



Original Article

Spectroscopic synthetic optimizations monitoring of silver nanoparticles formation from *Megaphrynium macrostachyum* leaf extract



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ABSTRACT

Nanobiotechnology is one of the most promising areas in modern nanoscience and technology. Metallic nanoparticles have found uses in many applications in different fields, such as catalysis, photonics, electronics, medicine and agriculture. Synthesized nanoparticles through chemical and physical methods are expensive and have low biocompatibility. In the present study, silver nanoparticles have been synthesized from *Megaphrynium macrostachyum* (Benth. & Hook. f.) Milne-Redh., Marantaceae, leaf extract. *Megaphrynium macrostachyum* is a plant with large leaves found in the rainforest of West and Central Africa. Synthetic optimizations following factors such as incubation time, temperature, pH, extract and silver ion concentration during silver formation are discussed. UV-visible spectra gave surface plasmon resonance for synthesized silver nanoparticles based *Megaphrynium macrostachyum* peaks at 400–450 nm. X-ray diffraction revealed the average size of pure crystallites composed from Ag and AgCl.

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Introduction

Nanomaterials with a characteristic dimension in the range of 1–100 nm are at the leading edge of nanosciences and nanotechnology (Masarovicová et al., 2014). In recent years, nanomaterials and specifically metal nanoparticles have received particular interest in diverse fields ranging from material sciences to biotechnology (Huang et al., 2007). High-density thin films of silver and copper nanoclusters have been produced in Middle-Age- and Renaissance-era glazed pottery, to exploit their peculiar optical properties for decorative purposes (Pérez-Arantegui et al., 2001). Nanometer-sized metallic particles in melt glasses have been used for centuries to produce colored glassware (Bamford, 1977). Because of extremely small size and high surface to volume ratio

of nanoparticles, the physicochemical properties of nanoparticles-containing materials are quite different to those of the bulk material (El-Sayed, 2001). Thus, nanomaterials have potential applications in electronics, photonics, information storage, chemical sensing, imaging, environmental remediation, drug delivery, and biological labeling (Huang et al., 2007).

The chemical synthesis of silver nanoparticles employs chemical reducing agents to convert Ag⁺ ions to Ag-nanoparticles. One of the most widely used chemical reducing agent is sodium borohydride. This process involves the undesired use of hazardous chemicals, and the biocompatibility of the resulting Ag-nanoparticles is too low for application in biological systems (Park, 2014). The biological method for the synthesis of nanoparticles employs use of biological agents like bacteria, fungi, actinomycetes, yeast and plants providing a wide range of resources for the synthesis of nanoparticles (Rai et al., 2008; Thakkar et al., 2010). The rate of reduction of metal ions using biological agents is found to be much faster and also at ambient temperature and pressure conditions (Rai et al.,

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2009). The process of green synthesis requires the use of water as an environmentally friendly solvent. Plant mediated synthesis of metal nanoparticles is gaining more importance owing to its simplicity, eco-friendliness, rapid rate of synthesis of nanoparticles of attractive and diverse morphologies and elimination of elaborate maintenance of cell cultures (Vadlapudi and Kaladhar, 2014). Earlier literature suggest that extracts from various plants leaves such as *Eucalyptus hybrid*, Myrtaceae (Dubey et al., 2009), *Helianthus annuus*, Asteraceae (Gebru et al., 2013), *Acalypha indica*, Euphorbiaceae (Krishnaraj et al., 2010), *Oryza sativa*, Poaceae (Leela and Vivekanandan, 2008), *Gliricidia sepium*, Fabaceae (Rajesh et al., 2009), *Mentha piperita*, Lamiaceae (Ali et al., 2011), *Atrocarpus heterophyllus*, Moraceae (Thombre et al., 2012), *Withania somnifera*, Solanaceae (Rajesh et al., 2013; Gregory et al., 2014), could be used for the Ag-nanoparticles synthesis.

Megaphrynium macrostachyum (Benth. & Hook. f.) Milne-Redh. belongs to family Marantaceae. The plant is found in the rainforest of West and Central Africa (Jennings et al., 2001). *M. macrostachyum* is a perennial semi-woody herb, rhizomatous, forming extensive clumps, with stems to 2½ m high bearing a single large leaf 30–60 (–90)cm long by 12–30 (–40)cm wide. The flowers, borne on the petiole below the leaf are whitish with red or purple calyx. The leaves are harvested from the forest and used fresh in wrapping food in order to preserve the food. In Central Africa, for instance, they are often used for wrapping cassava sticks before cooking (Ajayi and Ojelere, 2013). No significant anti-microbial activities of *M. macrostachyum* leaves over *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* in the doses considered were found. The phytochemical screening of the leaves shows presences of alkaloids, flavonoids, anthocyanins, saponins, reducing sugars and gallic tannins (Maloueki et al., 2013).

So far, there have been no reports on the synthesis of nanoparticles by using *M. macrostachyum*. The leaves are suitable for green synthesis and we present in this research the eco-friendly, simple and low cost synthesis of silver nanoparticles and the synthetic optimizations relate to incubation contact time, temperature, pH, extract and silver ion concentration. A study of the crystallinity and composition of the silver nanoparticles using X-ray powder diffraction is presented.

Materials and methods

Materials

Silver nitrate (AgNO₃) was obtained from Sigma–Aldrich chemicals Germany, H₂SO₄ 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. Fresh leaves of *M. macrostachyum* (Benth. & Hook. f.) Milne-Redh., Marantaceae, were procured from local market, Douala, Cameroon, and identified at the national herbarium of Cameroon by Tadjouteu Fulberg under number of deposit 10000/SRF Cam. All glass wares were washed with dilute nitric acid (HNO₃) and de-ionized water, and then dried in hot air oven. *M. macrostachyum* leaf was surface cleaned with running tap water followed by de-ionised water to remove all the dust and unwanted visible particles. Aqueous extract of *M. macrostachyum* was prepared by boiling 10 g of *M. macrostachyum* leaf in 200 ml de-ionized water for 5 min at 80 °C. The extract was filtered twice through Whatman No. 1 filter paper to remove particulate matter, get clear solutions and stored one week at 4 °C. Solutions of AgNO₃ 10^{–3} M, 10^{–2} M and 10^{–1} M were prepared in de-ionized water. Mixture solution of plant extract and silver ion was hand shaken during 1 min before incubation.



Fig. 1. *Megaphrynium macrostachyum* leaf: 32 cm large, 17 cm wide.

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 300 s. XRD measurements were carried out using a PANalytical Empyrean Serie 2 X-ray diffractometer (Cu K-Alpha1 [Å] 1.54060, K-Alpha2 [Å] 1.54443, K-Beta [Å] 1.39225) by preparing a thin film of silver-macrostachyum powder on silicium substrate.

Preparation of aqueous extract

Aqueous extract of *M. macrostachyum* was prepared using 10 g of freshly collected leaves (Fig. 1). The leaves were surface cleaned with running tap water, followed by distilled water and boiled with 100 ml of distilled water at 80 °C for 5 min. The extract was filtered 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 (Eya'ane Meva et al., 2016).

Green synthesis of silver nanoparticles

For the synthesis of the silver nanoparticles, a volume of *M. macrostachyum* leaf extract (5, 10, 15 ml) was added to 50 ml of 10^{–3} M, 10^{–2} M or 10^{–1} M aqueous AgNO₃ solution 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 measuring the absorbance at 5, 10, 20, 30, 40, 50 and 60 min. 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 AgNO₃ during 30 min. 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 H₂SO₄ and 0.1 N NaOH solutions. The contact time of incubation for all studied samples was varied from 1 h to 24 h and then 96 h.

Results and discussion

UV–visible Ag-nanoparticles formed 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. The color change of the mixture solution plant extract and silver ion is first recorded through visual observation. Colors of silver nitrate, *M. macrostachyum* leaf extract and silver nanoparticles solution is shown on Fig. 2. The evolution of the surface plasmon absorbance bands during the synthesis of

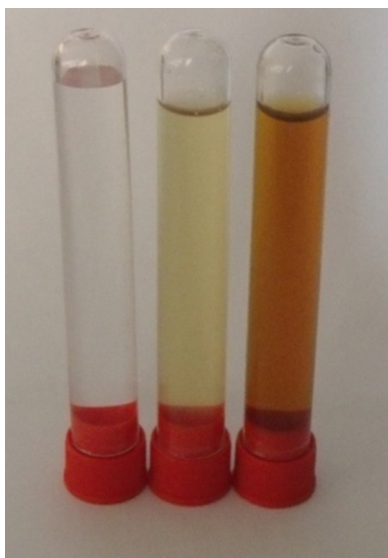


Fig. 2. Silver nitrate, *Megaphrynium macrostachyum* leaf extract, Ag nanoparticles solution.

silver nanoparticles using 50 ml of AgNO_3 10^{-3} M with 10 ml of extract concentration during the first hour is shown in Fig. 3. The solution color change within seconds to pale yellow, and then to yellowish brown, due to formation of plasmons at the colloid surface, indicating the synthesis of silver nanoparticles. The same sharp surface plasmon resonance absorbance band has been obtained with different extract concentrations (5, 10, 15 ml) at 10^{-3} M AgNO_3 . Then, 5 ml of extract is enough to reduce completely 50 ml of silver ion at 10^{-3} M concentration (Fig. 4). The plasmon resonance absorbance increases when a 10^{-2} M AgNO_3 solution is used with different extracts concentration, then more silver ion is available for

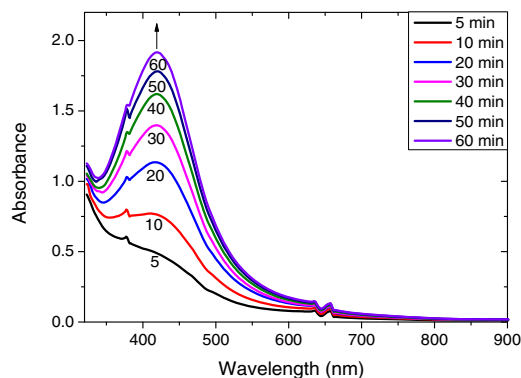


Fig. 3. Sixty minutes of reaction between 10 ml of *Megaphrynium macrostachyum* leaves extract and 50 ml AgNO_3 10^{-3} M.

reduction. At 10^{-1} M concentration of the silver ions with the different *M. macrostachyum* extract concentrations the nanoparticles are aggregate because of the deficiency of molecules of leaf extract to act as capping 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). As postulated by Mie's theory, spherical nanoparticles results in a single surface plasmon resonance (SPR) band in the absorption spectra. On the other hand, anisotropic particles provide two or more SPR bands depending on the particle shape (Mie, 1908). In the present study, reaction mixtures confirm single SPR bands disclosing spherical shape of Ag-nanoparticles which tend to become anisotropic with time. The plant leaf extract from *M. macrostachyum* act as reductant as well as capping agent, therefore mediate the synthesis as well as stabilization of the silver nanoparticles. The sharp surface plasmon resonance band increases with silver ion concentration as observed for olive leaf extract (Khalil et al., 2013). It

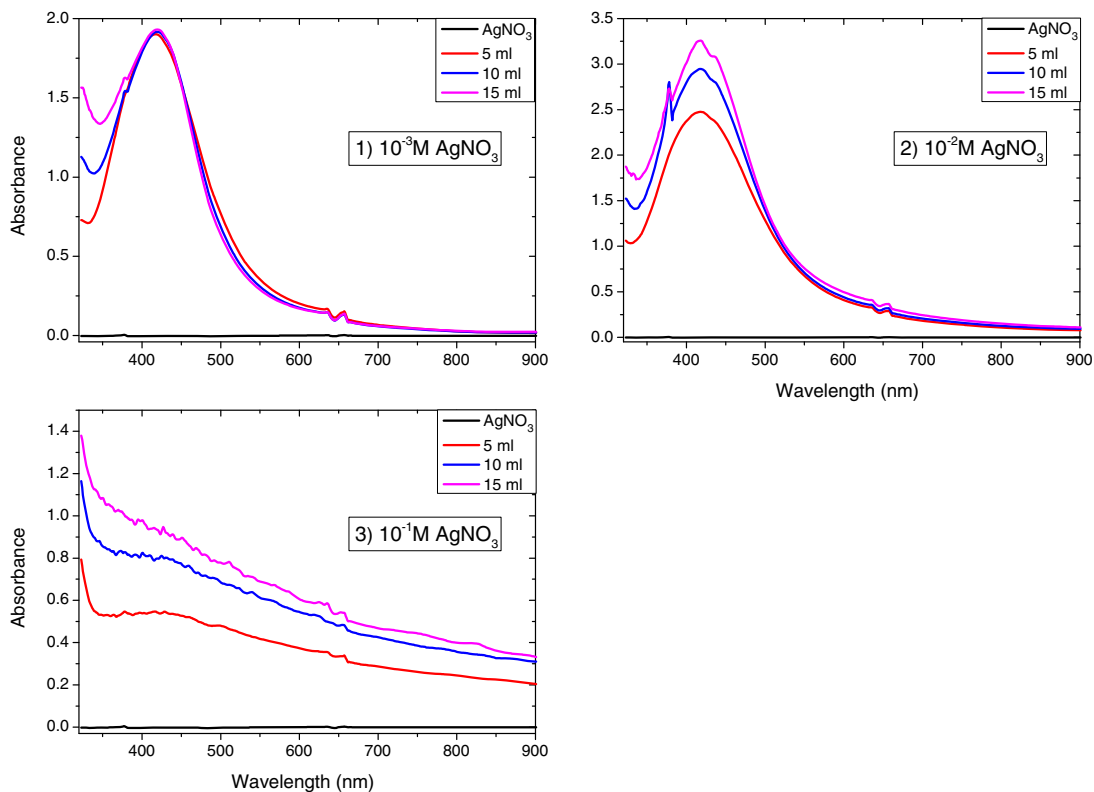


Fig. 4. UV-vis spectra of the variation of extract quantities 5, 10, 15 ml.

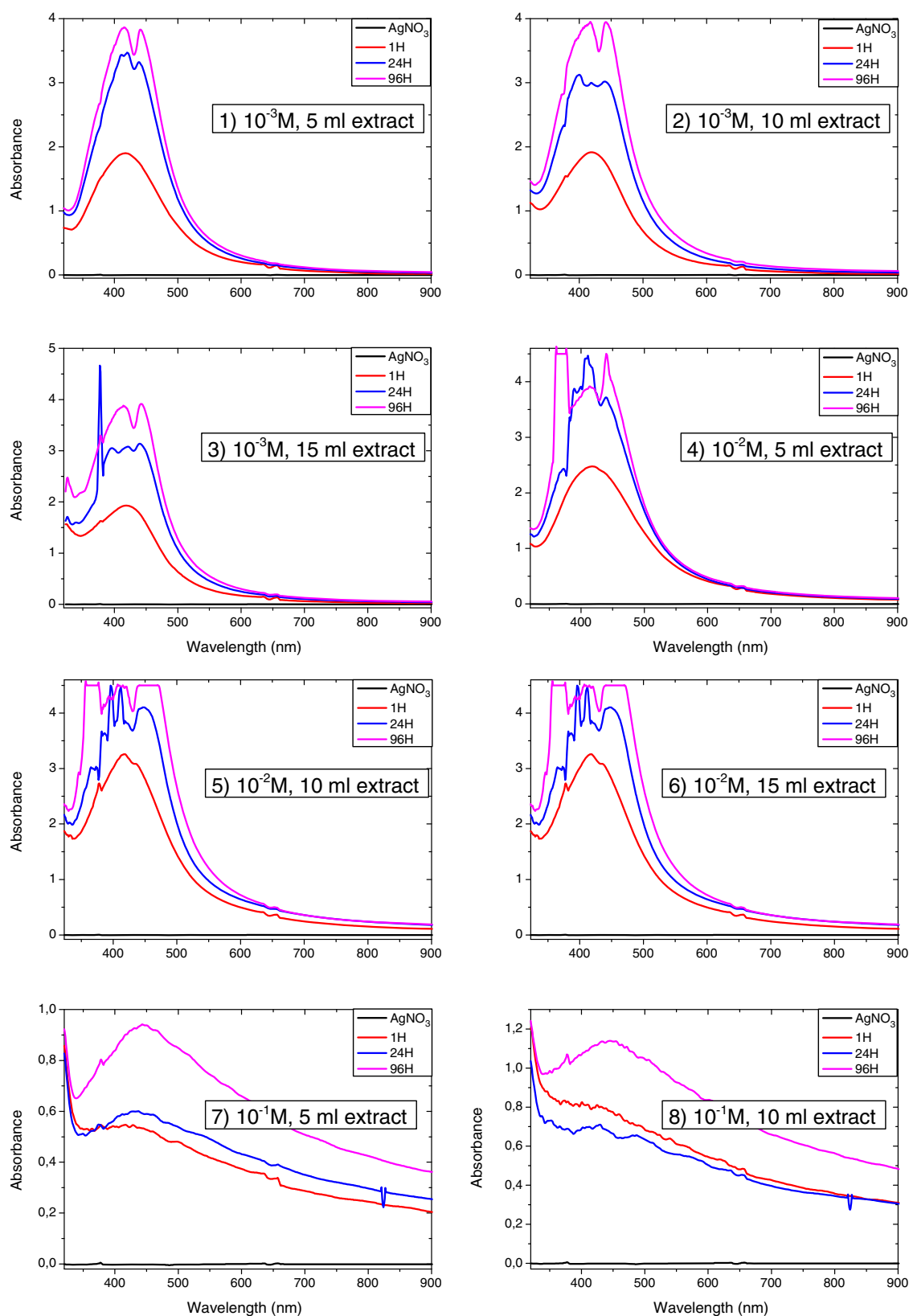


Fig. 5. UV-vis spectra of different quantities of extract and of silver nitrate in function of time.

can be seen that the absorbance band maxima of Ag-nanoparticles using *M. macrostachyum* is in the range 400–450 nm due to surface plasmon resonance (Mulvaney, 1996) of Ag-nanoparticles. The conduction electrons undergo oscillation due to the strong interaction of light with the silver nanoparticles (Thombre et al., 2012). As

the concentration of the *M. macrostachyum* extract increases, the absorption peak gets more intensity but tend to be polydispersed. Sharp narrow shape SPR band indicating the formation of spherical and homogeneous distribution of silver nanoparticles was generally observed. The UV-vis spectra also revealed that the formation

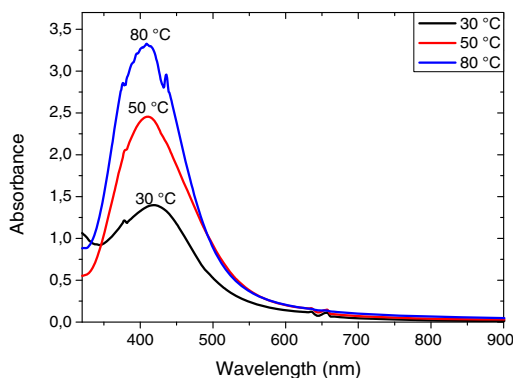


Fig. 6. UV-vis spectra of temperature variation from 30 to 80 °C during silver nanosynthesis.

of Ag-nanoparticles occurred rapidly within few minutes indicating that *M. macrostachyum* speeds up the biosynthesis of silver nanoparticles. Such of rapid reduction was observed for carob leaf extract with 2 min reduction time (Awwad et al., 2013).

The reaction between Ag^+ and the reducing material in the extract was followed for 96 h and UV-visible measurements were made at 1 h, 24 h and 96 h. Fig. 5 shows the UV-visible spectra of Ag-nanoparticles as a function of time after addition of different quantities of *M. macrostachyum* leaf extract. Reduction as well as nucleation and growing size of nanoparticles increases from 24 h to 96 h but poly-dispersions occur. The reaction time resulted in gradual increasing of absorbance bands. The color intensity of the solution change from light yellow to deep-brown at the end of the reaction because of increasing amount of silver nanoparticles as well as aggregation.

Effect of temperature

Fig. 6 shows UV-visible spectra of the Ag-nanoparticles prepared at 30, 50 and 80 °C. It can be seen that the absorbance increases with increasing temperature. This experiment suggests that the rate of nanoparticle synthesis at room temperature can be accelerated by increasing temperature of the reaction mixture. On the other hand the particles tend to be polydispersed at 80 °C.

Effect of pH

The UV-visible following the pH during the formation of silver nanoparticles from pH 2 to 12 is shown in Fig. 7. The variation of color is in Fig. 8. It can be seen that Plasmon absorbance bands increases with increasing pH from 2 to 12, which can be due to the

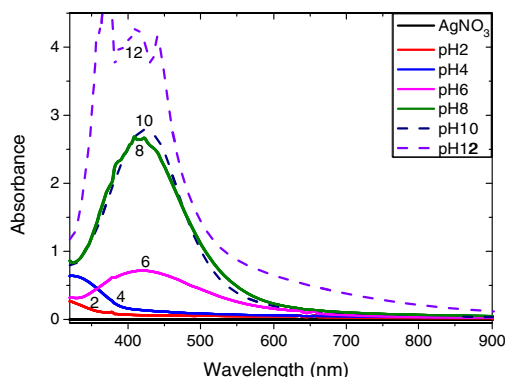


Fig. 7. UV-vis spectra of the variation of pH.

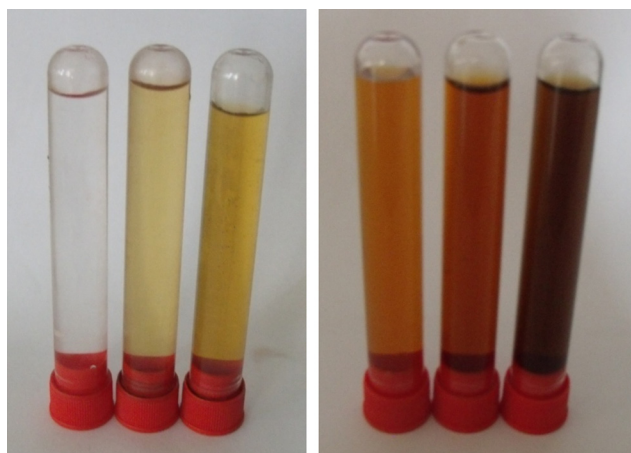


Fig. 8. Colors of Ag nanoparticles solution at pH 2, 4, 6 and 8, 10, 12.

increase in production of colloidal silver nanoparticles and reduction rate. The absorbance does not decrease at pH higher than 8 such as observed for olive oil leaf extracts (Khalil et al., 2013). Furthermore, it is observed that the brown color of the nanoparticles appeared shortly after mixing the AgNO_3 with the extract at pH 4–12. As observed in *Pinus eldarica* bark extract, pH affects the amount of nanoparticle production and their stability and is a critical factor of control of size and morphology of nanoparticles (Irvani and Zolfaghari, 2013). Furthermore, pH influenced the rate of the reduction reaction. The reaction mixture turned brown when silver was reduced, and the reaction mixture coloring accelerated when increasing pH. At pH 10, the sharp surface plasmon resonance band indicates that a monodisperse suspension occurs. In previous studies, it was shown that the size and shape of biosynthesized nanoparticles could be manipulated by varying the pH of the reaction mixtures (Khalil et al., 2013). A major influence of the reaction pH is its ability to change the electrical charges of biomolecules which might affect their capping and stabilizing abilities and subsequently the growth of the nanoparticles (Khalil et al., 2013). Then, high pH environment enhanced the reducing and stabilizing capability of the antioxidants in the *M. macrostachyum* leaf extract a situation found for olive leaf extracts. The number of nuclei increases with elevated pH maybe due to promoted reactivity of the *M. macrostachyum* leaf extracts reductants. Gardea-Torresdey et al. (2003) found that pH is an important factor in the biosynthesis of colloidal gold using alfalfa biomass and concluded that the size of nanoparticles varied with the change in pH. Mock et al. (2002) also have reached similar conclusions and reported that pH is responsible for the formation of nanoparticles of various shapes and size as different plant extracts and even the extracts coming from different parts of the same plant may have different pH values which further need optimization for the efficient synthesis of nanoparticles.

X-ray diffraction

The typical powder XRD pattern of the prepared nanoparticles is shown in Fig. 9. The pattern is compatible with the cubic phase of Ag with diffraction points at 2θ values of 38°, 44.2°, 64.4° and 77.4° and can be indexed to the (1 1 1), (2 0 0), (2 2 0), and (3 1 1) planes of the face centered cubic (FCC) structure, respectively (JCPDS file: 65-2871). The XRD pattern also showed the presence of the cubic phase of AgCl at 2θ values of 27.7°, 32.1°, 46.1°, 54.7° and 57.4°, corresponding to the (1 1 1), (2 0 0), (2 2 0), (3 1 1) and (2 2 2) planes, respectively (JCPDS file: 31-1238).

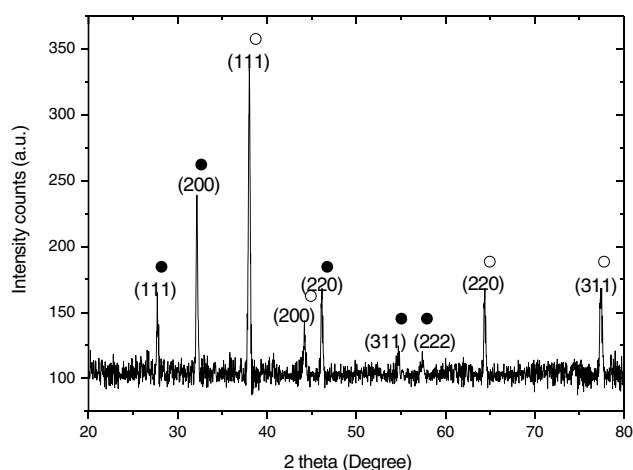


Fig. 9. XRD pattern of the nanoparticles from *Megaphrynium macrostachyum*, ○ represent Ag nanocrystallites and ● represent AgCl nanocrystallites.

The average crystallite size of the synthesized NP was determined using the Debye–Scherrer equation:

$$Dv = \frac{K\lambda}{\beta \cos \theta}$$

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 $K\alpha$; β is the full width at half-maximum of the diffraction peaks; and θ is Bragg's angle. No other characteristic peaks were found in the XRD spectra, indicating the high purity of the as-prepared Ag@AgCl nanoparticles. To calculate the average crystalline particle size of the synthesized Ag@AgCl nanoparticles, we preferred the most intense peaks of Ag and AgCl (Eya'ane Meva et al., 2016). We have selected the (1 1 1) and (2 0 0) lattice planes of Ag and AgCl to calculate the average crystalline particle size of the Ag@AgCl NPs. The calculated average crystalline particle size of the Ag@AgCl-*Megaphrynium* was found to be 33.7 nm and 44.2 nm for Ag and AgCl, respectively. The intense and narrow diffraction peaks revealed the crystalline nature of the synthesized nanoparticles (Wang et al., 2010).

Pattern identification shows the formation of pure crystals of Ag@AgCl. A similar observation was made using leaf extracts of *Corchorus olitorus* and *Ipomea batatas* or flowers extract of *Albizia julibrissin* (Eya'ane Meva et al., 2016; Awwad et al., 2015).

Conclusions

We have described a simple green method for silver nanoparticles synthesis using the reducing properties of *M. macrostachyum* leaf aqueous extract. The extract of *M. macrostachyum* leaves in contact with silver ions is capable of producing silver nanoparticles within 5 min. The extract act as reductant and stabilizer and the nanoparticles can be prepared easily, rapidly and in a cost-effectively manner. It was found that silver nanoparticles synthetic rate increase with extract and silver ion concentration, incubation contact time and temperature. UV–visible measurements shows that the synthesis is promoted at high pH with pH 10 been more favorable. Powder X-ray diffraction studies confirm the pure nature of the crystallites composed with Ag and AgCl nanocrystallites with size 33.7 nm and 44.2 nm for Ag and AgCl.

Authors' contributions

SML, AAN, and PBEK contributed in collecting plant sample and identification, confection of herbarium, running part of the

laboratory work. EMF and EMM carry analysis of the data, run laboratory work, provide chemicals and drafted the paper. All authors contributed to discuss the spectroscopy and powder diffraction. EMF and EMM designed the study, supervised the laboratory work and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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