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Synthesis, optimization and effect of condition reactions studies of seed kernel aqueous extract mediated silver nanoparticles from *Ricinodendron heudelotii* (Baill) Pierre Pax

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Abstract

Plant nanomaterial research becomes a main focus for developers working on biotechnological or biological fields. The use of seeds for the synthesis of nanoparticles is a simple route towards eco-friendly and sustainable nanotechnology area. Present work describes the synthesis, optimization and effect of condition reactions such as temperature, pH, incubation time and reactants quantities using seed kernels of *Ricinodendron heudelotii* (Baill) Pierre Pax. Ultraviolet-spectroscopy studies confirmed that the synthesis speed increases by affecting these factors. The silver nanoparticles exhibit an absorption peak around 415-442 nm.

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Introduction

Nanotechnology is concerned with the synthesis of nanoparticles of variable sizes, shapes and chemical composition and their use for human benefits (Kaur *et al.*, 2013). Nanoparticles are based on small well defined aggregates of the Noble metals in the zero valent state (Tauran *et al.*, 2013). Numerous techniques have been developed to synthesize Noble metal nanoparticles, including both chemical methods (*e.g.*, chemical reduction, photochemical reduction, coprecipitation, thermal decomposition, hydrolysis, *etc.*) and physical methods (*e.g.,* vapor deposition, laser ablation, grinding, *etc.*) The ultimate goal is to obtain nanoparticles with a high level of homogeneity and provide fine control over size, shape and surface properties (Feldheim and Foss, 2001). These methods suffer from various limitations such as low yield, cost ineffectiveness, toxicity and instability (Huang *et al.*, 2007; Sharma *et al.*, 2009).

Recently, plant mediated nanomaterial has drawn more attention due to its vast application in various fields due to their physic-chemical properties. The different metallic nanoparticles such as gold, silver, platinum, zinc, copper, titanium oxide, magnetite and nickel were synthesized from natural resources and have been studied exclusively. Silver nanoparticles have antibacterial activities. The AgNPs were effectively disrupting the polymer subunits of cell membrane in pathogenic organisms. The reciprocal action of nanoparticles subsequently breaks the cell membrane and disturbs the protein synthesis mechanism in the bacterial system (Sondi and Salopek-Sondi, 2004).

The different parts of plant such as stem, root, fruit, seed, callus, peel, leaves and flower are used to syntheses of metallic nanoparticles in various shapes and sizes by biological approaches (Kuppusamy *et al.*, 2015). Seed mediated biosynthesis of nanoparticles examples have been reported for the fenugreek (Mittal *et al.*, 2013), star anise *Illicium verum* (Luna *et al.*, 2015), *Foeniculum vulgare* (Showmya *et al.*, 2012), *Vigna radiata* (Choudhari *et al.*, 2015), *Mimusops elengi* (Kumar *et al.*, 2014), Olive (Khadri *et al.*, 2013) or *Tamarindus indica* (Edison *et al.*, 2015).

Ricinodendron heudelotii (Euphorbiaceae) is a fast-growing late secondary forest tree found in the Guinean Congolean humid forest of West and Central Africa (Plenderleith, 2006; Noumi, 1984). It is valued for its distinctively-flavoured seeds, most commonly called "*ndjanssang*", which are dried and ground and used as a flavouring and thickning agent in food (Plenderleith, 2006; Tchiegang, 1997; Mosso,1998). *Ricinodendron heudelotii* tree reach up to 50 m in height and 2.7 m in girth, or a diameter of 150 cm (Plenderleith, 2006). The fruit is an indehiscent drupe weighing about 20 g., flattened at the apex and the base, yellow-green in maturity, turning black, with 2 or 3 lobes, a fleshy exocarp and a woody endocarp containing 2-3 seeds (Ambe, 1997; Vivien and Faure, 1996). The seeds kernels are white (Dalziel, 1948) and soft, and cannot be freed easily from the shells. They are a food of interest for underdeveloped populations due to high levels of fats, 49.25-63.5% (more than cotton or soybeans), and proteins 49.89-65.16 % (Tiki *et al.*, 2000 ; Fondoun *et al.*, 2000 ; Tchiegang et Mezazjoug Kenfack, 2003). The fatty acids consist mainly of a high level of linoleic acid, a polyunsaturated fatty acid (60.32%), oleic acid, a monounsaturated fatty acid (14.66%), and two saturated fatty acids, stearic acid (12.95%) and palmitic acid (12.08%) (Tane, 1997; Kapseu *et al.*, 1998). In ndjanssang flour the percentage of essential amino acids in total amino acids is 35.5%, which is higher than the normal value for a well-balanced protein feed (Tchiegang *et al.*, 1998). To our knowledge, *Ricinodendron heudelotii* seeds kernel have not been used for the green silver synthesis of nanoparticles.

The present study deals with the biosynthesis of silver nanoparticles, using *Ricinodendron heudelotii* seeds kernels. The impact of reacting conditions such as pH, incubation time or temperature is demonstrated.

Materials and methods

Materials

Silver nitrate ($AgNO_3$) was obtained from Sigma-Aldrich chemicals Germany, H_2SO_4 98% from Merck KGaA Darmstadt Germany and NaOH from R. P. Normapur Prolabo Paris and used as received. De-ionized water was used throughout the reactions. Dry seed kernels of *Ricinodendron heudelotii* (Baill) Pierre Pax (figure 1) were procured from local market, Douala, Cameroon, and identified at the national herbarium of Cameroon by TADJOUTEU Fulberg under number of deposit 19695/SRF Cam. All glass wares were washed with dilute nitric acid (HNO_3) and de-ionized water, and then dried in hot air oven. Solutions of $AgNO_3$ 10^{-3} M, 10^{-2} M and 10^{-1} M were prepared in de-ionized water.

Instrumentation

The formation of Ag-nanoparticles was observed by measuring the UV-Vis spectrum of 2.5 mL of the reaction suspension at different time intervals. If absorbance higher than 4.5 u.a., the sample were dissolve by a factor of ½ with distilled water. An UV-visible Uviline 9100 spectrophotometer operated at with 1 nm resolution with optical length of 10 mm. UV-visible analysis of the reaction mixture was observed for a period of 300s. The UV-Vis spectrum, known as the surface Plasmon absorption band, is produced by the movement of the conduction electrons on the particles as a consequence of the incident electric field light, which results in a displacement of the negative and positive charges in the metal (Slistan-Grijalva *et al.*, 2005).

Aqueous extract preparation

Ricinodendron heudelotii seed kernels were surface cleaned with running tap water followed by de-ionisided water to remove all the dust and unwanted visible particles. Aqueous extract of *Ricinodendron heudelotii* was prepared by boiling 10g of grinded *Ricinodendron heudelotii* kernels in 100 mL de-ionized water for 5 min at 80 °C. The extract was centrifugated 10 minutes at 3500 rpm to remove particulate matter, get clear solutions and stored at -4°C for further use (1 week).

Biosynthesis of silver nanoparticles

The different experiments where done for three extract quantities named (5 mL, 10 mL and 15 mL), using 50 mL of different concentrations of $AgNO_3$ (10^{-3} M, 10^{-2} M, 10^{-1} M) at room temperature and pressure. The resulting solutions were 1 min hand shaken and incubate in the dark. The color of the solutions changed from initial white (which is the color of the *Ricinodendron heudelotii* extract) to yellowish brown after 24 h of incubation. In addition of room temperature (30 °C) the study was done at 50 and 80 °C to investigate the effect of temperature following the sample of 10 mL extract and 10^{-3} M $AgNO_3$ during 30 minutes. Different pH values 2, 4, 6, 8, 10 and 12 were chosen for investigation of pH effect in speed of silver nanoparticles formation. The pH of the solutions was adjusted using 0.1 N H_2SO_4 and 0.1 N NaOH solutions.

Visual observation

The formation of silver nanoparticles was preliminarily well known by changing of yellow to brown while adding leaf extract with silver ion solution due to the excitation of free electrons in the nanoparticles (Safaepour *et al.*, 2009). Using the extract of Ricinodendron heudelotii kernels, the colour change is from white to yellow brown (see figure 2) and the formation was occurs in 24 hours at room temperature (30°C).

Results and discussion

UV-visible Spectroscopic Ag-nanoparticles studies

Nanoparticle formation and incubation contact time

The absorption spectra of the synthesized silver nanoparticles were recorded against water in order to monitor the formation and stability of silver nanoparticles.

Fig. 1. Seed kernels of Ricinodendron heudelotii, each kernel measures above 1 cm diameter.

Fig. 2. Silver nitrate, *Ricinodendron heudelotii* seed kernel extract, Ag nanoparticles solution.

The color change of the mixture solution kernel extract and silver ion is first recorded through visual observation. Colors of silver nitrate, *Ricinodendron heudelotii* seed kernel extract and silver nanoparticles solution is shown on figure 2. The evolution of the surface plasmon absorbance bands during the synthesis of silver nanoparticles using 5 mL, 10 mL and 15 mL of kernel extract, and 50 mL of different concentrations of $AgNO_3$ (10^{-3} M, 10^{-2} M, 10^{-1} M) is shown on the chart on figure 3. The visible spectra have been recorded at 24 hours and 96 hours of incubation time in the dark to eliminate silver ions photoactivation.

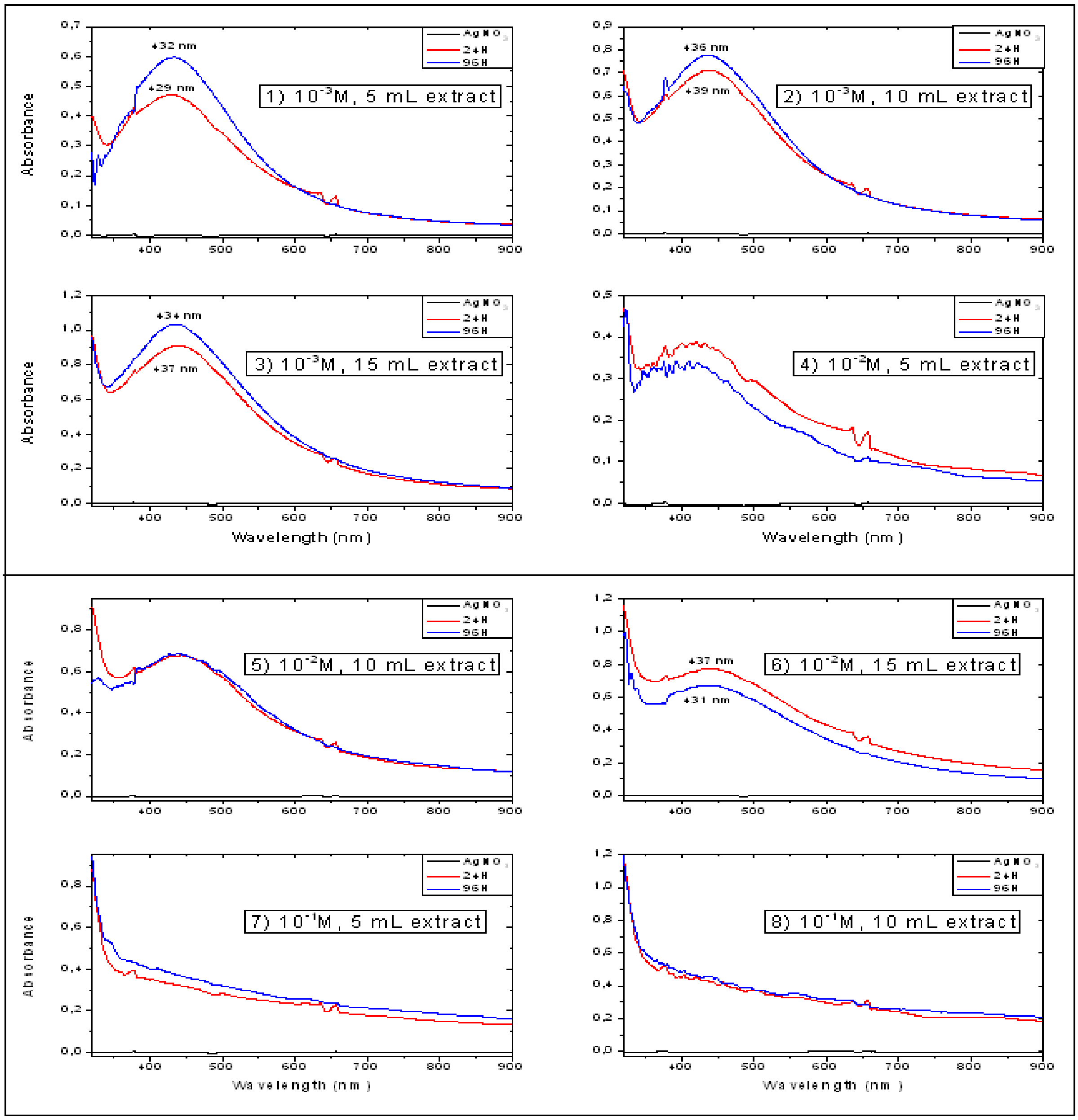

Fig. 3. UV-Vis spectra chart of different quantities of extract and of silver nitrate in function of time.

The solution color is changing within 24 hours to yellow brown due to vibration of plasmons at the colloid surface indicating the formation of silver nanoparticles. In previous studies, 6 hours of incubation time, have been observed for olive seeds (Khadri *et al.*, 2013), 3 hours for *Foeniculum vulgare* seeds (Showmya *et al.*, 2012) and Vigna radiate seeds (Choudhary *et al.*, 2015).

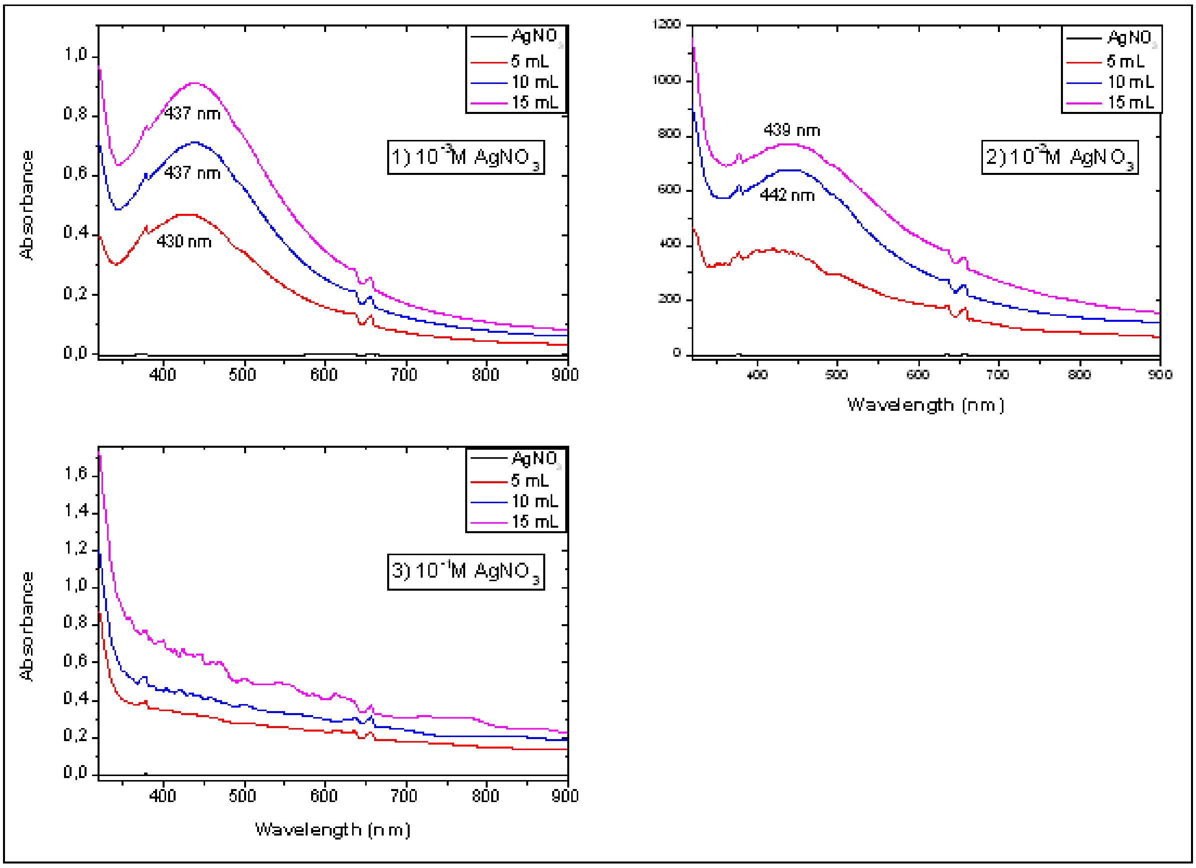

Fig. 4. UV-Vis spectra of the variation of extract quantities 5, 10, 15 mL.

At the 96 hour slight band increase is observed indicating continuous reduction of silver ions and concentration of silver nanoparticles. At 10^{-1} M and 10^{-2} M concentrations of the silver ions with the different *Ricinodendron heudelotii* extract concentrations the nanoparticles are generally aggregate because of the deficiency of molecule of leaf extract to act as protecting agents. The barrier potential developed as a result of the competition between weak Van der Waals forces of attraction and electrostatic repulsion is broken (Prathna *et al.*, 2011). The seed Kernel extract from *Ricinodendron heudelotii* act as reductant as well as capping agent, therefore mediate the synthesis as well as stabilization of the silver nanoparticles. It can be seen that the absorbance band maxima of Ag-nanoparticles using *Ricinodendron heudelotii* is in the range 429-442 nm due to surface plasmon resonance (Mulvaney, 1996) of Ag-nanoparticles. After 24 hour incubation, the absorbance increases as the concentration of the *Ricinodendron heudelotii* kernel extract increases (see figure 4).

Effect of temperature

Temperature is one of the important physical parameter for synthesis of nanoparticles. Synthesis of nanoparticles generally increases while increasing the reaction temperature (Bashir *et al.*, 2013). Figure 5 shows UV-visible spectra of the Ag-nanoparticles prepared at 30, 50 and 80 degree Celsius during 30 minutes. It can be seen that the absorbance increases with increasing temperature. This experiment suggests that the rate of nanoparticle synthesis using *Ricinodendron heudelotii* kernels at room temperature can be speed-up by increasing temperature of the reaction mixture. The Plasmon resonance bands slightly reduced (415-419 nm) due to the formation of smaller silver nanoparticles (see figure 5). Thus, it can be established that higher temperature is the optimal reacting condition for the *Ricinodendron heudelotii* kernel extract Ag nanoparticles synthesis.

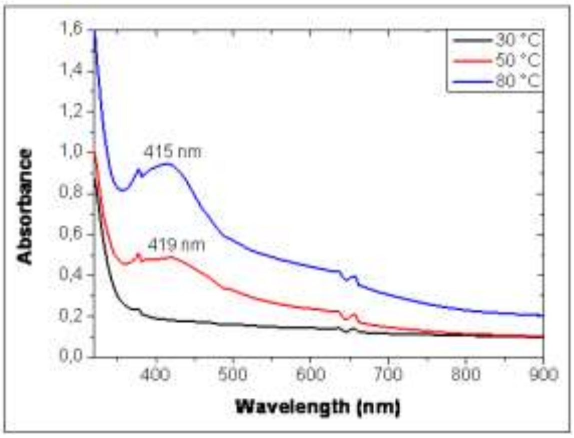

Fig. 5. UV-Vis spectra after 30 minutes of temperature variation from 30 to 80°C during silver nanosynthesis.

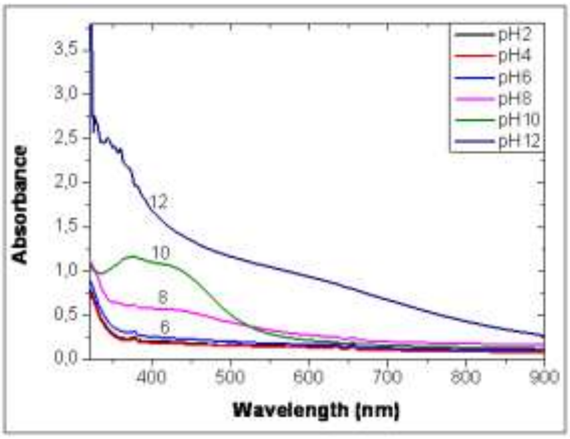

Fig. 6. UV-Vis spectra after one hour of reaction of the variation of pH.

Effect of pH

PH plays an important role in the nanoparticles synthesis, this factor induces the reactivity of extract with silver ions (Vanaja *et al.*, 2013). The UV-Visible following the pH during the formation of silver nanoparticles from pH 2 to 12 is shown on figure 6. The variation of color is on figure 7. It can be seen that Plasmon absorbance bands increases with increasing pH from 2 to 12, which can be due to the increase in production of colloidal silver nanoparticles and reduction rate. Furthermore, it is observed that the yellow-brown color of the nanoparticles appeared shortly after mixing the $AgNO_3$ with the extract at pH 8 to 12. At pH 10, the sharp surface Plasmon resonance band indicates that a monodisperse suspension occurs and a broad band at pH 12 indicating a polydispersion. In previous studies, no absorbance have been obtained at acid pH, and aggregation at higher pH for *Calendula officinalis* seed extract (Baghizadeh *et al.*, 2015).

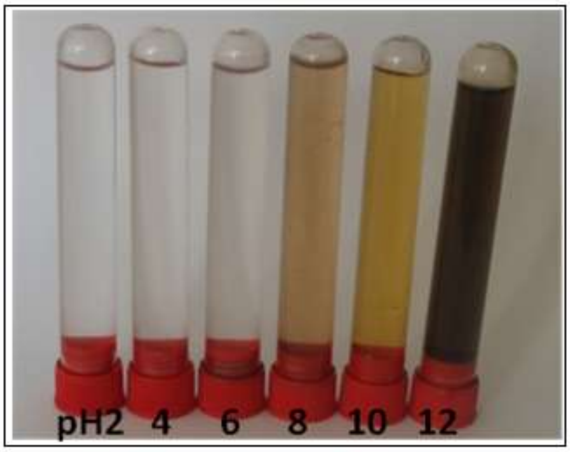

Fig. 7. Colors of Ag nanoparticles solution at pH 2, 4, 6, 8, 10, 12.

Conclusion

The present study is regarding the green synthesis of silver nanoparticles from *Ricinodendron heudelotii* (Baill) Pierre Pax seed kernels extract, the optimization and reaction condition studies. It is confirmed that silver nanoparticles could be obtained at increasing rate affecting factors such as temperature or pH as well as the extract concentration. The obtained nanoparticles are of great potential in health domains such as in the control of anti-infectious diseases or antimicrobial agent.

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