-Lc and AgNPs-Mm show silver contents of 54,2 %9 and 19.80 % respectively. This may be due to a better reduction power of phytochemicals from *L. camara* leaf extract leading to the formation of more silver nanoparticles or, simply to a better recovery of AgNPs-Lc by centrifugation if these particles were bigger than those of AgNPs-Mm. Apart from silver, a significant amount of carbon, oxygen and chlorine in both samples suggesting the presence of organic matter, was found as illustrated in figures 3 and 4. These last ones came probably from phytochemicals in the leaf plant extracts that are supposed to play the role of capping and stabilizing agents for silver nanoparticles as reported in the literature [14], [28], [29], [30]. These results are in agreement with results those reported by FTIR analysis.

These phytochemicals should facilitate the dispersion of green-synthesized silver nanoparticles in water. However, AgNPs-Lc and AgNPs-Mm showed an evident “insolubility” in water and were water-dispersed only by sonication. It seems that phytochemicals from *Lantana camara* and *Morinda morindoides* leaf extracts have not been able to facilitate the dispersion of silver nanoparticles in water probably because the particles obtained were strongly bonded together into agglomeration and thus, the spontaneous dispersion of AgNPs-Lc and AgNPs-Mm became difficult.

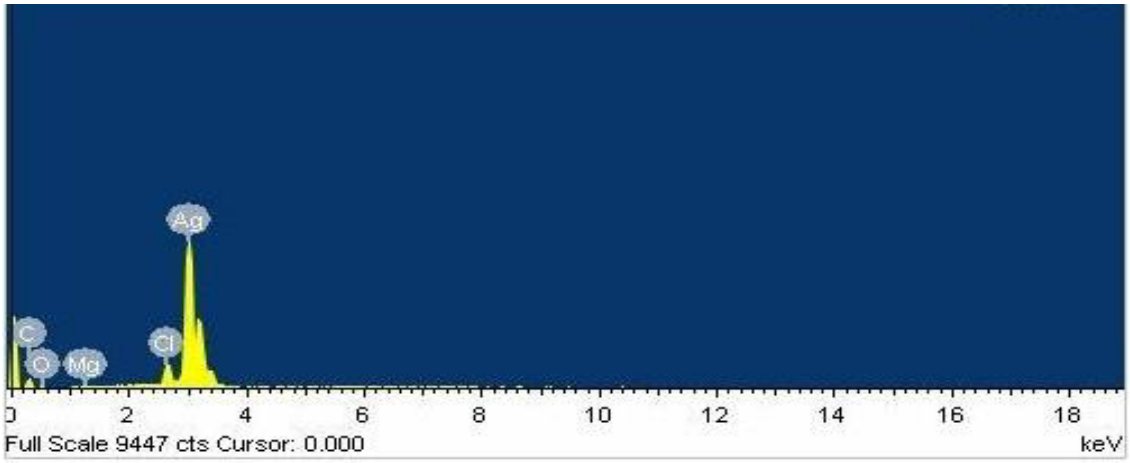

Figure 3: *EDX spectra of Ag-NPs-Lc*

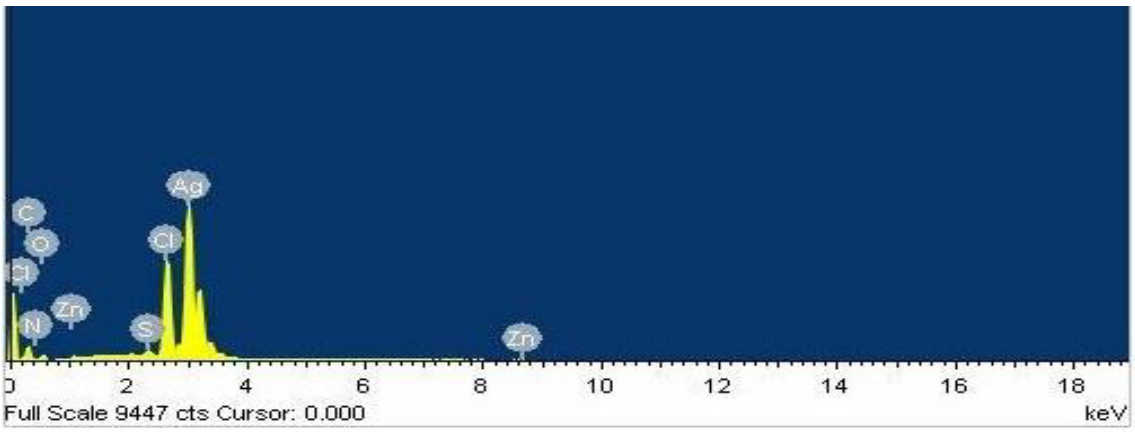

Figure 4*: EDX spectrum of Ag-NPs-Mm*

Average particle size and Zeta Potential

The DLS results summarized in **Table 2** revealed that all the prepared nanoparticles were in nanoscale but AgNPs-Lc synthesized were bigger than AgNPs-Mm. Table 2 shows the average values of size and zeta potential but also the polydispersity index of both products. All the particles exhibited negative zeta potential close to -30 mV suggesting the presence of relatively instable particles [31], [32]. The size distribution is shown in *Figure 5* (repeated 3 times for more precision) for both AgNPs-Lc and AgNs-Mm.

Table 2: *Size, Zeta Potential and PDI of the AgNPs-Lc and AgNPs-Mm*

Sample	Average size (nm)	Zeta Potential (mV)	Polydispersity index (PDI)
AgNPs-Lc	240 ± 28	- 31 ± 2	0.42
AgNPs-Mm	127 ± 1	- 32 ± 1	0.286

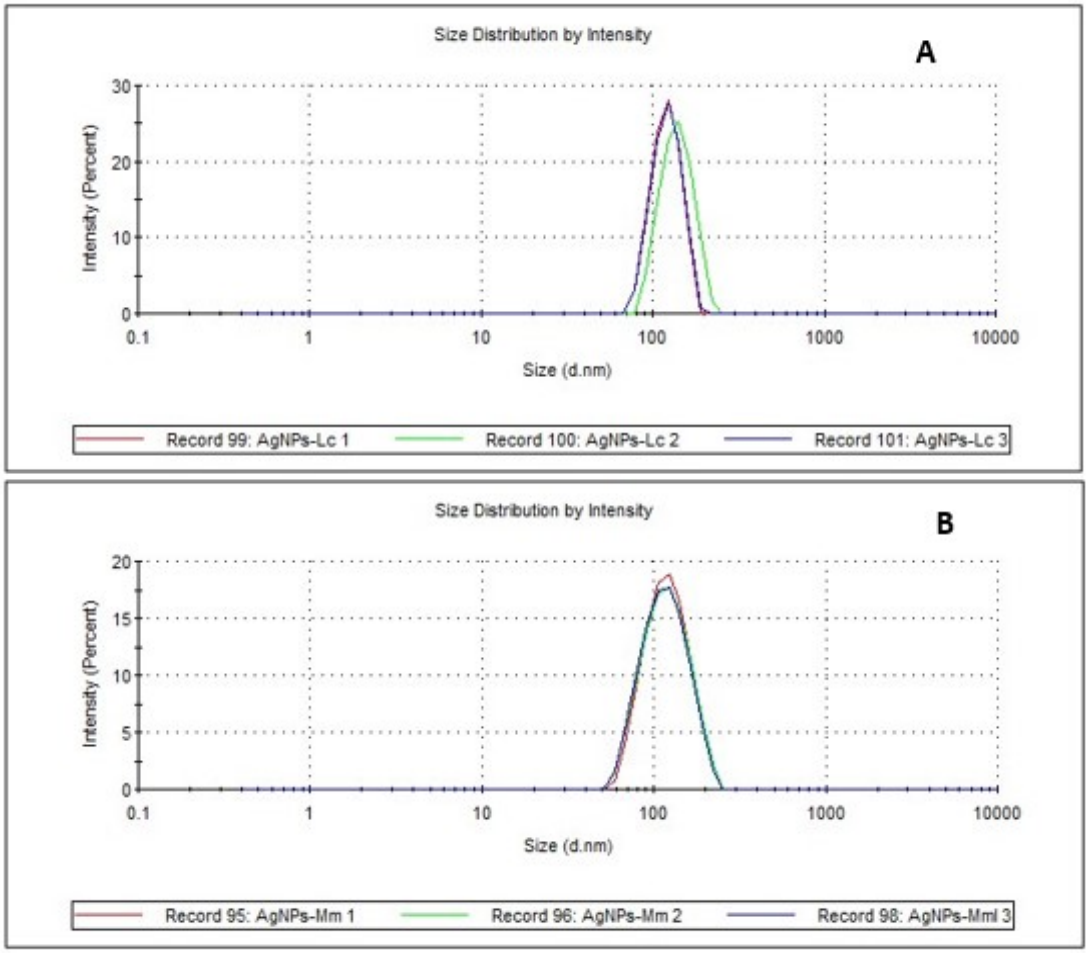

***Figure** 5: Size distribution of AgNPs synthesized by Lc (A) and Mm (B)*

TEM

TEM images for AgNPs-Lc (**Figure 6a**) show oval agglomerated nanoparticles whereas AgNPs- Mm (**Figure 6b**) were irregular in shape but, also regrouped into agglomeration. This behavior of both AgNPs-Lc and AgNPs-Mm is in agreement with the zeta potential values reported previously suggesting a significant instability and thus, a strong tendency to agglomeration. The size of silver nanoparticles suggested by DLS could not be confirmed by TEM results due to the strong agglomeration.

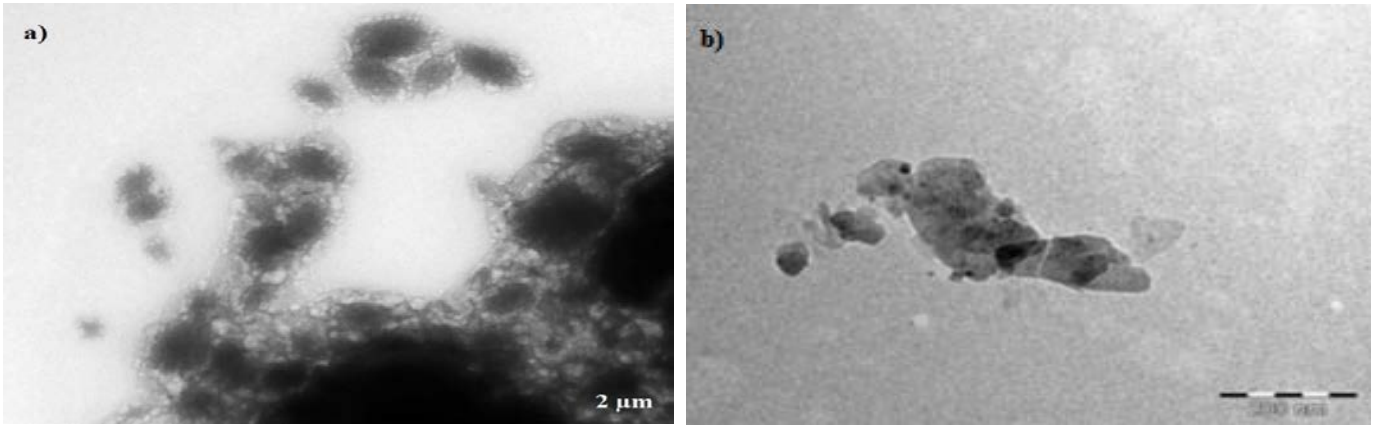

***Figure 6:** TEM of a) AgNPs-Lc and b) AgNPs-Mm*

Antibacterial activity

Both AgNPs-Lc and AgNPs-Mm presented significant antibacterial activities against *S. aureus* and *E. coli*. Inhibition zones of each sample for different concentrations are given in Table 3. Ag-Nps Mm showed a better antibacterial activity against the two tested strains than Ag-NPs Mm. This result is probably due to the lower average size of Ag-NPs Mm reported by DLS studies. Such behavior is in agreement with the hypothesis of many researchers which says that antibacterial activity of silver nanoparticles is inversely proportional to their size [14], [33], [34]. *L. camara* leaf extract showed an antibacterial activity against *S. aureus* but none for *E. coli* whereas no zone of inhibition was observed for *M. morindoides* leaf extract.

***Table 2**: Different concentrations of Ag-nps Lc and Ag-nps Mm tested for antibacterial activity.*

Samples	**AgNPs- Lc**		**AgNPs-Mm**	
Concentration en µg/mL	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*
150	12	13	20	14
100	11	12	18	13
75	10	12	18	13
50	9	12	16	12
20	-	-	12	10
Plant extract	15	-	-	-
Distilled water	-	-	-	-

Catalytic activity

The behavior of characteristic peaks of methylene blue during the evaluation of catalytic activities are shown in **figure 5**, **6** and 7 respectively for the first (mixture of plant extract and methylene blue), second (mixture of plant extract, methylene blue and silver nanoparticles) and third (mixture of methylene blue and silver nanoparticles) reactions. No significant decrease in the intensity of the characteristic maximal absorption of methylene blue was observed in the first reaction for both plant extracts. It is suggested that both *L. camara* and *M. morindoides* leaf extracts do not have the ability to reduce methylene blue into leucomethylene. Surprisingly, the same behavior was observed for the second reaction for both samples. Even in the presence of AgNPs-Lc and AgNPs-Mm that are supposed to catalyze the reduction of methylene blue in the presence of plants extracts as reduction agents as reported by Ajitha *et al.* 2015b, Edison and Sethuraman 2012 [23], [24], no decrease in the intensity was observed. This result may be attributed to the agglomerated state of AgNPs-Lc and AgNPs-Mm that has probably a negative influence on their catalytic activity.

Unexpected results were observed in the third reaction for AgNPs-Lc and AgNPs-Mm samples. The characteristic peaks of methylene blue showed significant decreases in intensity of 53,1% and 45,2 % respectively at 664 nm after 3 hours and half. It was then suggested that AgNPs and AgNPs-Mm alone have the ability of degrading methylene blue.

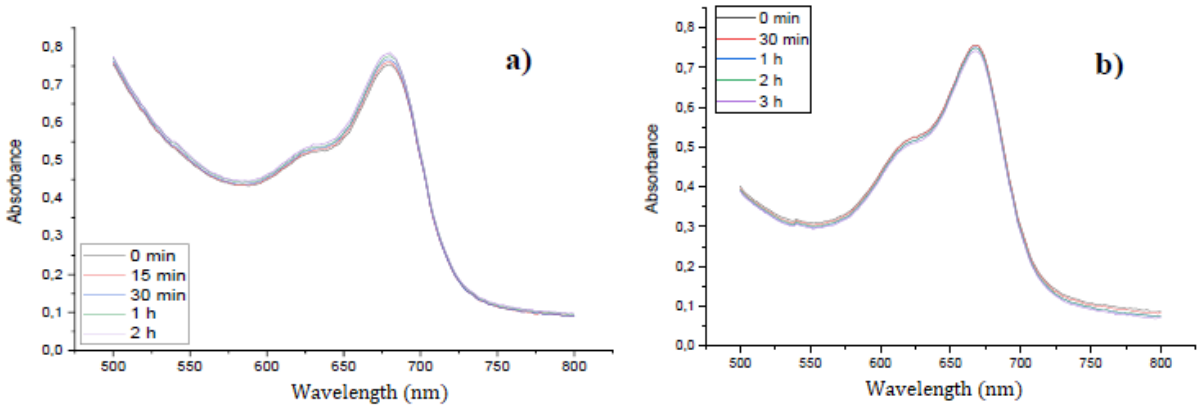

***Figure 6**: UV-visible spectra of the first reaction of catalytic activity evaluation: a) L. camara and b) M. morindoides*

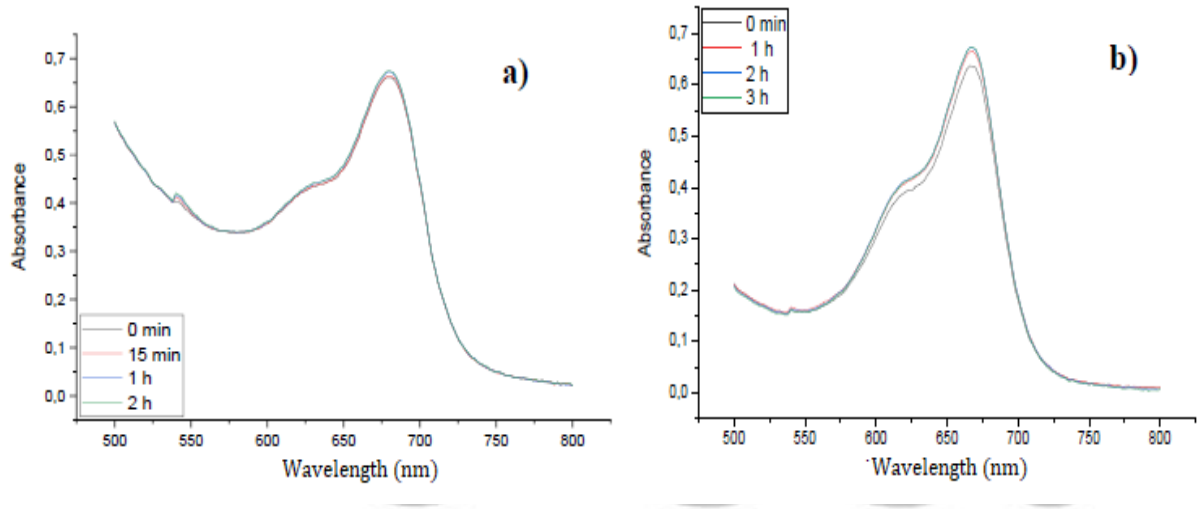

***Figure 7**: UV-visible spectra for the second reaction of catalytic activity evaluation: a) L. camara-AgNPs-LC and b) M. morindoides-AgNPs-Mm*

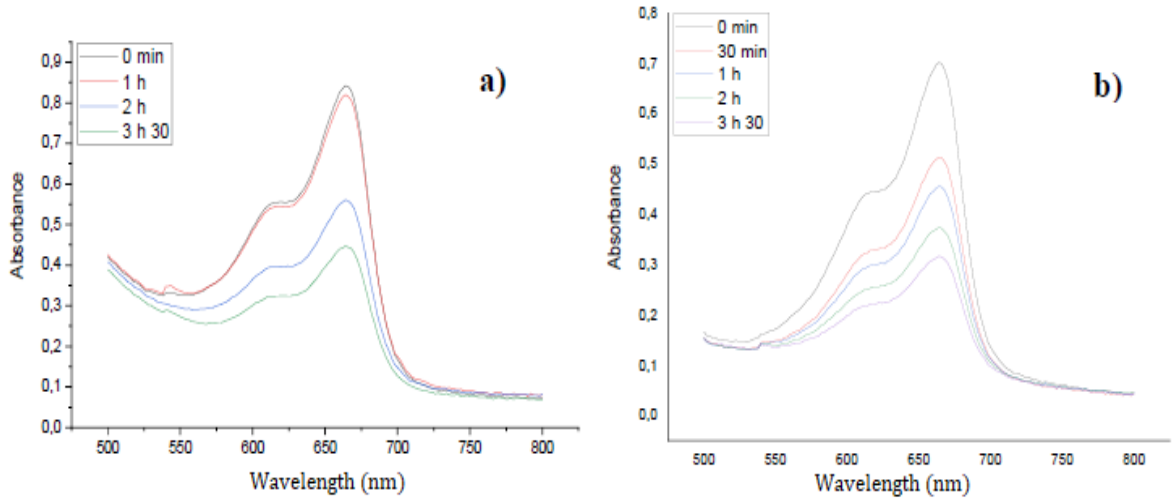

***Figure 8**: UV-visible spectra for the third reaction of catalytic activity evaluation: a) AgNPs-Lc and b) AgNPs- Mm*

CONCLUSION

In this study, a green synthesis method was used to prepare silver nanoparticles from aqueous leaf extracts of two medicinal plants widely used in D.R. Congo. Physicochemical characterization (UV-Vis, FTIR, EDS, DLS and TEM) results confirmed the formation of agglomerated nanoscale silver particles with average size of 127 and 240 nm. Both synthetized silver nanoparticles presented a significant antibacterial activity again *S. aureus* and *E. coli* strains and an interesting ability to discolor methylene blue solution in the absence of a reducing agent. Nevertheless, the operating conditions used appear not to be optimal for the green synthesis as they have led to instable agglomerate particles. Thus, further comparative studies are needed to identify optimal conditions of green synthesis of silver nanoparticles using *L. camara* and *M. morindoides* leaf extracts.

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