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Green synthesis and Electrochemical Characterizations of Gold Nanoparticles Using Leaf Extract of *Magnolia kobus*

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Currently, the mechanism of biological nanoparticles synthesis is not fully understood. We proposed an ecofriendly method for gold nanoparticles (AuNPs) synthesis using plant extractsand electrochemical characterization. The reduction of the metal ions through leaf extract leading to the formation of AuNPs of fairly well-defined dimensions. This green chemistry approach toward the synthesis of AuNPs has many advantages such as, ease with which the process can be scaled up, economic viability, etc. Leaves extract is found suitable for the green synthesis AuNPs. Average crystal size calculated from SEM, respectively. The particle size ranging from 100 to 300 nm and the shape of the plate and spherical structures could be controlled by changing the reaction temperature and leaf broth concentration. The concentrations of leaves extract and metal ion are playing an important role in the green synthesis of AuNPs. The spectroscopic characterizations from Cyclic voltammetry (CVs), Electrochemical impedance spectroscopy (EIS) and UV-visible absorption spectra support the formation and stability of the biosynthesized AuNPs. This simple, efficient and rapid green synthesis of AuNPs can be used in various biomedical and biotechnological applications. This environmentally friendly method of biological AuNPs synthesis can potentially be applied in various products that directly come in contact with the human body, such as cosmetics, foods, and consumer goods, besides medical applications. More elaborate studies are required to elucidate the mechanism of biological nanoparticles synthesis. This low cost and greener method for development of AuNPs may be valuable in environmental, biotechnological, electrochemical and biomedical applications.

Keywords: bionanotechnology, gold nanoparticles (AuNPs), electrochemical, green chemistry, leaves extract.

1. INTRODUCTION

Nanoparticles exhibit completely new and improved properties based on specific characteristics such as size, distribution and morphology, if compared with larger particles of the bulk material they are made of. Nanomaterials have a long list of applicability in improving the human life and its environment. Nanoparticles present a higher surface to volume ratio with decreasing size of nanoparticles. As specific surface area of nanoparticles is increased, their biological effectiveness can increase due to the increase in surface energy. Nanoparticles of noble metals, such as gold [1-4], silver [5-7], and platinum [8], are widely applied in products that directly come in contact with the human body, such as shampoos, soaps, detergent, shoes, cosmetic products, and toothpaste, besides medical and pharmaceutical applications.

Gold nanoparticles (AuNPs) have found use in diagnostic and drug delivery applications [9]. Metal nanoparticles have been of great interest due to their distinctive features such as catalytic, optical, magnetic and electrical properties [10]. We have been used for the synthesis of gold nanoparticles. A number of methods including physical and chemical processes [11-17], electrochemical reduction [18-19] photochemical reduction [20-21] and heat evaporation [22-23] for synthesis of metal nanoparticles were developed considering the real life application of nanoparticles in the area of medicine [24], catalysis [25] detection [26], etc. Chemical synthesis methods lead to the presence of some toxic chemical species adsorbed on the surface that may have adverse effects in medical applications. Therefore, there is a growing need to develop environmentally friendly processes for nanoparticle synthesis without using toxic chemicals. Biological methods for nanoparticle synthesis using microorganisms, enzymes, and plants or plant extracts have been suggested as possible ecofriendly alternatives to chemical and physical methods [34]. The green chemistry approach was reported for the use of plant broths as an efficient route for the synthesis of pure nanomaterials [27]. Recently the studies started under green chemistry for the search of benign methods for the development nanoparticles and searching antibacterial, antioxidant, and antitumor activity of natural products. Biosynthetic processes have received much attention as a viable alternative for the development of metal nanoparticles where plant extract is used for the synthesis of nanoparticles without any chemical ingredients [28-32]. Bioinspired synthesis of nanoparticles provides advancement over chemical and physical methods as it is a cost effective and environment friendly and in this method there is no need to use high pressure, energy, temperature and toxic chemicals. The use of environmentally benign materials like plant leaf extract, bacteria and fungi for the synthesis of nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and biomedical applications as they do not use toxic chemicals in the synthesis protocols. However, simple and more greener procedures for the synthesis of nanoparticles will be beneficial, without accumulating an enormous quantity of toxic and redundant chemicals in solid, liquid and gaseous form in the environment. Some of biomimetic processes have been used for the synthesis of gold nanoparticles [33-35].

Using plants for nanoparticle synthesis can be advantageous over other biological processes because it eliminates the elaborate process of maintaining cell cultures and can also be suitably scaled up for large-scale nanoparticle synthesis [36]. Leaf extracts of neem, geranium, hibiscus, cinnamon,

tamarind and coriander have also found suitable for the biosynthesis of silver and gold nanoparticles [37-41]. Among various metal nanoparticles, AgNPs and AuNPs have several effective applications as antibacterial, sensors and detectors besides their biomedical applications [42-45]. Although the biosynthesis of nanoparticles by plants such as fungus [46-47], soluble starch, aloe vera [48], geranium leaf, *cinnamomum camphora* [49], neem, emblica officianalis [50], lemongrass, tamarind, barbated skullcup (BS) herb extract, bark extract, *tanacetum vulgare* (tansy) [51], parthenium [52], employing coriander leaves, phyllanthin extract [53], from henna leaves [54] and pear fruit [55] has been reported, however possibilities in plant-mediated biological synthesis of nanomaterial have to be fully explore [56]. This simple, low cost and greener method for development of AuNPs may be valuable in environmental, biotechnological, electrochemical [57-60], and biomedical applications [61].

2. EXPERIMENTAL

2.1. Materials and Apparatus

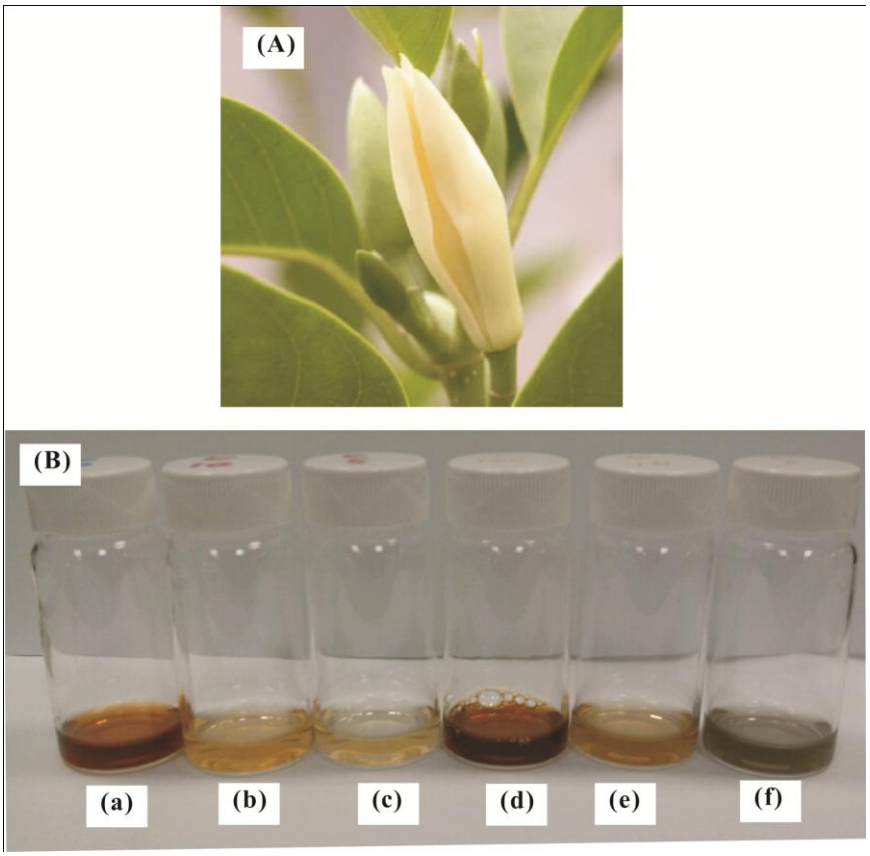

Figure 1. (A) shows Magnolia (*Magnolia kobus*). (B) shows different concentrations of leaf broth (a) 100 %, (b) 10 % and (c) 5 % in pH 7.0 PBS; (d) 100 %, (e) 10 %, (f) 5 % in pH 7.0 PBS containing 1 mM $KAuCl_4$.

Magnolia (*Magnolia kobus*) were obtained from domestic plant shows in Fig.1 (A). Potassium

tetrachloroaurate ($KAuCl_4$) was purchased from Sigma–Aldrich (St. Louis, U.S.A.). All other chemicals used were of analytical grade and used without further purification 0.1 M pH 7.0 phosphate buffer solutions (PBS) was used as supporting electrolyte. Aqueous solutions were prepared using doubly distilled deionized water.

Cyclic voltammetry (CVs) was performed in an analytical system model CHI-1205A and CHI-1205B potentiostat. A conventional three-electrode cell assembly consisting of an Ag/AgCl reference electrode and a Pt wire counter electrode were used for the electrochemical measurements. The working electrode was glassy carbon electrode (GCE) (area 0.07 cm^2). In these experiments, all the potentials have been reported versus the Ag/AgCl reference electrode. Electrochemical impedance spectroscopy (EIS) measurements were performed using an IM6ex Zahner instrument (Kroanch, Germany). The morphological characterizations of the films was examined by scanning electron microscope (SEM) (Hitachi S-3000H). The UV-visible absorption spectra were checked by using a U3300 Spectrophotometer (HITACHI).

2.2. Preparation of leaf broth from Magnolia kobus

The fresh leaves of *Magnolia kobus* were washed several times with ultra pure water to remove the dust and dried for 2 d at room temperature. The plant leaf broth solution was prepared by taking 5 g of thoroughly washed and finely cut leaves in 100 mL of pH 7.0 PBS and then boiling the mixture for 5 min before finally decanting it. The leaf broth concentrations were also varied between 0 % to 100 % by volume in pH 7.0 PBS. Leaf broth was added 1 mM $KAuCl_4$ for the reduction of Au^{3+} ions. The effects of temperature on the synthesis rate and particle size/shape of the prepared gold nanoparticles (AuNPs) were studied by carrying out the reaction in a water bath at 95 ^oC with reflux. The AuNPs solution thus obtained was purified by repeated centrifugation at 15,000 rpm for 20 min followed by redispersion of the pellet in deionized water.

3. RESULTS AND DISCUSSIONS

3.1. Morphological characterization of Au nanoparticles

Stable gold nanoparticles were formed by treating solution using the plant leaf extracts as reducing agents. The leaf broth concentrations were also varied between 0 % to 100 % by volume in pH 7.0 PBS. Fig.1 (B) shows different concentrations of leaf broth (a) 100 %, (b) 10 % and (c) 5 % in pH 7.0 PBS; (d) 100 %, (e) 10 %, (f) 5 % in pH 7.0 PBS containing 1 mM $KAuCl_4$. We observed externals of (a) to (c) the color variation by different concentrations of leaf broth. Obviously color change from (f) 5 % in pH 7.0 PBS containing 1 mM $KAuCl_4$, explained that the plant leaf extracts as reducing agents. Successfully biosynthesized nanoplates were observed, elegantly assembled with gold nanoparticles. Nanostructure size, crystal nature, purity and morphologies were characterized by SEM. Prior to modification, ITO surfaces were cleaned and ultrasonicated in acetone–water mixture for 15

min and then dried. SEM images of biosynthesized AuNPs at different leaves extract quantities were observed in Fig. 2. Different concentrations of leaf broth (A) 0 %, (B) 5 % , (C) 10 % and (D) 100 % in pH 7.0 PBS containing 1 mM $KAuCl_4$. With an increase in leaves extract quantity, AuNPs possess different shape and particle size of both the synthesized metal nanoparticles. Without leaf broth treating has smooth surface, no particle was synthesized. 5 % leaf broth condition shows batter synthesized, the particle size ranging from 100 to 300 nm.

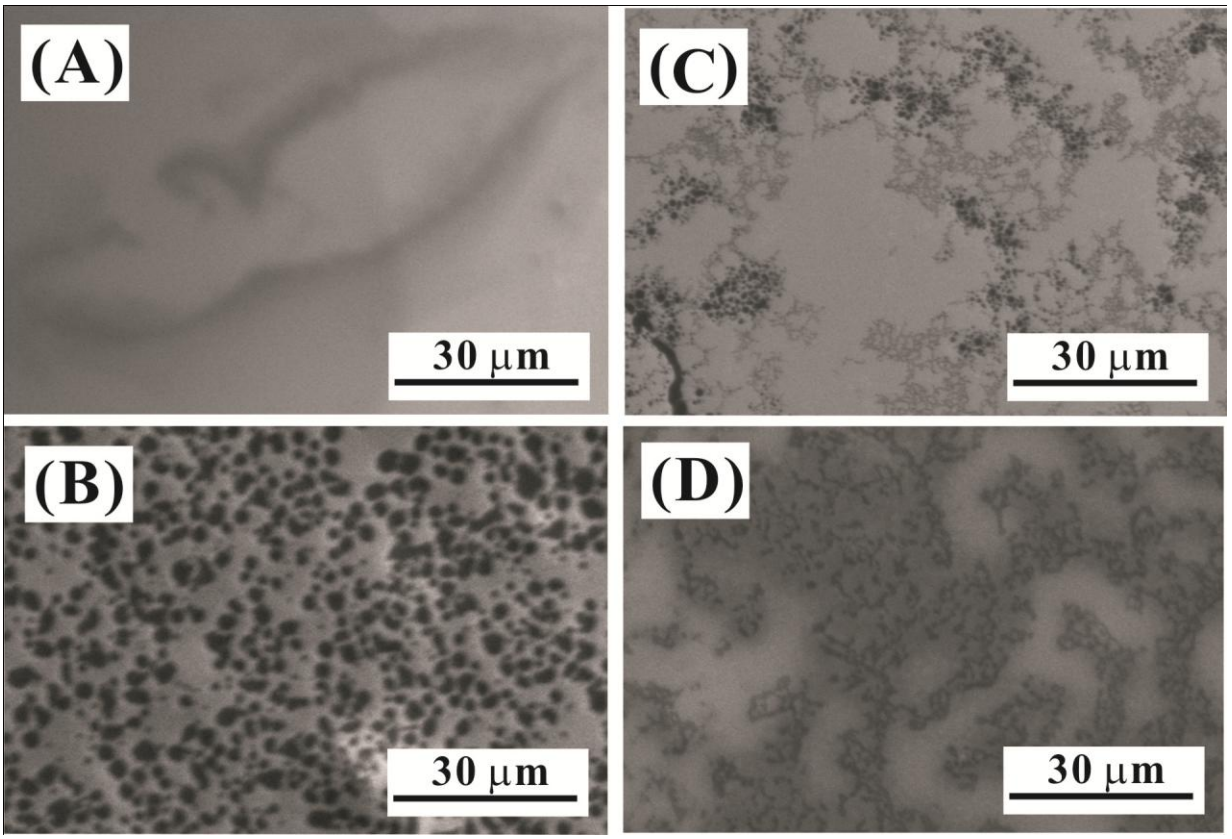

Figure 2. Different concentrations of leaf broth (A) 0 %, (B) 5 % , (C) 10 % and (D) 100 % in pH 7.0 PBS containing 1 mM $KAuCl_4$.

The stability of particles was evaluated at different concentrations of leaf broth without any additional stabilizing chemicals. We also investigated the effects of reaction conditions such as reaction temperature, leaf broth concentration and reaction time on synthesis rate and particle size of the gold nanoparticles. At room temperature, there was on synthesis product. As the reaction temperature increased, the gold nanoparticle synthesis rate increased. The conversion at 95 ^{o}C was 79% after 1 min and reached almost 100% after 5 min [61]. Heating is key point for suitable reaction temperature at 95 ^{o}C.

3.2. UV-visible absorption spectra of Au nanoparticles

Reduction of the Au^{3+} to gold nanoparticles during exposure to the plant leaf extracts could be

detected by the color change. Synthesis of AuNPs by reducing respective metal ion solution with leaves extract may be easily observed by UV–vis spectroscopy. The absorption spectra at different leaves extract quantities and metal concentrations were measured spectrophotometer in 300–900 nm range. Naturally synthesized AuNPs of diameters (100–300 nm) gave sharp peaks in the visible region of the electromagnetic spectrum. Fig. 3 shows the UV–vis spectra recorded from the aqueous solution of 1 mM $KAuCl_4$ as a function of the reaction time using leaf broth at 95 ^oC, 5 min.

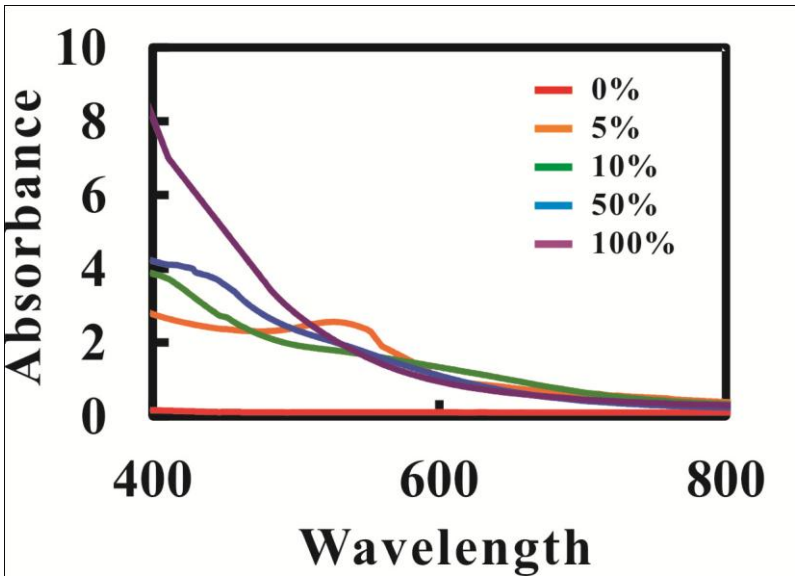

Figure 3. UV-visible absorption spectra of 0 %, 5 %, 10 %, 50 % and 100 % leaf broth in the presence 1 mM $KAuCl_4$ pH 7.0 PBS.

The maximum absorbance was observed to occur at ca. 520 - 540 nm, and the intensity steadily increased to saturation as a function of the reaction time. It was reported that flat gold nanoparticles absorb the wavelength in the NIR region at 0 % (red line) of leaf broth, proved leaf extracts as reducing agents for synthesia AuNPs. The 5 % (orange line) of leaf broth has peak at 520 nm, and the intensity of 2.28. In case of AuNPs, absorbance and sharpness increases at higher leaf broth concentration showing but no significant peak was found in visible or NIR region at higher concentrations. The 100 % (purpule line) of leaf broth shows highest background curve, but no significant peak was found. 10 % (green line) and 50 % (blue line) of leaf broth shows significant peak at 539 nm and 535 nm, the intensity of 1.8 and 1.88. Gold nanoparticles are known to exhibit a ruby-red color in aqueous solutions due to excitation of the surface plasmon vibrations in the gold nanoparticles [62].

3.3. Electrochemical impedance spectra (EIS) of Au nanoparticles

Electrochemical Impedance Spectroscopy (EIS) or ac impedance methods have seen tremendous increase in popularity in recent years. EIS, a relatively new and powerful method of characterizing electrochemical properties of materials and their interfaces, is now the method of choice

for characterizing interfaces in which the physical and chemical behavior is dependent on several different processes occurring at different rates. Initially applied to the determination of the double-layer capacitance and in ac polarography, they are now applied to the characterization of electrode processes and complex interfaces. In theory, any intrinsic property that influences the conductivity of a nanoparticles/solution interface can be examined by impedance measurements. The ratio of the Laplace transforms of potential and current, $E(s)/i(s)$ is expressed in the units of resistance, Ω, and is called *impedance*, $Z(s)$. The inverse of impedance is called *admittance*. They are *transfer functions* which transform one signal, *e.g.* applied voltage, into another, *e.g.* current. Both are called *immittances*. In order to simplify the calculations of impedances, the result obtained for the periodic perturbation of an electrical circuit may be represented using complex notation. In the the system impedance, $Z(j\omega)$ and the real and imaginary parts of the impedance are: $Z' = R$ and $Z'' = -1/\omega C$, respectively. The result may be represented graphically using two types of plots: *complex plane* (also known as *Nyquist plots*) and *Bode plots*. The complex plane plot is a plot of *Z''* versus *Z'*, that is, the imaginary versus the real components, plotted for various frequencies.

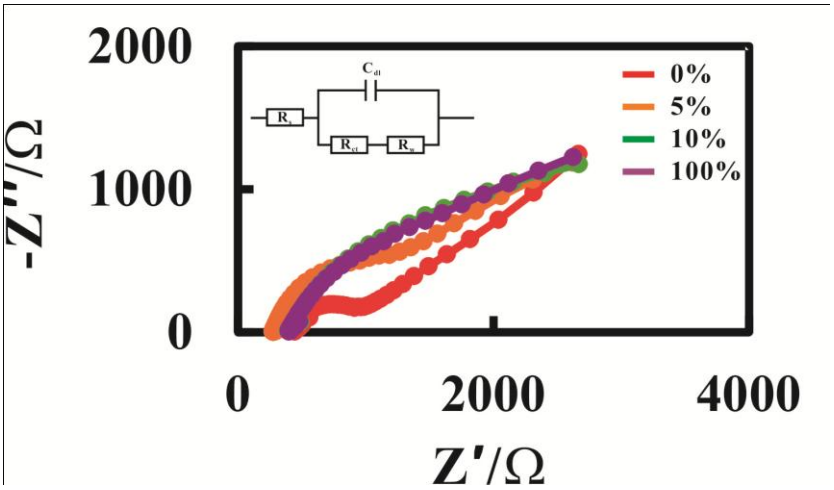

Figure 4. Electrochemical impedance spectroscopy (EIS) of 0 %, 5 %, 10 % and 100 % of leaf broth in the presence pH 7.0 PBS of equimolar 5 mM $[Fe(CN)_6]^{3-/4-}$ and 1 mM K_2AuCl_4. The insert displayed the equivalent circuit (Randles model) was used to fit Nyquist diagrams.

Fig. 4 shows the results of EIS for different concentrations of leaf broth in the presence pH 7.0 PBS of equimolar 5 mM $[Fe(CN)_6]^{3-/4-}$ and 1 mM $KAuCl_4$. The 0 % (red line) of leaf broth exhibited almost a straight line with a very small depressed semicircle arc (R_{et} = 895.7 (Z'/Ω)) represents the characteristics of diffusion limited electron-transfer process on the electrode surface. On the same conditions, the 5 % (orange line) of leaf broth shows like a depressed semicircle arc (R_{et} = 1268.8 (Z'/Ω)) clearly indicated the higher electron transfer resistance behavior comparing with 0 %. The 10 % (green line) and 100 % (purpule line) of leaf broth shows dispersed semicircle. We consider semi-infinite diffusion to a sphere of radius with both oxidized and reduced forms soluble in the solution. The mass transfer impedance may be obtained. Assuming a reversible dc process one obtains, similar to the case of linear diffusion. The insert displayed the equivalent circuit (Randles model) was used to

fit Nyquist diagrams. It constitutes a distributed element which can only be approximated by an infinite series of simple electrical elements.

3.4. Cyclic voltammetry of Au nanoparticles

Glassy carbon electrode was polished with 0.05 μm alumina on Buehler felt pads and then ultrasonically cleaned for about a minute in water. Finally, the electrode was washed thoroughly with double distilled water and dried at room temperature.

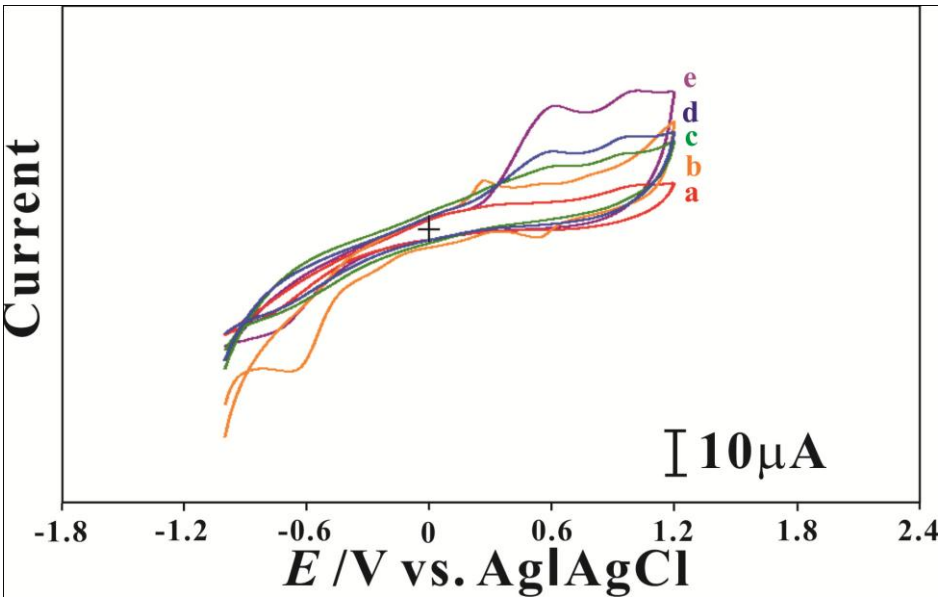

Figure 5. Cyclic voltammograms of (a) 0 %, (b) 5 %, (c) 25 % and (d) 100 % of leaf broth in the presence 1 mM $KAuCl_4$ pH 7.0 PBS. The corresponding CVs have been obtained at 100 mVs^{-1} scan rate in the potential range of -1.0 to 1.2 V.

After that electrode was immersed in different concentrations of leaf broth (a) 0 %, (b) 5 %, (c) 25 %, (d) 50 % and (e) 100 % pH 7.0 PBS solution containing 0.1 mM $KAuCl_4$ from -1.0 V to 1.2 V, scan rate 100 mVs^{-1} by cyclic voltammetric in Fig 5. Curve (a) 0 % of leaf broth shows flatness graph. With increase concentrations of leaf broth from 5 % to 100 %, two irreversible peak current increased occur at 0.93 V and 0.6 V. Curve (e) 100 % of leaf broth shows highest background current was 30.61 μA and 28.04 μA. Particularly, curve (b) 5 % of leaf broth shows reversible peak at 0.25 V and 0.54 V, we supposed that characterization of Au peak [1]. The 5 % concentrations of leaf broth have been already proved to be suitable to synthesized AuNPs present in this paper. This environmentally friendly method of biological AuNPs production provides rates of synthesis faster or comparable to those of chemical methods and can potentially be used in various human contacting areas such as cosmetics, foods and medical applications.

4. CONCLUSIONS

Metal nanostructures have unusual physicochemical properties and biological activities compared to their bulk parent materials. Thus in recent years a number of physical, chemical and biological techniques were applied for the development of metal nanoparticles (NP). Here we have green synthesized gold nanoparticles (AuNPs) by using leaves extracts. In this work, a single-step biosynthetic route for producing AuNPs using leaves extract. The preparation of nanostructured AuNPs provides an environmentally friendly option, as compared to currently available chemical and/or physical methods. The process for the synthesis of AuNPs is rapid, novel and ecofriendly.

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