GREEN SYNTHESIS OF SILVER NANOPARTICLES USING PSIDUMGUAJAVALEAF EXTRACT

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ABSTRACT

In this paper, the green synthesis of silver nanoparticles (AgNPs) using Psidium guajava leaf extract at room temperature. The prepared silver nanoparticles were characterized using UV-Vsible Spectroscopy, Transmission Electron Microscopy (TEM), X-ray diffraction (XRD), Fourier transform infrared (FTIR) and Thermal gravimetric analysis (TGA). The effects of extract concentration, extract pH and contact time on the size and shape of silver nanoparticles are studied. The data revealed that the rate of formation of nanosilver increased significantly in the basic medium and with increasing temperature. TEM studies showed that the particles to be of various shapes and sizes. X-ray diffraction (XRD) study confirmed crystalline nature of the synthesized nanoparticles. FTIR measurement showed that the silver nanoparticles having a coating of the extract compounds indicating a possible role of groups of phenolic compounds that act as a capping and stabilizing agents for the formed silver nanoparticles. Thermo gravimetric analysis (TGA) was carried out to determine the amount of capping phytochemicals on the silver nanoparticles. Supposed mechanism for the formation of silver nanoparticles is discussed.

Keywords: Green synthesis, Psidium guajava, leaf extract, Ag NPs.

INTRODUCTION

Silver nanoparticles are one of the most heavily used nano-material in the world. They are also widely prepared and stored as a metal particle dispersion. Colloidal suspensions of spherical silver nanoparticles are bright

yellow, typically showing maximum absorption around 420 nm. They are most commonly prepared by reduction of silver salt into zero valence state using a reducing agent. Silver NPs (AgNPs) have known to be the most effective against bacteria and viruses (Galdiero *et al*., 2011; Lara *et al*., 2010).

In addition, microbes are unlikely to develop resistance against silver, because the metal attacks a broad range of target sites in the organisms (Pal *et al.*, 2007). AgNPs target both the respiratory chain and the cell-division machinery while releasing silver ions (Ag+) that enhance bactericidal activity and finally leading to cell death (Prabhu and Poulose, 2012). AgNPs are utilized in the filtration membranes of water due to the slow release rate of the membrane to be utilized as a protective bulkhead against different bacteria and other microbes present in the water (Krutyakov *et al*., 2008). Metallic nanoparticles can be synthesized using a number of routinely used physical and chemical methods. But most of these methods are usually expensive and employ toxic chemicals and nonpolar solvents during synthesis followed by addition of synthetic additives or capping agents as stabilizers thereby limiting their applications in clinical and biomedical fields. Therefore, there is a growing need for the development of eco-friendly, benign, biocompatible, reliable and synthetic methods to avoid any undesired environmental and health effects (De *et al*., 2008). Using plants for nanoparticles synthesis offers important advantages over other biological systems. The low cost of cultivation, short production time, safety, simplicity and can also be suitably used for large-scale nanoparticles synthesis (Noruzi, 2015; Shankar *et al*., 2003; Njagi *et al*., 2011).

Plant extracts that were recently reported in biosynthesis of silver nanoparticles included Datura stramonium (Gomathi *et al*., 2017), Moringa stenopetala (Mitiku and Yilma, 2017), Clitoria ternatea L (Vanaraj *et al*., 2017) Cymbopogon citrates (Ajayi and Afolayan, 2017), Cassia roxburghii (Moteriy *et al*., 2017), Bergenia ciliate (Phull *et al*., 2017), Cardiospermum halicacabum (Sundararajan *et al*., 2016), Carica papaya (Banala *et al*., 2016), Eclipta alba (Premasudha *et al*., 2015), olive leaf extract (Khalil *et al*., 2014), and many others.

P. guajava (Myrtaceae) is widely used in Mexico to treat gastrointestinal and respiratory disturbances and is used as an anti-inflammatory medicine (Aguilar *et al*., 1994). Commonly roots, bark, leaves and immature fruits, are used in the treatment of gastroenteritis, diarrhoea and dysentery. Leaves are applied on wounds, ulcers and for rheumatic pain, while they are chewed to relieve toothache (Heinrich *et al*., 1998). A decoction of the new shoots is taken as a febrifuge. A combined decoction of leaves and bark is given to expel the placenta after childbirth (Mart´ınez and Barajas, 1991). A water leaf extract is used to reduce blood glucose level in diabetics (Aguilar *et al*., 1994).

Flavonoids, and saponins combined with oleanolic acid have been isolated from the leaves (Arima and Danno, 2002). In mature leaves, the greatest concentrations of flavonoids were found in July: Myricetin (208.44 mg kg−1), quercetin (2883.08 mg kg-1), luteolin (51.22 mg kg-1) and kaempferol (97.25 mg kg-1) (Vargas *et al*., 2006).

It was clearly seen that quercetin is the most active principle in Thai guava leaves, followed by quercetin-3-O-glucopyranoside and morin,

respectively. The higher antioxidant activity being related to the greater number of hydroxyl groups on the flavonoid nucleus. The antioxidant activity of flavonoids was considered dependent on the presence of ortho phenolic functions (Foti *et al*., 1996). In this paper, green synthesis of silver nanoparticles using Psidium guajava leaf extract under different conditions is described.

EXPERIMENTAL

1) **Materials:** Silver nitrate AgNO3was obtained from Sigma–Aldrich chemicals and used as received. Deionized water was used throughout the reactions. All glass wares were washed with dilute nitric acid HNO3 and distilled water, then dried in hot air oven. 2.0 g of guava leaf broth was boiled for 15 min, filtrated and completed to 100 ml to get the extract. The filtrate used as reducing agent was kept in the dark at 100C to be used within one week. A stock solution of AgNO3(2 × 10-2 M) was prepared by dissolving 0.34 g/100 ml de-ionized water.
2) **Instrumentation:** The UV–vis spectra were recorded at room temperature using a k-Helios SP Pye-Unicam spectrophotometer. Photoluminescence spectra were recorded on a Perkin Elmer LS 50B luminescence spectrophotometer. Transmission electron microscopy (TEM) studies were performed using a JEOLJEM 1200 electron microscope operating at an accelerating voltage of 90 kV. For the TEM measurements, a drop of a solution containing the particles was deposited on a copper grid covered with amorphous carbon. After allowing the film to stand for 2 min, the extra solution was removed by means of blotting paper and the grid

allowed drying before the measurement. Fourier transform infrared (FTIR) spectra were recorded at room temperature on a Nicolet 6700 FTIR spectrometer. For the FTIR measurements of capped silver nanoparticles, a small amount of silver nanoparticles (0.01 g) dried at 60 0C for 4 h was mixed with KBr to form a round disk suitable for FTIR measurements. To obtain the FTIR spectrum of the extract, an appropriate amount of the extract was mixed with KBr. Thermo gravimetric analyses were carried out with a heating rate of 10 0C/min using a Shimadzu DT-50 thermal analyzer. X-ray diffraction (XRD) pattern was obtained using a Shimadzu XRD-6000 diffractometer with CuKa (k= 1.54056 Å) to confirm the biosynthesis of AgNPs.

3) **Synthesis of silver nanoparticles:** For the synthesis of the silver nanoparticles, a certain volume of the Psidium guajava extract (0.2–6) ml was added to the AgNO3 solution and the volume was adjusted to 10 ml with de-ionized water. The final concentration of Ag+ was 1×10-3 M. The reduction process Ag+ to Ag0 nanoparticles was followed by the color change of the solution from yellow to brownish-yellow to deep brown depending on parameters studied such as the extract concentration, silver ions concentration, temperature and pH. The nanoparticles were prepared at different pH values; the pH of the solutions was adjusted using 0.1 N H3PO4 or 0.1 N NaOH solutions.

RESULTS AND DISCUSSION

1) **UV–visible and TEM of AgNPs formed at room temperature (effect of leaf extract concentration):** For monitoring the formation and stability of

silver nanoparticles, the absorption spectra of the synthesized silver nanoparticles were recorded against water. Fig. (1) shows the UV–visible spectra of silver nanoparticle formation using constant AgNO3 concentration (1 × 10-3 M) with different extract concentrations at room temperature. The AgNPs exhibit changing in the colour from yellow to dark brown, depending on the size of nanoparticles; the colour arises due to the excitation of Surface Plasmon resonance (SPR) of the AgNPs and blue shift was observed from 428 to 422 nm. This blue shift indicates the reduction in mean diameter of the AgNPs. This blue shift indicates to homogenous distribution of spherical silver nanoparticles. This was confirmed by TEM images of leaf extract mixed samples using 0.4 and 5 ml extract at room temperature, Fig. (2). The average of particle size affected by concentration of leaf extract. As the leaf extract concentration increases the particle size decreases in the reaction mixture. Reduction of silver ions will take place at lower concentration of leaf extract (0.4 ml) but do not protect them from the aggregation because of deficiency of biomolecules and some quasi spherical nanoparticles were formed in the range from 40 to 60nm, Fig. (2) a. By increasing the leaf extract concentration in the reaction this lead to decreasing in particle size. On the other hand at higher extract concentration (5 ml) the most of silver nanoparticles that were formed are in the range from 14 to 35 nm, Fig. (2) b.

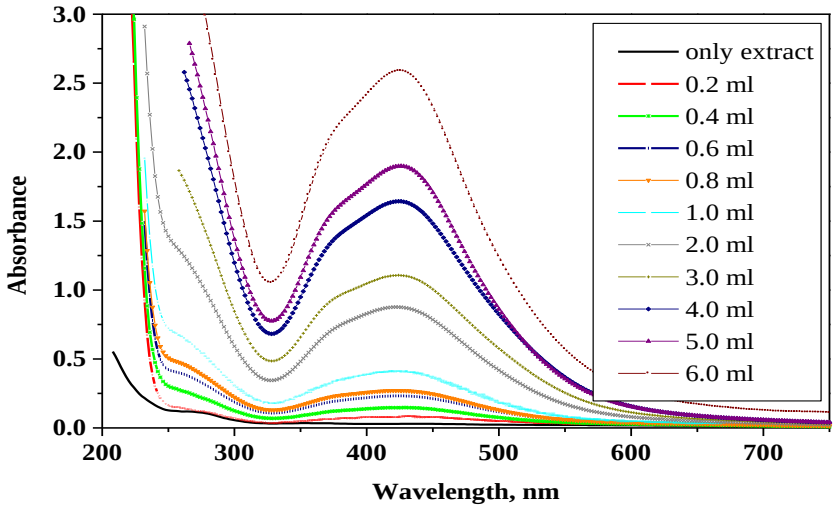

Figure (1): UV–Vis spectra of silver nanoparticles at different concentrations of leaf extract

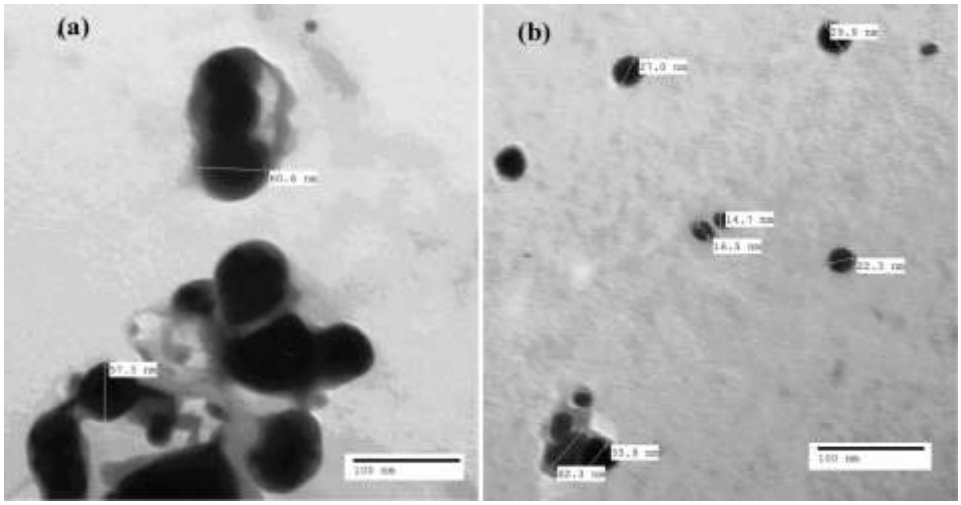

Figure (2): TEM micrograph of the silver nanoparticles (a) 0.4 ml of leaf extract (b) 5ml of leaf extract

2) Effect of contact time at room temperature: UV-vis spectra were recorded at different time intervals after addition of 2ml of guava leaf extract for 199 days to study the formation rate and stability of the formed AgNPs. The reaction start immediately within 2 min and the absorbance increases sharply within 24 h and increases slowly till it reach the maximum within 2 months, Fig. (3). The stability of the reaction mixture was studied by UV-vis spectroscopy for 199 days where the AgNPs showed peak is at the same wavelength. Thus, it was found that the colloidal mixture was stable for 199 days, which was very supportive and convenient for the synthesis of AgNPs. This result implies that the silver nanoparticles prepared by this green synthesis method is very stable without aggregation and the extract is very good capping agent.

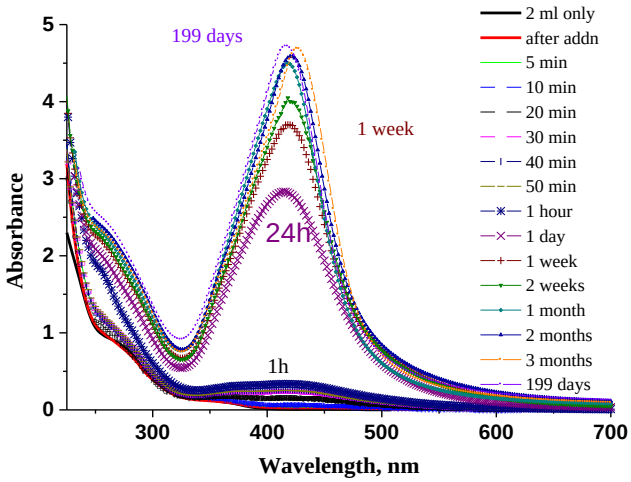

Figure (3): UV–vis spectra of Ag NPs as a function of time at room temperature (0.2ml of AgNO3 and 2 ml guava leaf extract)

3) Effect of temperature: Fig. (4) shows UV–visible spectra of the Ag NPs prepared at different temperature. It can be seen that there is an increasing in absorbance with increasing temperature. This experiment suggests that the slow rate of Ag NPs at room temperature can be accelerated by increasing temperature of the reaction mixture. Increasing of the reaction temperature led to a rapid reduction rate of the Ag+ ions and the nucleation of silver nuclei-allowing for the formation of AgNPs with small size.

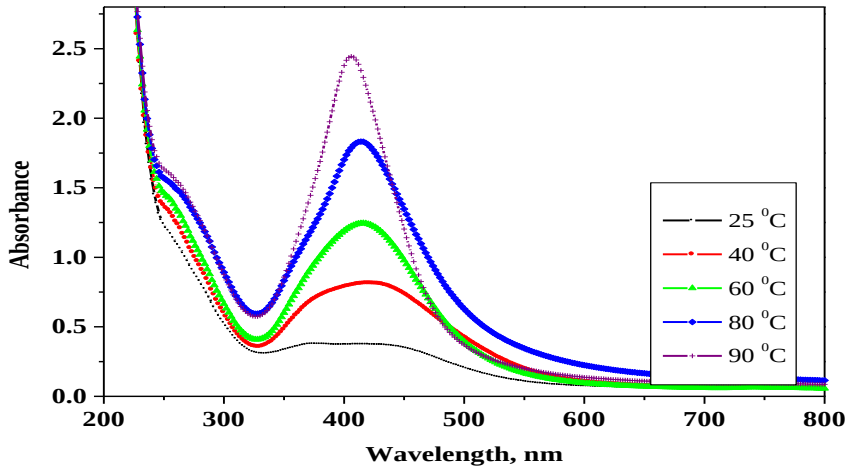

Figure (4): UV–Vis spectra of Ag NPs as a function of temperature (0.2 ml of AgNO3 and 3 ml guava leaf extract)

4) Effect of PH: Fig. (5) shows the effect of pH on the formation of AgNPs. The reaction was studied at different pH using 2 ml leaf extract and 0.2 ml AgNO3. The major effect of the PH of reaction 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. The particle size is expected to be larger in acidic medium than in basic medium. TEM measurement confirms this results Fig. (6) a, b. There is no SPR peaks at pH 1.19 and 2.15 PH, which suggests that acidic is not favorable for the AgNPs synthesis. The SPR peak increases at alkaline pH of 4.35, 5.35, and 6.46 till 8.49 PH due to the increased formation of AgNPs. However, at pH 9.53, there is an agglomeration of particles due to poor stability of AgNPs. These reports are in well agreement with the earlier reports (C Krishnaraj *et al*., 2012).

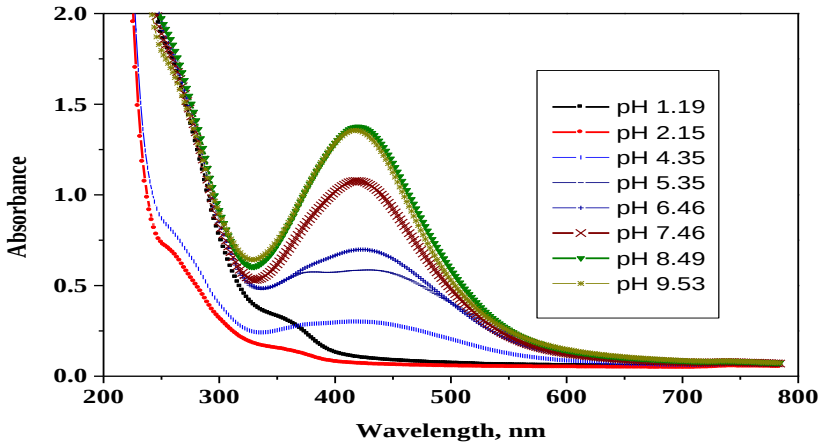

Figure (5): Effect of pH on the formation of Ag NPs at room temperature

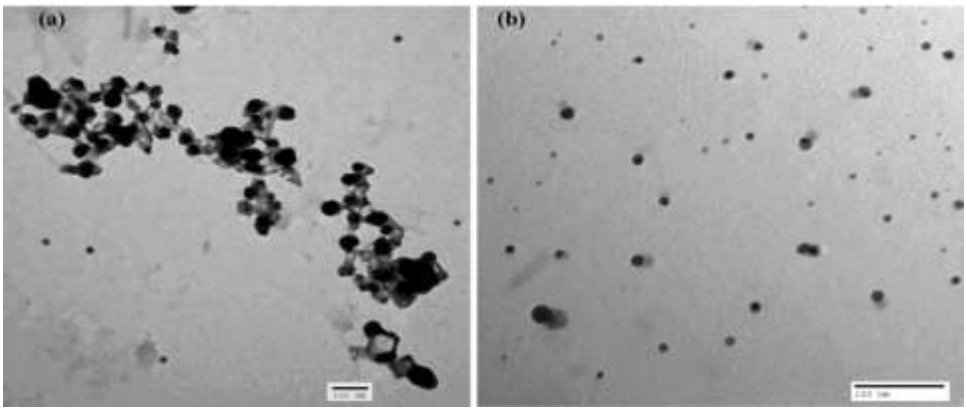

Figure (6): TEM micrograph of the silver nanoparticles (a) at pH 4.4; (b) at pH 8.49

5) X-ray diffraction (XRD): Analysis of AgNPs using X-ray diffraction confirmed the crystalline nature of particles Fig. (7). The XRD peaks at 2θ= 38, 44, 64, 77, which clearly indicates the presence of (111), (200), (220) and (311) sets of lattice plane and indexed as the band for face centered cubic structure (fcc) of silver. The mean grain size of the AgNPs was calculated using the Debye-Scherrer's equation D= 0.94ƛ/βcosθ, where D is the particle diameter size perpendicular to the reflecting planes, ƛ is the X-ray wavelength, β is the full width at half maximum (FWHM), and θ is the diffraction angle. XRD pattern thus clearly illustrates that the silver nanoparticles formed in this present synthesis are crystalline in nature. In addition, some small peaks were also observed suggesting that the presence of mixed phase of Ag/Ag2O of the nanoparticles (Firdhouse and Lalitha, 2016). Hence, from XRD pattern, it is clear that AgNPs formed using Psidium guajava (leaf) broth were essentially crystalline.

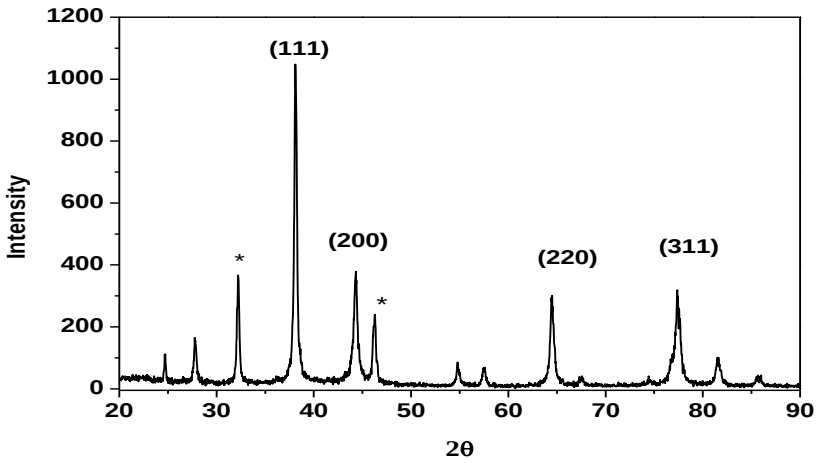

Figure (7): X-ray diffraction pattern of Ag nanoparticles prepared with aqueous guava leaf extract

6) Fourier transform infrared spectroscopy (FTIR): FTIR analysis was determined to identify the function groups of some molecules associated with AgNPs, from Psidium guajava leaf extract which is responsible for reduction, capping and efficient stabilization of the AgNPs. FTIR spectra of Psidium guajava leaf and capped AgNPs are shown in Fig. (8). Previous studies showed that Psidium guajava leaf water extract contain mainly tannins, eugenol and flavonoids as quercetin. FTIR spectra of Psidium guajava leaf showed a strong broad band at 3430 cm-1 (-OH) and shoulder at 1730 cm-1 due to(C=O) and 1620 cm-1 (amide), 1450 (C-C=C), 1363 (N-O), 1040 (C-O), 835 (alkenes), and 702 (aromatic rings), represents the different functional groups present in the P. guajava leaf extract. FTIR peaks similar to that of the extract with respective shift due to attachment of the extract biomolecules on the AgNPs surface. It should be mentioned

that the band at 3440 cm-1 appeared sharper in AgNPs than extract due to consumption of a -OH groups in the reduction process.

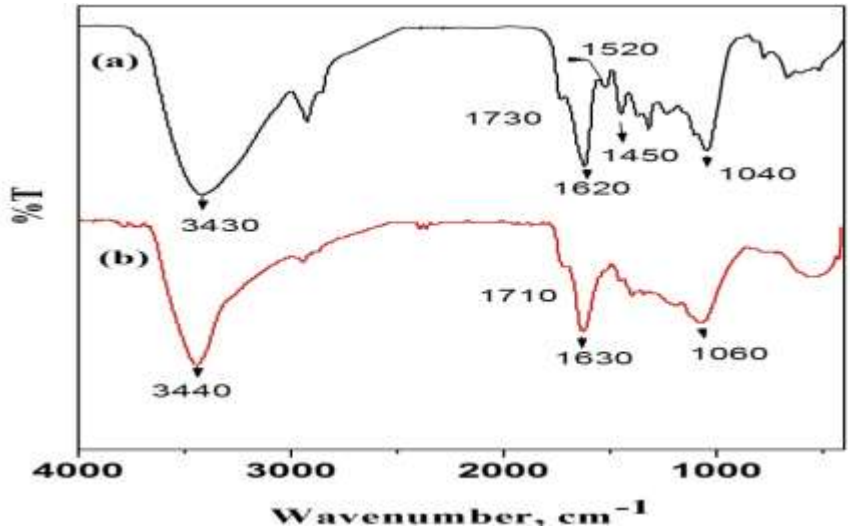

Figure (8): FTIR spectra of (a) a green guava leaf and (b) capped AgNPs

7) **Thermal gravimetric analysis:** The TGA plot of the capped Ag NPs prepared using 5 ml guava leaf extract Fig. (9) showed a steady weight loss in the temperature range of 160–600 0C. The weight loss of the nano-powder due to desorption of bioorganic compounds in the AgNPs was 32.2%.

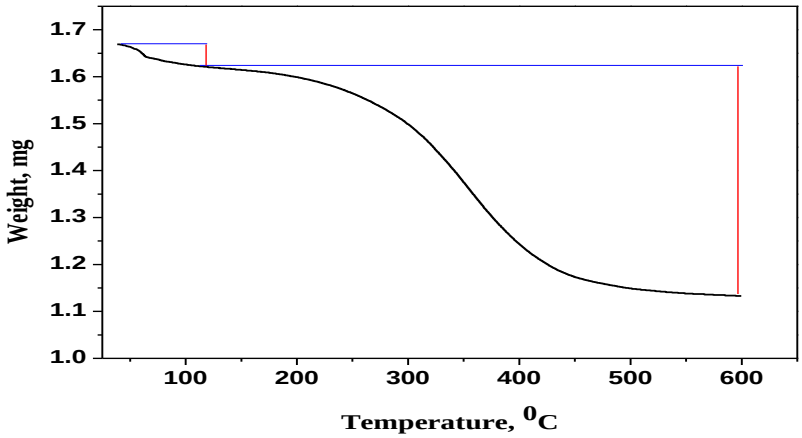

Figure (9): TGA of capped Ag NPs prepared using guava leaf extract

8) Possible mechanism of green AgNPs synthesis: The guava leaf extract has already investigated and showed the presence of flavonoids (Arima H and Danno, 2002; Begum *et al.*, 2004). The possible reduction of Ag+ ions and the consumption of –OH groups during reduction have been discussed in the above-proposed model, Fig. (10) (Jain and Mehata, 2017). It has suggested that the enol form present in tannins and flavonoids is changed to the quinonoid form after reduction this causes the shifting of the –OH group peak to a higher frequency from 3430 to 3440 cm−1.

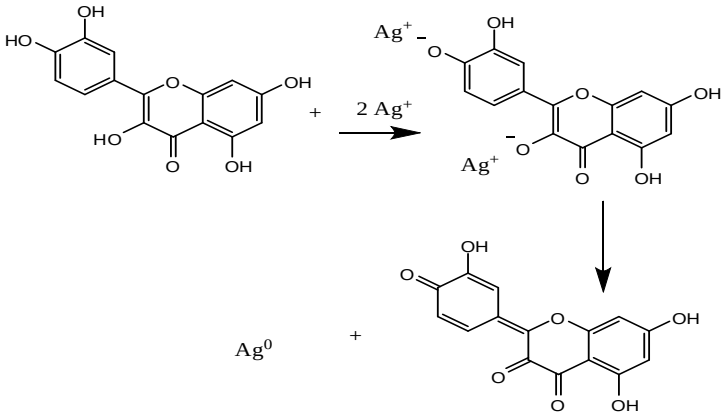

Figure (10): Proposed mechanism for bio-reduction and synthesis of AgNps

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تحضير جزيئات الفضة النانومترية باستخدام مستخلص ورقة نبات الجوافة [١]

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المستخلص

ويتضمن هذا الفصل التخليق الحيوي لجسيمات الفضة النانوية باستخدام مستخلص أوراق الجوافة كعامل إختزال. وقد تم توصيف جزيئات الفضة النانومترية باستخدام ,UV-Spectroscopy Transmisson Electron microscope, Thermal gravimetric analysis and X-ray diffraction. وقد تم دراسة تأثير بعض العوامل علي تكوين جسيمات الفضة مثل تركيز مستخلص النبات والاس الهيدروجينيي والوقت علي الشكل والحجم للجسيمات المتكونة من جزيئات الفضة.

واوضحت النتائج ان معدل تكوين جزيئات الفضة النانومترية يزداد في الوسط القاعدي وزيادة درجة الحرارة. واوضحت صور الميكروسكوب الالكتروني ان الجسمات المتكونة ذات اشكال واحجام مختلفة. ومن خلال ميكانيكية الاشعة السينية تم التأكد من ان جسيمات الفضة المتكونة لها شكل بللوري. واوضحت الاشعة التحت حمراء ان جسيمات الفضة قد تم تغليفها من خلال مركبات المستخلص النباتي. وهذا يشير الي احتمالية تغليف الجسيمات المتكونة بالمركبات الفينولية من خلال مجموعات خاصة بها وهذا يساهم بدوره في مدي استقرارية الجسيمات المتكونة. كمية المواد الممتصة على سطح جسيمات الفضة تم تقديرها عن طريق قياس التحلل الحرارى للجسيمات.