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Research Article

Natural Substances for the Synthesis of Silver Nanoparticles against *Escherichia coli*: The Case of *Megaphrynium macrostachyum* (Marantaceae), *Corchorus olitorus* (Tiliaceae), *Ricinodendron heudelotii* (Euphorbiaceae), *Gnetum bucholzianum* (Gnetaceae), and *Ipomoea batatas* (Convolvulaceae)

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The development of drug-resistant strains is rising and the search for new and novel ways of fighting new or reemerging microbes goes on. A hope of treating such multidrug-resistant infections came from plants mediated nanoparticles since nature is a generous source which provides a variety of chemical compounds that can be used for new drug discovery. Silver nanoparticles are reported to possess antiviral, antibacterial, antifungal, antiparasitic, larvicidal activity and anticancer properties. We reported green synthesis of silver nanoparticles mediated food plants *Megaphrynium macrostachyum*, *Corchorus olitorus*, *Ricinodendron heudelotii*, *Gnetum bucholzianum*, and *Ipomoea batatas* and their antibacterial efficacy against the Enterobacteriaceae *Escherichia coli*. The nature and size of the obtained nanoparticles are discussed as well as their Minimum Inhibitory Concentration (MIC) and the Minimum Bactericide Concentration (MBC) values considering their application in medical industry.

1. Introduction

Increasing resistance against antibiotics is a burning health problem and there is an urgent need to improve the existing drugs or find new, novel strategies to overcome this problem [1]. Nanoparticles, generally considered as particles with a size of up to 100 nm, exhibit completely new or improved properties as compared to the larger particles of the bulk material that they are made up of [2]. One of the most challenging goals in nanoparticle research is to develop successful protocols for the large-scale, simple, and (possibly) low-cost preparation of morphologically pure nanoparticles with identical properties. Additionally, nanoparticles should be easily stored and manipulated without losing their properties

Table 1: Biochemical profile of selected *Escherichia coli* strain.

Character	ODC	IND	ADH	βNAG	LDC	βGAL	URE	GLU	LARL	SAC	GAT	LAR	5KG	DARL	LIP	αGLU
Result	−	+	−	−	+	−	−	+	−	+	+	+	−	−	−	−
Character	RP	αGAL	βGLU	TRE	MAN	RHA	MAL	INO	ADO	CEL	PLE	SOR	MNT	ASPA	βGUR	αMAL
Result	+	+	−	+	+	+	+	−	−	−	−	+	−	−	+	−

ODC: ornithine decarboxylase; ADH: arginine; LDC: lysine decarboxylase; URE: urease; LARL: L-arabitol; GAT: galacturonate; 5KG: 5-ketogluconate; LIP: lipid; RP: red phenol; βGLU: beta-glucosidase; MAN: mannitol; MAL: maltose; ADO: adonitol; PLE: palatinose; βGUR: beta-glucuronidase; MNT: malonate; IND: indole; βNAG: N-acetyl-beta-glucosaminidase; βGAL: beta-galactosidase; GLU: glucose; SAC: saccharose; LARA:L-arabinose; DARL: D-arabitol; αGLU: alpha-glucosidase; αGAL: alpha-galactose; TRE: trehalose; RHA: rhamnose; INO: inositol; CEL: cellobiose; SOR: sorbitol; αMAL: alpha-maltosidase; ASPA: L-aspartic arylamidase acid.

Table 2: Susceptibility profile of selected *Escherichia coli* strain.

ATB	AMP	AMOX	TIC	PIP	CEFA	CEFO	CEFTA	ERTA
Result	R	R	R	R	R	R	R	S
ATB	IMI	AMI	GENTA	TOBRA	Ac NA	CIPRO	OFLO	NITRO
Result	S	R	R	S	R	R	R	R

AMP: ampicillin, AMOX: amoxicillin, TIC: ticarcillin, PIP: piperacillin, CEFA: cefalotin, CEFO: cefotaxime, CEFTA: ceftazidime, ERTA: ertapenem, IMI: imipenem, AMI: amikacin, GENTA: gentamicin, TOBRA: tobramycin, Ac NA: nalidixic acid, CPRO: ciprofloxacin, OFLO: ofloxacin, and NITRO: nitrofurantoin.

[3]. With the rise in antibiotic resistance in recent years and the development of fewer new antibiotics, research has begun to focus on these antibacterial nanoparticles as potential new medical tools [4, 5]. The most common methods for preparing most of these nanoparticles are wet-chemical techniques, which are generally low-cost and high-volume. However, the need for toxic solvents and the contamination from chemicals used in nanoparticle production limit their potential use in biomedical applications [6]. Therefore a "green," nontoxic way of synthesizing metallic nanoparticles is needed in order to allow them to be used in a wider range of industries. This could potentially be achieved by using biological methods [4].

Research and development in biomedicine utilizes past cited history of natural products used in ancient world [9]. The antimicrobial effect of various metals and their salts has been known for centuries [10]. The Phoenicians used silver vessels to preserve water, wine, and vinegar during their long trips, while ancient Egyptians believed that silver powder provided beneficial healing and antidisease properties [11]. Recent efforts have been focused on developing new *green chemistry* methods of silver nanoparticles synthesis with the advantage of using natural products and avoiding toxic reducing agents, organic solvents, and wasteful purifications with high cytotoxic residuals [10]. Molecules produced by living organisms such as bacteria, fungi, or plants can replace the reducing and capping agents for the production of nanoparticles.

Herbs have been an integral part of our therapeutic consideration since thousands of years but are still under investigation and have become a part of biomedical research laboratories. Several medicinal plants and their bioactive molecules has been studied and tested for their efficacy against various diseases [9]. A number of secondary metabolites like phenols, flavonoids, glycosides, alkaloids, saponins, triterpenes, and so forth produced by plants are pharmacologically active. The added advantage of using natural products therapeutically is that they are safe, economical, and with lesser side effects [1]. The plants used in this research are *Megaphrynium macrostachyum* (Marantaceae), *Corchorus olitorus* (Tiliaceae), *Ricinodendron heudelotii* (Euphorbiaceae), *Gnetum bucholzianum* (Gnetaceae), and *Ipomoea batatas* (Convolvulaceae). They have been selected due to their involvement in food supply. The synthetics optimizations and the suitability of these plants parts to produce silver nanoparticles have been described in our previous reports [7, 8, 12]. We describe herein the uses of the obtained silver nanoparticles as antimicrobial agent against *Escherichia coli*.

2. Material and Methods

2.1. Selection of Bacteria. *Escherichia coli* strains were isolated from urine sample in the bacteriology unit, of the General Hospital laboratory, Douala, Cameroon. These strains were identified by automatic colorimetric reading on VITEK 2™ (BIOMERIEUX SA, France) after plating into Eosin Methylene Blue agar and incubation at 37°C for 24 hours. Susceptibility tests were performed by dilution in cards, an automatic turbidimetric reading on VITEK 2. The biochemical profile and susceptibility are presented in Tables 1 and 2, respectively.

2.2. Plant Extracts. Fresh leaves of *Megaphrynium macrostachyum* (Benth. & Hook. f.) Milne-Redh. (Marantaceae) deposit number 10000/SRF Cam, *Corchorus olitorus* Linn (Tiliaceae) deposit number 14725/SRF Cam, *Gnetum bucholzianum* Engl. (Gnetaceae) deposit number 59887/HNC, and *Ipomoea batatas* (L.) Lam (Convolvulaceae) deposit number 26429/SRF Cam and seed kernels of *Ricinodendron heudelotii* (Baill) Pierre Pax (Euphorbiaceae) deposit number 19695/SRF Cam were procured from local market, Douala, Cameroon, and identified at the National Herbarium of Cameroon. The organic materials were surface cleaned with running tap water followed by deionized water to remove

Table 3: Silver nanoparticles dilutions (μg/mL).

Silver organic nanoparticles	C1	C2	C3	C4	C5	C6	C7
Ag-*Megaphrynium macrostachyum*	4,12	2,06	1,03	0,515	0,257	0,128	0,0643
Ag-*Corchorus olitorus*	16,5	8,25	4,125	2,06	1,031	0,515	0,257
Ag-*Gnetum bucholzianum*	54	27	13,5	6,75	3,37	1,687	0,843
Ag-*Ipomoea batatas*	21,2	10,6	5,3	2,65	1,325	0,662	0,331
Ag-*Ricinodendron heudelotii*	13,5	6,75	3,37	1,687	0,843	0,421	0,21

all the dust and unwanted visible particles. Aqueous plant extracts were prepared by boiling 10 g organic material in 200 ml deionized water for 5 min at 80°C. The extract was filtered through Whatman number 1 filter paper to remove particulate matter and get clear solutions and stored at 4°C for further use, being usable for one week due to the gradual loss of plant extract viability for prolonged storage [8].

2.3. Synthesis of Silver Nanoparticles. Silver nitrate ($AgNO_3$) was obtained from Sigma-Aldrich chemicals Germany. Deionized water was used throughout the reactions. All glass wares were washed with dilute nitric acid (HNO_3) and deionized water and then dried in hot air oven. A solution of $AgNO_3$ 10^{-3} M was prepared in deionized water. As a standard procedure, 10 mL of organic material extract was added to 50 mL of 10^{-3} M aqueous $AgNO_3$ solution, hand shaken during 1 min, and incubated at room temperature in the dark to minimize the photoactivation of silver nitrate. The reactions were made under static conditions. First hour of reaction was monitored by ultraviolet visible spectroscopy of 2.5 ml of the reaction suspension using an UV-visible Uviline 9100 spectrophotometer operating at 1 nm resolution with optical length of 10 mm. Concentrations were determined by centrifugation after 24 hours of incubation. UV-visible analysis of the reaction mixture was observed for a period of 300 s. Powder X-ray diffraction measurements were carried out using a PANalytical Empyrean Serie 2 X-ray diffractometer (Cu K-Alpha1 [Å] 1.54060, K-Alpha2 [Å] 1.54443, and K-Beta [Å] 1.39225) by preparing a thin film of the silver-organic nanopowder on silicon substrate.

2.4. Determination of the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericide Concentration (MBC). The silver nanoparticles solutions have been screened for sterility by incubation 48 hours at 37°C on Eosin Methylene Blue (EMB) agar plates. Twenty-four hours cultures of the *Escherichia coli* strains diluted in 4 ml of saline water were used to prepare a suspension of 0,5 McF according to the breakpoints of the French Society of Microbiology. This suspension was diluted to 1/10th to obtain a concentration of inoculum test for about $1{,}5 \times 10^6$ germs/mL [13]. The microdilution broth method was used to obtain the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericide Concentration (MBC) of colloidal silver nanoparticle solution. The MIC is defined as the smallest concentration of silver nanoparticles for which no growth is visible to the naked eye [14] and the MBC the smallest concentration of silver nanoparticles for which no bacterial growth was observed on Eosin Methylene Blue agar [13]. In a microplaque numbered from C1 to C7 and a control Tc, 100 μl of the inoculums test, 100 μL of Mueller Hinton broth, and, with the exception of the control tube T, concentrations of silver nanoparticles following a dilution of geometric progression of reason 1/2 as reported in Table 3 were introduced successively. The microplaque was incubated at 37°C and after 24 hours, ten μL of each dilution was then inoculated on Eosin Methylene Blue agar and incubated at the time $t_0 = 0$ hours, $t_1 = 24$ hours, and $t_2 = 48$ hours, at 37°C, at the rate of two plates per dilution [14].

The cloudiness was then assessed visually. The lowest concentration at which the cloudiness occurred was taken as the MIC value. To obtain MBC values, samples were taken from the pots showing no growth, spread onto nutrient agar plates, and incubated at 37°C for 24 hours. MBC was determined based on 3log decrease in the viable population of the pathogens.

3. Results and Discussion

3.1. Synthesis. A green chemistry approach, which protects the environment, has been used to generate the silver nanoparticles. Thus, all organic materials were cleaned with running tap water followed by deionized water to remove all the dust and unwanted visible particles. Aqueous plant extracts of dissolved reductants and capping molecules were prepared by boiling 10 g organic material in 200 ml deionized water for 5 min at 80°C. The method is generally used to synthetize silver nanoparticles and has been retained for this work [7, 8, 12]. The silver nanoparticles mediated leaves of *Megaphrynium macrostachyum*, *Corchorus olitorus*, *Gnetum bucholzianum*, and *Ipomoea batatas* and seed kernels of *Ricinodendron heudelotii* are therefore obtained in the same synthetic conditions. Hours are required for an ionic silver reduction using the seed kernels of *Ricinodendron heudelotii.* The other plant material extract reacts completely above one hour of reaction time.

3.2. Ultraviolet Visible Spectroscopy. Ultraviolet visible spectroscopy is a valuable tool to characterize nanoparticles. The technique is fundamental to ascertain the formation of nanoparticles giving indications such as formation, size growth, or shape. Generally, the formation of nanoparticles begins immediately after the contact of plant extract and $AgNO_3$ solution. The colour of the mixture extracts and silver nitrate changes from clear to yellow and dark brown due to formation of plasmons at the colloid surface, thus

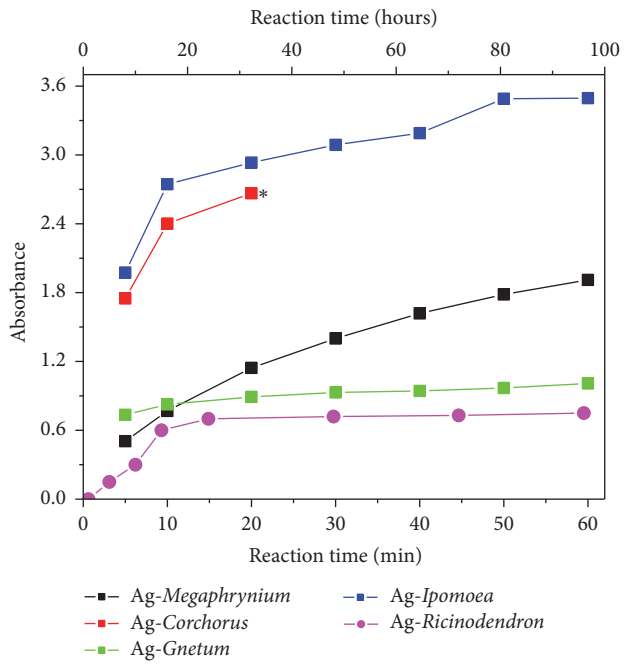


Figure 1: Evolution of the maximum of absorbance of the synthetized nanoparticles from *Megaphrynium macrostarchyum*, *Corchorus olitorus*, *Gnetum bucholzianum*, and *Ipomoea batatas* during one hour of reaction. *Ricinodendron heudelotii* mediated silver nanoparticles synthesis requires hours of contact time (magenta). *Ag-*Corchorus* silver nanoparticles absorbances are over 4 a.u. after 20 minutes of reaction.

indicating the synthesis and growth of silver nanoparticles. Based on visual observation, few seconds are necessary to reduce ionic silver to silver nanoparticles when the reaction is mediated with extracts of *Megaphrynium macrostarchyum* and *Corchorus olitorus*. Such a rapid reductions in two minutes as been observed in 2013 by Awwad et al. using a carob leaf extract [15]. The lowest reduction rate is found for the synthesis of silver nanoparticles from *Ricinodendron heudelotii* seed kernel extract where twenty-four hours is necessary for the reduction. Figure 1 presents the monitoring of the maximum absorbance of the synthetized nanoparticles from *Megaphrynium macrostachyum*, *Corchorus olitorus*, *Gnetum bucholzianum*, and *Ipomoea batatas* during the first hour of reaction. In the case of the synthesis of silver nanoparticles mediated *Corchorus olitorus* leaf extract the absorbance is above 4, the limit of the spectrometer after 20 minutes reflecting a very rapid reduction of the ionic silver. The plant extracts used are able to reduce ionic silver and to stabilize the obtained nanoparticles which have been usable over 3 months.

3.3. X-Ray Diffraction. Powder XRD, one of the most important characterization tools used in solid state chemistry, is used to prove the formation of crystalline phases and to calculate the particle size. The patterns of the prepared nanoparticles from *Ricinodendron heudelotii* seed kernels extract and *Gnetum bucholzianum* leaf extract are shown in Figures 2 and 3. Both pattern is one of generated silver

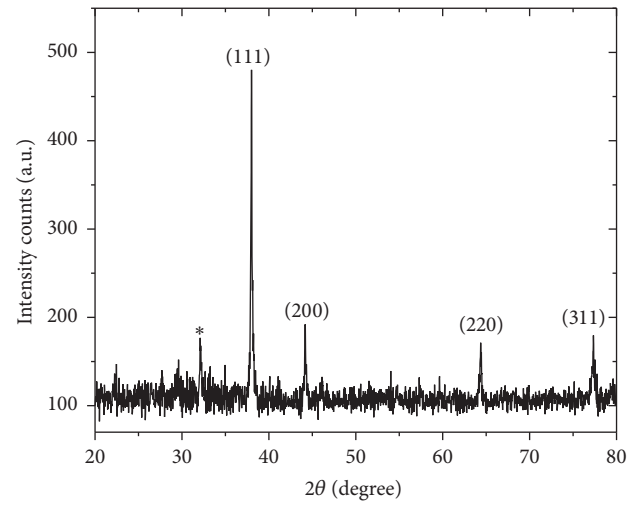


Figure 2: XRD pattern of the silver nanoparticles from *Ricinodendron heudelotii*. *Undefined silver-extract phase.

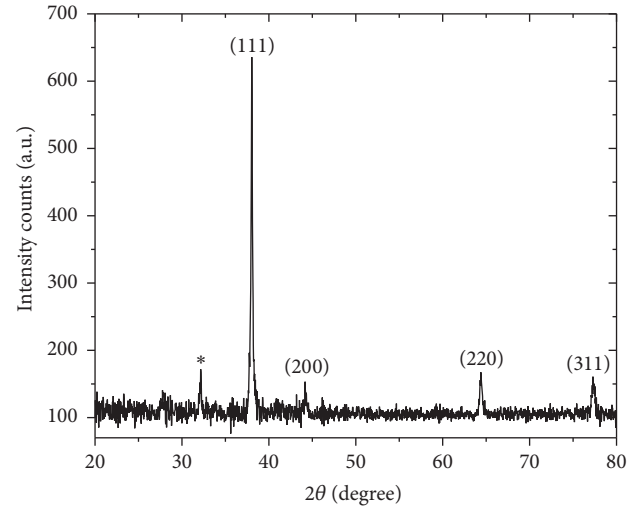


Figure 3: XRD pattern of the silver nanoparticles from *Gnetum bucholzianum*. *Undefined silver-extract phase.

nanoparticles compatible with the cubic phase of Ag with diffraction points at 2θ values of 38°, 44.1°, 64.4°, and 77.3° which can be indexed to the (111), (200), (220), and (311) planes of the face centered cubic (FCC) structure, respectively (JCPDS file: 65-2871). The average crystallite size of the synthesized NP was determined using the Debye-Scherrer equation:

$$D_v = \frac{K\lambda}{\beta \cos\theta}, \quad (1)$$

where Dv is the average crystalline size; K is a dimensionless shape factor, with a value close to unity (0.9); λ is the wavelength of Cu Kα; β is the full width at half-maximum of the diffraction peaks; and θ is Bragg's angle.

To calculate the average crystalline particle size of the synthesized nanoparticles, the most intense peaks of Ag have

been preferred [8]. The calculated average crystalline particle size of the Ag-*Ricinodendron* was found to be 89.0 nm, while 67.4 nm has been found for the Ag-*Gnetum* nanoparticles. The intense and narrow diffraction peaks revealed the crystalline nature of the synthesized nanoparticles [16].

Both patterns identification shows the formation crystals of silver. The signal at the 2θ value of 32.1 in both diffractograms could be attributed to the crystallization of bioorganic phase that occurs on the surface of the nanoparticle [17]. Silver nanoparticles pattern of *Megaphrynium macrostachyum* (Marantaceae), *Corchorus olitorus* (Tiliaceae), and *Ipomoea batatas* (Convolvulaceae) has been obtained in previous work [8, 12]. Using these leaf extracts, silver nanoparticles of the type Ag@AgCl have been obtained. The calculated average sizes are inside Table 4. Why Ag@AgCl or Ag nanoparticles are generated using the different organic materials is unclear and has not been further investigated in this report.

3.4. Microbiological Assays. The resistance of human pathogens to the commercially available antimicrobial agents and antibiotics has raised the need to explore new natural and inorganic substitutes to overcome the problem [17]. Le et al. [18] demonstrated that Ag nanoparticles get attached to the cell surface of *Escherichia coli* and then penetrated into the cell, destroyed the cell cytoplasm, and killed the organism. Le et al. [18] also found that Ag nanoparticles significantly increase the cell permeability and affect the proper transport through plasma membrane. It seems that sulphur and phosphorus containing proteins or enzymes or phosphorous moiety of DNA of bacterial system may be affected by the Ag nanoparticles which lead to the inhibition of enzyme system of the organism [19].

In this study, no bacterial growth was observed on Eosin Methylene Blue (EMB) agar plates after 48 hours of incubation of all synthesized silver nanoparticles, evidence that the suspension contained no germs before the test. Table 4 presents the results of the in vitro toxicity test of the silver-organic nanoparticles on *Escherichia coli*. The value of MIC and MBC shows that the silver nanoparticles are highly effective to annihilate the apparition of *Escherichia coli* strain. The lowest value of MIC was obtained from silver nanoparticles mediated *Megaphrynium macrostachyum* leaf extract (0.515 μg/mL) while the lowest MBC was obtained with silver nanoparticles mediated *Gnetum bucholzianum* leaf extract (1,687 μg/mL). The MIC and MBC values are identical for the silver nanoparticles obtained from *Ipomoea batatas* and *Gnetum bucholzianum*. All values are consistent with the literature on antibacterial activity of plant mediated silver nanoparticles [20, 21]. Silver chloride nanoparticles have proved efficacy as antibacterial agent [22]. Silver nanoparticles generated in this work, alone and in mixtures with silver chloride nanoparticles, are effective antibacterial agents too. The antibacterial activity exhibited by silver nanoparticles depends on $AgNO_3$ concentration. A low metal concentration enhances the antibacterial activity because smaller particles have larger surface area available for interaction and will give more bactericidal effect than the larger particles [23]. No reasonable significant efficacy differences were here observed between the sites of 30 nm and 90 nm using different silver nanoparticles mediated plants extracts. The CMI and CMB data of the synthesized nanoparticles are shown in Table 5.

Table 4: Type and powder diffraction based-size of the silver nanoparticles used.

Organic material	Type and size of particles	References
Megaphrynium macrostarchyum	Ag 33.7 nm AgCl 44.2 nm	[7]
Corchorus olitorus	Ag 30.0 nm AgCl 37.9 nm	[8]
Ipomoea batatas	Ag 67.3 nm AgCl 37.9 nm	[8]
Ricinodendron heudelotii	Ag 89.0 nm	This work
Gnetum bucholzianum	Ag 67.4 nm	This work

Table 5: CMI and CMB of the synthesized nanoparticles (μg/mL).

Nanoparticles	CMI	CMB
AgNPs from *Megaphrynium macrostachyum*	0,515	4,12
AgNPs from *Corchorus olitorus*	8,25	16,5
AgNPs from *Ipomoea batatas*	5,3	5,3
AgNPs from *Ricinodendron heudelotii*	1,68	6,75
AgNPs from *Gnetum bucholzianum*	1,687	1,687

4. Conclusion

Nanomaterials are providing solutions to many technological and environmental challenges in the areas of solar energy conversion, catalysis, medicine, and water treatment. In our study, we have investigated the synthesis of silver nanoparticles in a simple, cost-effective, and green approach using plants extracts of *Megaphrynium macrostachyum*, *Corchorus olitorus*, *Ricinodendron heudelotii*, *Gnetum bucholzianum*, and *Ipomoea batatas*. The obtained nanoparticles were composed of crystalline silver for *Ricinodendron heudelotii* and *Gnetum bucholzianum* and silver chloride and silver for the other plant extracts. Antimicrobial activity of the nanoparticles ranged from 30 nm to 80 nm against *Escherichia coli* and was evaluated by obtaining the MIC and MBC values of few μg/mL. Thus, biosynthesized silver nanoparticles can find immense application in the field of biomedical appliances and formulation of antimicrobial agents and in combination with antibiotics.

Competing Interests

The authors declare no competing interests.

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