Biosynthesis and characterization of gold nanoparticles using extracts of tamarindus indica L leaves

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Abstract. This study reports the biosynthesis of gold nanoparticles using an extract of Tamarindus indica L. leaves. Phenols, ketones and carboxyls were present in the leaves of T. indica. These organic compounds that allowed the synthesis of nanoparticles were identified by gas chromatography coupled to mass spectrometry (GC/MS) and High Pressure Liquid Chromatographic (HPLC). Synthesis of gold nanoparticles was performed with the extract of T. indica leaves and an Au³⁺ aqueous solutions (HAuCl₄) at room temperature with one hour of reaction time. Characterization of gold nanoparticles was performed by UV visible spectroscopy, scanning electron microscopy (SEM) and EDX. The results indicated the formation of gold nanoparticles with a wavelength of 576nm and an average size of 52±5nm. The EDX technique confirmed the presence of gold nanoparticles with 12.88% in solution.

1. Introduction
A wide variety of physical and chemical procedures have been developed for the synthesis of nanoparticles with different compositions, sizes and shapes. However, physicochemical techniques for nanoparticles production like photochemical reduction, ablation laser, electrochemistry, aerosol technologies and ultrasound field, are highly expensive and require the use of toxic substances such as sodium borohydride, hydroxylamine, tetrakis (hydroxymetil) phosphonium chloride (THPC), and N, N-dimethylformaldehyde [1].

Synthesis through biological methods results attractive due to the environmentally friendly nature of the process. These biological procedures have been developed from several routes involving the use of metal salts precursors along with microorganisms, plants, fruits tissues and marine algae [2,3]. Such methodologies avoid the generation of negative impacts in the form of dangerous wastes, unlike mechanical, chemical and physical procedures [4-6].

Large amounts of agricultural residues including bagasse, leaves of trees and husks that are generated in monoculture regions of Colombia, could be used for the generation of nanoparticles through biological methods. This research was focused on the synthesis and characterization of gold nanoparticles from extracts of Tamarindus indica L. leaves as a biological method [7]. The main purpose of this study was to identify the compounds that allow the formation of gold nanoparticles. In order to do that, Ultraviolet-visible spectroscopy (UV-vis), Fourier Transform Infrared Spectroscopy (FTIR), Gas Chromatographic/Mass spectrometry (GC/MS) and High-Performance Liquid Chromatography (HPLC) techniques were implemented. Nanomaterials characterization was performed by Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDX).
2. Methodology
Leaves of Tamarindus indica L. were obtained from the region of Arroyo Grande (Bolivar – Colombia). Biosynthesis of gold nanoparticles required the preparation of aqueous extracts from this raw material. To this end, infusion technique was performed by adding 1L of water to 100g of the plant, then heating the mixture to 90°C, subsequently filtering it and finally concentrating the obtained liquid until reaching a volume of 100mL. The extracts were then diluted in order to perform the UV-vis analysis in such a way that there was no interference with the wavelength of the gold nanoparticles. Consecution of functional groups belonging to reducing organic compounds (flavonoids, terpenes, tannins) was achieved by heating the extract samples to 80°C under constant agitation at 200rpm and concentrating them until 10mL of the extract were obtained. These samples were analysed by FTIR in an 8201 Shimadzu spectrophotometer.

A liquid-liquid extraction with HPLC grade ethanol was carried out on the organics extracts with the purpose of establishing the presence of organic compounds that act as bioreducing agents. The organic phase-extracts were concentrated in a Stuart Vertical Condensor rotary evaporator at 60°C for 15 minutes. The extracts were analysed by GC/MS and HPLC for the identification of flavonoids and terpenes. This was achieved by using an AT6890 Series Plus Agilent Technologies gas chromatograph coupled to a AT MSD 5975 Inert XL selective mass detector operating in radiofrequency full scan mode with a DB-5MS (J&W Scientific) 60mx0.25mmx0.25µm column with 5% phenyl-poly (dimethylsiloxane). The HPLC analysis was realized by Agilent Technologies 1200 Series (Palo Alto, California, USA), with a diode array detector (DAD) at λ=254nm using a DB-5MS (J&W Scientific) 60mx0.25mmx0.25µm column with 5% phenyl-poly (dimethylsiloxane).

Biosynthesis of the gold nanoparticles was carried out at room temperature on the basis of G.M. Nazeruddin methodology with some modifications [11]. It consisted of a simple, relatively fast procedure where 10mL of the chloroaauric acid (HAuCl₄) precursor salt with a concentration of 0.27mM were mixed to sodium hydroxide (NaOH) at a concentration of 2M. After that, 1mL of the vegetal extract was added to the mixture, which proceeded to experience agitation for one hour and centrifugation at 6000rpm for 10 minutes. The settled material (gold nanoparticles) was washed with distilled water and submitted to an additional centrifugation. The solid material was extracted with 2mL of distilled water in order to generate a homogeneous solution of gold nanoparticles. A drop of NaOH 2M was added to the final solution, so that a pH of 10 was achieved, along with higher stability for the nanoparticles.

Formation of gold nanoparticles was verified by diluting the synthesized extracts of each biomass and using UV-Vis at a wavelength close to 550nm. Size and distribution of the nanoparticles were measured by SEM with a Quanta FEG 650 microscope (FEI, Netherlands). EDX analysis was performed using Apolo X equipment in high vacuum mode with a backscattered electron detector for secondary electrons with a resolution of 126.1eV (EDAX Inc, N.Y., USA). This allowed the identification of the elemental composition of T. indica extracts.

3. Results
3.1. Reducing agents identified by FTIR, GC/MS and HPLC
Functional groups in the leaves extracts of T. indica were determined by FTIR. Figure 1 shows the results of this analysis. There, it can be observed that a wide variety of functional groups were present in the extracts, including carbonyl compounds (1716.54cm⁻¹), aromatic rings (1559.37cm⁻¹), nitro compounds (1540.32cm⁻¹), alkanes (1394.81cm⁻¹), alkenes (1650.19cm⁻¹), amines (1254.59cm⁻¹), alcohols (3307.91, 1126.74, 1072.36cm⁻¹), phosphates (1072.36cm⁻¹) and alkyl halides (557.22cm⁻¹) X. These groups are commonly found as part of promoter agents for the bioreduction of gold nanoparticles, such as –OH and –COOH.
Figure 1. (a) Infrared spectrum of the obtained Tamarindus indica L leaves extract. (b) Chromatogram of the samples of Tamarindus indica L leaves obtained by HPLC.

Figure 1(b) presents the identification of bioreducing compounds for the leaves extracts of Tamarindus indica L by GC/MS and HPLC/DAD. Organic compounds with aromatic rings such as phenols were identified in the T. indica sample. These included benzyl alcohol, o-guaiacol, 2,3-dihydrobenzofuran, p-vinilguaiacol and diethyl phthalate. Phenolic compounds act as bioreducing agents for nanoparticles by wrapping them and providing an excellent robustness in order to avoid agglomeration [7]. For the T. indica leaves extracts more compounds were detected using HPLC than the GC/MS.

3.2. Biosynthesis of gold nanoparticles
The synthesis of gold nanoparticles (AuNPs) was suggested by a pink coloration, and UV-Vis analysis confirmed the presence of this material at a wavelength of 576nm. Figure 2 shows the UV-Vis spectra that were obtained in the sample of original Tamarindus indica L. leaves extracts and the one with nanoparticles [7-9].

Figure 2. UV-Vis absorption spectra for 1mL of the original T. indica L. leaves extract and for nanoparticles that were synthesized with this extract.

3.3. Electron scanning microscopy (SEM)
The obtained nanoparticles exhibited an average size of 52±5nm according to SEM. An optimum size distribution was observed in the HAuCl₄ sample with an initial concentration as low as 0.27mM. Figure 3 shows the micrograph that was obtained by SEM, along with the analysis by EDX.
These results show the achievement of uniform, spherical sizes, with some agglomeration sectors. EDX confirmed the presence of gold nanoparticles, which accounted for a 12.88% of the weight in the analysed sample. This recognition was made because of the registered energy, which is characteristic of gold nanoparticles.

4. Conclusion
Tamarindus indica L leaves extract is an efficient raw material for the synthesis of spherical gold nanoparticles with an average size of 52nm. FTIR analysis allowed the identification of carbonyl groups that can intervene in the reduction process and help stabilize the generation of nanoparticles. GC/MS and HPLC results confirmed the presence of these nanoparticles-producing compounds. UV-Vis, SEM and EDX techniques made it possible to identify the gold nanoparticles that were synthesized by biological methods. The resulting biomass from agricultural cultivation can be used to synthesize this material. Applications of gold nanoparticles continue to increase, and many of them play a vital in human and environmental health regarding mercury control and quality of water and air.

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