

Facile synthesis of gold nanoparticles using *Mimusops coriacea* leaves extract

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Abstract: Gold nanoparticles have attracted great interest from the scientific community in recent years due to their wide variety of applications in various science areas. The green synthesis shows a sustainable alternative to obtain nanomaterials and aims to use biological materials to replace chemical reagents, traditionally used in these processes. In this work gold nanoparticles (AuNP) were synthesized using aqueous leaf of *Mimusops coriacea* (McAuNP). The obtained nanoparticles were characterized by different techniques, such as UV-Vis; fluorescence; FTIR; transmission electron microscopy (TEM); dynamic light scattering (DLS) and Zeta potential. Variations in the pH of the medium, concentration of the reagents and pH were applied, aiming to optimize the synthesis processes. The method to synthesize gold nanoparticles using *Mimusops coriacea* leaves extract presented high reproducibility and is in accordance with the principles of green chemistry and sustainability, presenting a low-cost and biocompatible alternative to obtain AuNPs. Antimicrobial action of McAuPs was tested using minimum inhibitory concentration test.

Keywords— Green Chemistry, Gold nanoparticles, *Mimusops*, Antimicrobial action

I. INTRODUCTION

One of the firsts applications of colloidal gold synthesized using plant extracts was attributed to the physician and theologian Paracelsus (1493-1541)[1]. At that time gold was considered the “Elixir of Life” that could cure diseases and restore youth [2]. After studying the works of Paracelsus, Michael Faraday prepared the first pure colloidal gold, in 1857, and many applications were found for his "gold activated" solution. In 1890, Robert Koch discovered that gold compounds inhibited the growth of bacteria [3].

Nowadays, gold nanoparticles (AuNPs) can be obtained by various physical, chemical or biological processes and can be synthesized in various shapes such as spheres, cubes, rods, prisms, and many others [4-6]. Each shape has different physical properties – electrical, magnetic, catalytic and optical – which can be adjusted by modifying, for example, the ratio between length and diameter of the nanoparticle. Different physical properties, in turn, enable different commercial and medical applications [7-9].

Several plants have been successfully used for the efficient and fast synthesis of AuNPs[10, 11]. The eco-friendly aqueous plant extracts act as a stabilizer and make the synthesis method more advantageous over other synthetic methods due to the low toxicity, simple synthetic route and possibility to obtain huge volumes of nanoparticles [12-15].

Kumar et al reported in 2013[16] [16]the green synthesis of AuNPs by reduction of AuCl_4 using *Mimusops elengi* (*M. elengi*) pericarp extract. Majumdar et al in 2016[17] [17]demonstrated the green synthesis of colloidal gold nanoparticles using the *M. elengi* bark extract.

Mimusops coriacea (*Mc*), (from Greek *mimo* ape, and *ops* resembling or “looks like a monkey”) is a tree originally from Madagascar and well established in the tropics[18]. The leaves are coriaceous thus the name of the plant. Fruits produce latex when they are green, present antimicrobial activity and are indicated to remove warts and mycoses. Ripe is yellow. Its pulp is white and sweet, without presenting a specific aroma. The seeds are sub-spherical drupe and dark brown and very hard. *Mimusops* are rich in polyphenolic compounds that can be utilized for the AuNPs synthesis [19].

This paper presents a facile method to synthesize gold nanoparticles using *Mimusops coriacea* leaves extract (McAuNPs). The McAuNPs formation mechanisms were formulated based in the results obtaining by UV-vis spectra, TEM and FTIR. The McAuNP antibacterial activity was studied.

II. MATERIALS AND METHODS

A. Synthesis of gold nanoparticles

Chloroauric acid ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) was purchased from Sigma-Aldrich. The leaves of *M. coriacea* were collected in Prainha Branca, located in the Environmental Preservation Area, Serra do Guararu, Guarujá-SP, latitude-23.869296 and longitude-46.137139.

The *M. coriacea* leaves were washed with deionized water, dried and chopped. 2.5 or 5.0 g of chopped leaves were mixed with 100 mL of deionized water and boiled ~for 5 min to get the extract as shown in Figure 1. The extract was filtered using filter paper.

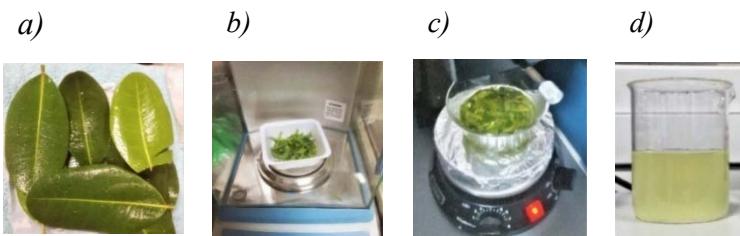


Fig. 1: Preparation of *M. coriacea* leaves extract. a) washed and dried leaves; b) weighing of the chopped leaves; c) infusion; d) filtered extract.

To prepare McAuNPs, 100 mL of the filtered Mc extract was mixed with HAuCl₄ (0.2%) aqueous solution according to Tables 1 and 2.

After the reaction, the suspensions become acidic ($\text{pH} \approx 2.5 \pm 0.3$) (Table 2). The pH was adjusted to neutral or basic.

TABLE 1. VARIATIONS IN THE SYNTHESIS CONDITIONS.

Reagents	Values
Volume of leaves in extract preparation (g)	2.5 or 5.0 (± 0.1)
HAuCl ₄ (mM)	1.0 – 2.0 (± 0.1)
pH	Acid (2.0-3.0) – Neutral (6.5-7.5) – Basic (9.5-11.5)

TABLE 2– VARIATIONS IN THE CONDITIONS APPLIED IN THE PREPARATION OF MCAUNPS.

Name	Mc leaves(g) (± 0.10)	HAuCl ₄ (mmol/L) (± 0.05)	pH (± 0.10)
McAu 1	2.5	1.0	2.90
McAu 2	2.5	1.0	2.50
McAu 3	5.0	1.0	2.71
McAu 4	5.0	1.0	2.73

B. Characterization of gold nanoparticles

McAuNPs were characterized by UV–Vis spectra with Shimadzu AVW 220D spectrophotometer. The gold particles were analyzed by high-resolution transmission electron microscopy (HR-TEM; JEOL-2010), and Fourier-transform infrared spectroscopy (FTIR; IR Prestige 21- Shimadzu). The extract and nanoparticles fluorescence spectra were obtained with a Jobin Yvon Fluorolog 3 in the range 550 -750 nm with excitation at 430 nm. The average particle size and distribution were measured using a particle analyzer (Malvern Zetasizer NanoZS).

C. Minimum inhibitory concentration analysis of nanoparticles

Staphylococcus aureus (*S. aureus*) and *Escherichia coli* (*E. coli*) were cultured in *Luria–Bertani* (LB) broth media during 24 h at 37 °C. Antimicrobial activities of the synthesized McAuNPs against *S. aureus* and *E. coli* were

analyzed by determining the minimum inhibitory concentration (MIC), using different concentrations of McAuNPs (McAu 1 pH ~ 7). McAu 1 solution was serially diluted with culture medium (LB) and distributed in 12 of the 96 wells microplates (1 - the highest concentration and 10 - the lowest concentration), according the method described by Hess et al. (2007) [20]. 10 µL of the inoculum (*E. coli* or *S. aureus*), in known concentration (10⁴ MPN/mL), was added in all the wells, except well 11. The microplates were incubated at 37 °C, in a rotational shaker (150 rpm), during 24 h. The wells corresponding to the columns 11 and 12 were adopted as positive (no antimicrobial agent was added) and negative controls (no bacteria were inoculated), respectively. Positive and negative controls were used to validate the accuracy of McAuNPs tested. The obtained results in this method are qualitative in relation to inhibition and indicate whether there has been microbial growth in each dilution of the antimicrobial agent.

III. RESULTS AND DISCUSSION

A. *McAuNPs synthesis and characterization*

In the Figure 2a, is possible to observe the aspect of McAuNP extract (2.5g of leaves in 100ml distilled water-up). After the addition of HAuCl₄ (1mM), the color of the solution changed from yellow (middle) to violet (down) after ~15 min. This color characterizes the presence of gold nanoparticles in solution.

B. Reaction time

UV-vis spectra presented in the Figure 2b shows that the reaction starts after 5 minutes, as indicated by the increase of the band at ~ 550nm due to the surface plasmon resonance (SPR) of gold nanoparticles and stabilizes at 60 minutes.

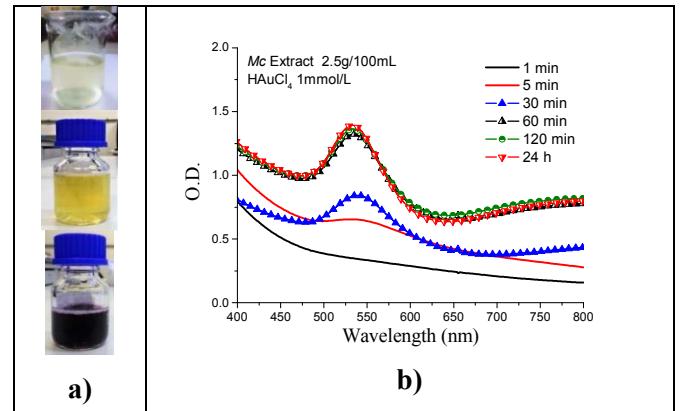


Fig. 2 – a)Change in the color of the *Mimusops coriacea* extract: Mc extract (2.5g/100mL distilled water)(up); with addition of HAuCl₄(middle); after 15 minutes (down).**b)** Changes in the McAuNPs SPR band intensity due to time of reaction (Diluted to 50%).

C. Synthesis parameters

Changes in leaf volume and HAuCl₄ concentration as shown in Table 2 resulted in changes in UV-vis spectra (measured after 60 minutes) of suspensions as shown in Figure 3a, indicating differences in particle concentration, shape and size distribution. McAu4 and McAu 2 have SPR band around 540nm, while McAu 3 around 590nm, indicating the presence of spherical AuNPs. McAu 1 shows the band around 520nm, characteristic of spherical NPs, and has a

band at 750nm, indicating the presence of triangles and prisms.

DLS analyses, summarized on Table 3 indicate greater monodispersity and presence of smaller particles when used extract with lower volume of leaves (McAu 1 and 2), a result compatible with that indicated in the UV-vis spectra and observed by Chandran et al.[13]for aqueous extract of *Aloe being*. They observed in the TEM images that that the increase in the concentration of the extract in the solution led to a reduction of the average size of nanotriangles and the formation of a higher volume of spherical nanoparticles.

A comparison between the McAu 1 and McAu 4 suspensions through TEM images (Figure 3b), indicates that McAu 1 presents planar structures with mostly triangular and some hexagonal formats, as well as spherical nanoparticles and some three-dimensional structures with diamond or some irregular forms, justifying the band in $\lambda \sim 750\text{nm}$. McAu 4 also has similar structures, but in smaller numbers.

The zeta potential value below 20mV (Table 3) indicates particles with a high tendency to aggregate.

As summarized in Table 2, after the reaction, the suspensions become acid ($\text{pH} \pm 2.5 \pm 0.3$). A pH adjustment to values between 7.0-8.0 and 9.5-12.0 was performed with the addition of NaOH in the suspensions. The results are shown in Figure 3c, d e and f. Except for McAu 3, there is a reduction in absorbance intensity around 550nm after pH adjustment. In addition, there is a decrease in the intensity of the band present at longer wavelengths. These changes in the optical properties may be related to the fast reduction of gold occurred in the acidic medium, tendency to form aggregates in the presence of Na^+ ions or changes in particle shapes under these conditions. McAuNPs in neutral pH are stable at least for 6 months.

D. Changes in properties of leaves extract in the presence of nanoparticles

The Figures 4a, 4b, and 4c show *Mc* extract and McAu1 absorption spectra (from 300-1000nm), emission spectra ($\lambda \sim 680\text{nm}$, exc. 430 nm) and FTIR (from 4000-500 cm^{-1}), respectively. The absorption spectra showed that *Mc* leaf extract absorbs strongly throughout the ultraviolet (UV) and the band around 250 and 360 nm is characteristics of phenolic compounds. The excitation of *Mc* extract around 430 nm results in a peak at ~ 685 nm which is attributed to fluorescence emission of chlorophyll (PS II) and in the region at 720-740 nm emitted by PS I. For McAu 1 this fluorescence is suppressed probably due to a quenching process[21].

The FTIR spectra of *Mc* extract present peaks around 3436 cm^{-1} , 2929 cm^{-1} , 1615 cm^{-1} , 1444 cm^{-1} and 1040 cm^{-1} . The band at 3436 cm^{-1} occurs due to the OH stretch. The band 2929 cm^{-1} occurs due to the presence of CH aldehyde and was shifted to a lower frequency (2928 cm^{-1}) in McAu1. The band 1615 cm^{-1} in the *Mc* leaf extract, due to the presence of the vibrations of the amide I was shifted to 1624 cm^{-1} in McAu1 probably due to the presence of proteins that possibly connected to gold nanoparticles through the amine groups. The band 1040 cm^{-1} due to the C-O-C stretch could be

attributed to the reduction of Au^{3+} since the band was shifted to 1037 cm^{-1} in McAu1[22].

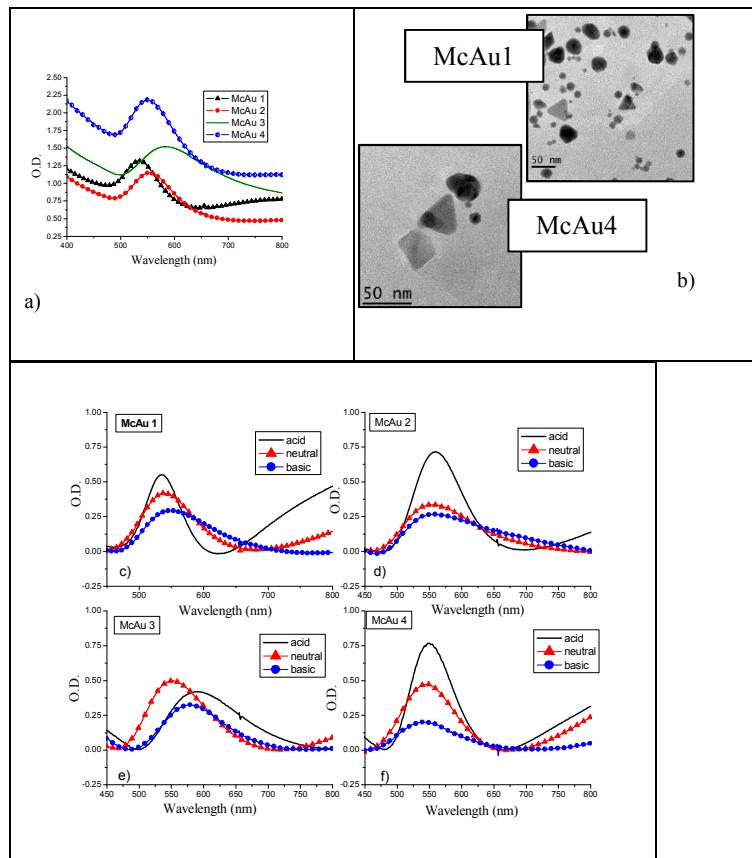


Fig. 3 – a) UV-Vis spectra of the McAuNPs extract with HAuCl_4 concentrations presented in the Table 2. b) TEM images of McAuNP McAu 1.c, d, e,f) Influence of pH in the McAuNPs.

TABLE 3 – RESULTS OF THE DLS ANALYSES OF THE MCAUNPS.

Name	Hydrodynamic diameter (nm)	Vol. %	PdI	Zeta potential (mV)
McAu 1	50.83 (± 7.92)	99.1		
	1148 (± 273.3)	0.8	0.458	-17.3
	4871 (± 972.0)	0.1		
McAu 2	53.06 (± 11.89)	13.2		
	412.1 (± 169.7)	49.2	0.352	-19.2
	1118 (± 347.7)	32.8		
McAu 3	218.7 (± 32.73)	10.5		
	3205 (± 470.1)	33.3	0.606	-12.1
	675.3 (± 104.8)	22.9		
McAu 4	1006 (± 358.2)	75.6		
	106.3 (± 24.88)	1.7	0.598	-15.2
	44.26 (± 10.68)	19.4		

E. McAuNPs antimicrobial activity

The McAu1 antimicrobial activity was evaluated using the MIC method. The antimicrobial effectiveness was determined against the final microorganism concentration of 10^4 MPN/mL and the results are presented in Table 4.

An antimicrobial activity against *S. aureus* and *E. coli* was found for McAu1 corresponded to a MIC value of 50 % of the concentration of the McAu1.

F. McAuNPs formation mechanism

This study shows the use of extract of *Mimusops coriacea* leaves for synthesis of gold nanoparticles. The synthesis process reaches a maximum after 60 min of addition of HAuCl₄ in the extract solution (Figure 2b).

TABLE 4. MIC OF MCAUNPS AGAINST S. AUREUS AND E. COLI.

	1	2	3	4	5	6	7	8	9	10	11	12
	100.00	50.00	25.00	12.50	6.25	3.13	4.56	0.78	0.39	0.20	control	- control
<i>S. aureus</i>	-	+	+	+	+	+	+	+	+	+	+	-
<i>E. coli</i>	-	+	+	+	+	+	+	+	+	+	+	-

(+) Showed growth of microorganism. (-) No growth of microorganisms. 11 positive control/12 negative control.

No reports of the synthesis of nanoparticles with aqueous extract of *Mimusops coriacea* leaves, not even a characterization on the compounds present in the extract were found in the literature. However, other species of the same genus have already been characterized and the presence of tannins, flavonoids, triterpenoids and saponins has been reported [23]. Polyphenolic compounds present in the extract (Figure 4a), can be attributed to the reduction of metal ions and particle encapsulation [24, 25].

The action of tannins and other polyphenolic compounds present in aqueous extracts of plants, in the synthesis of nanoparticles by a redox reaction is well described in the literature[26, 27]. These compounds are indicated as responsible for the reduction of metal ions and the coating of the nanoparticles avoiding their agglomeration [28-30].

A possible reduction mechanism occurs by the hydroxyl groups present in the benzene ring. This process begins with the oxidation of tannins in the presence of ions Au³⁺, forming an intermediary complex. The ions Au³⁺ are then reduced to Au⁰, that collide into clusters. The stabilization of the growth of the nanoparticles possibly occurs through their encapsulation promoted by the polyphenolic compound.

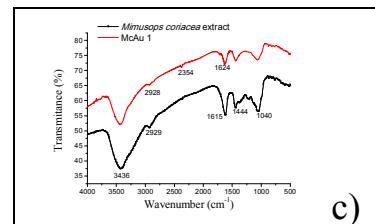
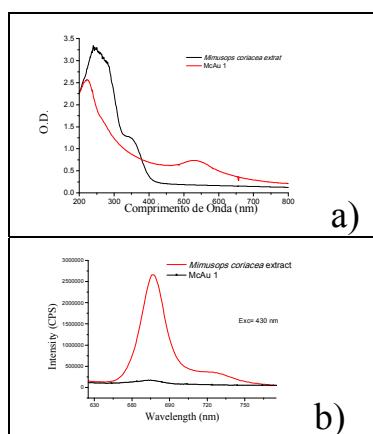


Fig. 4. a) Spectra UV-vis. Mc extract and McAu1; b) Chlorophyll and fluorescence spectra McAuNP; c) FTIR spectra of the McAu1..

The results have shown that the variations in the synthesis conditions directly influence the particle shape. As observed in the Figures 2 and 3 the increase in the concentration of the extract in the solution led to a reduction of the absorption band in the infrared, favoring the majority formation of spheres

Changes in the solution pH also causes important changes in shapes and stability of AuNPs. This fact is related to the speciation of gold in aqueous solution. When HAuCl₄ is added in the plant extract, different species of gold as [AuCl(OH)₃]⁻, [Au(OH)₄]⁻, [AuCl₄]⁻ and [AuCl₃(OH)]⁻ are present. When [AuCl(OH)₃]⁻ and [Au(OH)₄]⁻ dominate speciation, the nucleation process occurs slowly and the growth of AuNPs leads to the formation of larger colloids of gold, that is the case observed for McAu 1 in pH 2.9 (Figure 3c). The control of gold species can be done by changing the solution pH. Neutral pH favors [AuCl₄]⁻ and [AuCl₃(OH)]⁻ speciation[31] and nucleation process proceeds rapidly facilitating the formation of small and well dispersed AuNPs.

IV. CONCLUSIONS

The synthesis of gold nanoparticles using HAuCl₄ as a metallic precursor and aqueous extract from *Mimusops coriacea* leaves, was possible by a redox reaction, where the polyphenolic compounds present in the extract of the leaves of *Mimusops coriacea*, oxide and reduce the metal ions to neutral atoms, which agglomerate forming nanoparticles. These molecules are also responsible for their encapsulation, stabilizing growth. The procedures used were simple, fast, low cost and high yield. The McAuNPs showed antibacterial activities against *S. aureus* and *E. coli*. Many applications in various fields of biotechnology deserve to be tested.

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