Kufa Journal for Veterinary Medical Sciences Vol.(7). No.(1) 2016

Kufa Journal for Veterinary Medical Sciences
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Biosynthesis of Silver Nanoparticles using *Cinnamon zeylanicum* Plants Bark Extract

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Abstract

Nanoparticles are a special group of materials with unique features and extensive applications in diverse fields. The use of nanoparticles of some metals is common and useful in several fields due to the good properties of these nanoparticles. In this work, silver nanoparticles synthesized by extract of *Cinnamon zeylanicum* plants barks were investigated. 1ml of *Cinnamon zeylanicum* plants barks extract was added to 50 ml of 1mM aqueous silver nitrate ($AgNO_3$) solution at the room temperature for (1hr -8hrs) to produce silver nanoparticles. A change in the color was observed. The color intensity increased with increasing reaction time. After 2 hrs, the color intensity was even higher; and giving a darker looks after 8 hrs. Separation by centrifugation, characterization using UV-VIS spectrophotometry and electron microscopy analysis were performed. The centrifugation lead to formation of 0.35 gm silver nanoparticles by 100 ml of *Cinnamon zeylanicum* plants barks extract. The UV-Vis spectral analysis showed silver surface plasmon resonance band at 400 nm. Electron microscopy showed that the particles were crystalline in nature, most of the nanoparticles were roughly spherical or circular in shape with smooth edges were observed, while the average size of the nanoparticles ranged between 8 and 20 nm. The approach of green synthesis seems to be cost effective, eco-friendly and easy alternative to conventional methods of silver nanoparticles synthesis.

التصنيع الحيوي لدقائق الفضة النانوية باستخدام مستخلص سيقان نبات القرفة

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الخلاصة:

ان الاجسام النانوية هي مجموعة خاصة من المواد ذات المواصفات الفريدة والتطبيقات الواسعة في المجالات المختلفة . لقد وجد ان استعمال الاجسام النانوية لبعض المعادن شائعا وذو فائدة في عدة مجالات نتيجة للخواص الجيدة التي تمتلكها هذه الاجسام النانوية. تم في هذه الدراسة تصنيع دقائق الفضة النانوية بواسطة مستخلص

سيقان نبات القرفة. اذ تم مزج 1 مل من المستخلص النباتي مع 50 مل من محلول نترات الفضة المحضرة بتركيز 1 ملي مولاري بدرجة حرارة الغرفة ولمدة 1-8 ساعات لانتاج دقائق الفضة النانوية حيث لوحظ زيادة كثافة اللون مع تقدم الوقت وكان اكثر كثافة بعد 8 ساعات. لفصل الدقائق المتشكلة تم استعمال الطرد المركزي اما تشخيصها فتم باستخدام المقياس الطيفي والمجهر الالكتروني. ادى الطرد المركزي الى تكوين 0.35غم من دقائق الفضة النانوية باستخدام 100مل من مسخلص سيقان نبات القرفة. ظهرت حزمة التصوير الطيفي عند القراءة 400 نانوميتر واظهر المجهر الالكتروني بان جزيئات الفضة كانت بتركيب كروي دائري الشكل وبمعدل حجم يتراوح بين 8-20 نانوميتر. بذلك يعد تصنيع دقائق الفضة النانوية بهذه الطريقة البايولوجية الصديقة للبيئة سهلا وغير مكلفا ويقود نحو استخدامها كطريقة بديلة للطرق التقليدية المستخدمة في تصنيع دقائق الفضة النانوية.

Introduction

Nanotechnology is the newest and one of the most promising areas of research in modern medical science. Nanoparticles are usually a cluster of atoms ranging between 1-100 nm in size and exhibit new and improved properties based on size, distribution and morphology than larger particles of the bulk materials from which the nanoparticles are made [1,2,3,4]. Silver nanoparticles have found wide applications in the area of catalysis, optoelectronics, detection and diagnostic, antimicrobials and therapeutics [5,6,7,8]. Silver has long been recognized as an effective antimicrobial agent that exhibits low toxicity in humans and has diverse in vitro and in vivo applications [9]. Recently, silver-based topical dressings are widely used to treat infections in open wounds and chronic ulcers [10]. Several approaches are out there for the synthesis of silver nanoparticles for example, chemical reduction [11], photochemical [12], thermal decomposition [13], radiation assisted [14], electrochemical [15], and recently via green chemistry method [16].

Biological method of nanoparticles synthesis using microorganisms [17], enzyme [18], and plant or plant extract offers numerous benefits over chemical and physical method [16]. Among the various known synthesis methods, plant mediated nanoparticles synthesis is preferred as it is cost-effective, environmentally friendly, and safe for human therapeutic use [19, 20]. Many reports are available on the biogenesis of silver nanoparticles using several plant extracts [21].

Cinnamomum zeylanicum is a small, tropical, evergreen tree most noted for its bark, which provides the world with the commonly known spice, cinnamon. *Cinnamomum zeylanicum* is the scientific name, which refers to the specific species of tree that cinnamon is harvested from. The name cinnamon is in fact the shortened version of its true common name, Ceylon cinnamon. Ceylon is an extinct term for the country Sri Lanka, the native country of *Cinnamomum zeylanicum* [22,23].

C. zeylanicum bark is widely used as a spice. It is principally employed in cooking as a condiment and flavoring material. In In folk medicine, it acts like other volatile oils and was once used as a cure for colds. It has also been used to treat diarrhea and other problems of the digestive system. *C. zeylanicum* bark is high in antioxidant activity [24,25]. The essential oil of cinnamon also has antimicrobial properties, which can aid in the preservation of certain foods [26,27] . *C. zeylanicum* bark has been reported to have remarkable pharmacological effects in the treatment of type II diabetes and insulin resistance [28].

C. zeylanicum bark is rich in terpenoids, including linalool, eugenol and methyl chavicol, and in chemicals, including resinous compounds, cinnamaldehyde, ethyl cinnamate and caryophyllene, Cinnamic acid, L-borneol, L-bornyl acetate, E-nerolidol, and cinnamyl acetate which contribute to its distinct aroma [29,30]. In addition, some protein is also present in the bark. Terpenoids are believed to play an important role in silver nanoparticle biosynthesis through the reduction of silver (Ag) ions [31].This work was aimed to Biosynthesis of silver nanoparticles by plant extract, and characterization of silver nanoparticles.

Materials and methods

Plant Materials:

Cinnamon zeylanicum barks that were collected from local market in Baghdad were dried naturally in room temperature at shade for a week for complete moisture removal**.** The barks were cut into small pieces, crushed to a fine powder by an electrical grinder then sieved using a 20-mesh sieve to get uniformed size range. The final sieved powder was used for all further studies (figure: 1) [32]. The plant classification was done in the Ministry of Agriculture/ State Board for Seeds Testing and Certification S.B.S.T.C in Abu Graib /Baghdad at certificate No. 1077 in 26 / 3 / 2014.

Figure (1): Barks and powder of *C. zeylanicum*.

Biosynthesis of Silver Nanoparticles Using *Cinnamon zeylanicum* Plants:

A- Preparation of *Cinnamon zeylanicum* Plants Barks Extract:

The extract was produced by adding 2.5gm of *Cinnamon zeylanicum* plants barks powder into 100 ml of distilled water and boiled for 5 minutes in 500 ml flask and after cooling was filtered using whatman No.1 filter paper (figure: 2). The final extract was kept at 4◦C [32,33].

B-Preparation of 1mM Silver Nitrate Solution:

For the preparation of 1mM Silver nitrate ($AgNO_3$) 0.0421gms of $AgNO_3$ was dissolved in 100 ml of double distilled water. The solution was mixed thoroughly and stored in yellow colored bottle to prevent auto oxidation of silver [34] .

C-Synthesis of Silver Nanoparticles using *Cinnamon zeylanicum* Plants Barks Extract:

One (1ml) of *Cinnamon zeylanicum* plants barks extract was added to 50 ml of 1mM aqueous silver nitrate ($AgNO_3$) solution and kept at room temperature for 8hrs to produce silver nanoparticles [32]. The solution initially appeared yellowish in color and upon reduction of nitrate from silver Ag^+ to free reduced form change to dark color. The changing of color solution was measured at every 1hrs. The changing in the color intensity after the reduction of Ag+ to silver nanoparticles by *Cinnamon zeylanicum* plants barks extracts with increasing time of reaction was recorded.

Figure (2): ***Cinnamon zeylanicum* plants barks extract.**

Separation and Identification of Silver Nanoparticles:

1-Spectrophotometery:

Formation and stability of silver nanoparticles in sterile distilled water is confirmed with UV-vis spectrophotometer in a range of wavelength from 100 to 700 nm. For the UV-Vis spectral analysis. (1ml *Cinnamon zeylanicum* plants barks extract treated with 50 ml of 1mM aqueous silver nitrate). This solution was kept under UV light at 250 nm for 1-8hrs.Absorbance was measured at 1hr interval and the changing in the color was observed gradually as it turned dark brown at the end of 8hrs of exposure to UV radiation [(35)].

2-Centerfugtion:

Silver nanoparticles solution which was obtained from (1ml *Cinnamon zeylanicum* plants barks extract /50 ml of 1mM aqueous silver nitrate) was centrifuged at 10,000 rpm for 30 min. The pellet was washed three times with 20 ml of distilled water, and finally dried at 60°C, to get rid of the free proteins/ enzymes that are not capping the silver nanoparticles. The centrifugation process were done at time (1-8hrs). This was done in order to know the effect of time incubation on amount of silver nanoparticles synthesized [(36)].

3-Electron Microscopy:

Huang *et al.*, [(37)] reported that there was no marked difference in the shape and size of SNPs at various initial biomaterial concentrations, therefore the samples for electron microscopy were prepared from the concentration (ml of extract/50ml of 1mM aqueous silver nitrate) and various time of reaction. The biomass had settled at the bottom of the conical flasks and the suspension above the precipitate was sampled for transmission electron microscopy (TEM) observation. Transmission electron microscopy samples of the aqueous solution of silver nanoparticles prepared by placing a drop of the solution on carbon-coated copper grids and the films on the TEM grids allowed standing for 2 min, after which the extra solution removed using a blotting paper and drying the grid. The size distribution of the resulting nanoparticles estimated on the basis of TEM micrographs [38].

Results:

Synthesis of Silver Nanoparticles Using *Cinnamon zeylanicum* Plants Barks Extract:

For the green synthesis of silver nanoparticles by *C. zeylanicum* plant extracts were carried by 1 ml of extract was added to 50 ml of 1mM $AgNO_3$ solution.

We adopted a simple procedure to synthesize silver nanoparticles from cinnamon bark extract. On mixing the plant extract of *C. zeylanicum* with silver nitrate solution (1mM), the color of the reaction mixture started changing to yellowish within 1hr and to dark brown after 8 h, indicating the generation of silver nanoparticles, due to the reduction of silver metal ions Ag+ into silver nanoparticles Ag0 via the active molecules present in the *C. zeylanicum* plant extracts. Changing in color after the reduction of Ag+ to silver nanoparticles is shown in (Figure: 3). The reduction rate and formation of nanoparticles can be increased further by increase in incubation time.

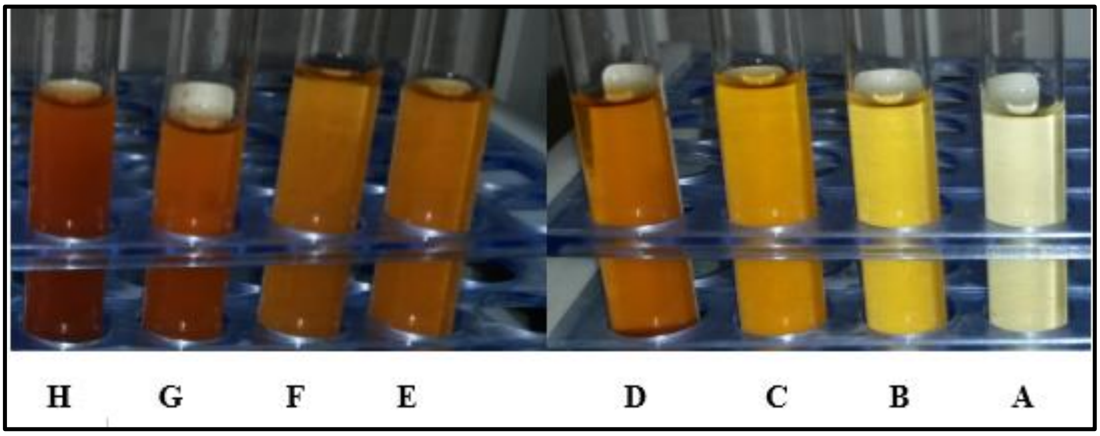

Figure (3): Change in color after the reduction of Ag+ to silver nanoparticles by *Cinnamon zeylanicum* plants barks extracts 1ml(CBPE) /50 ml(AgNO3), at: (A) 1hrs, (B) 2hrs, (C) 3hrs, (D) 4hrs, (E)5hrs, (F)6hrs, (G)7hrs and (H)8hrs.

Separation and Identification of Silver Nanoparticles:

Spectrophotometry.

The formation of silver nanoparticles was monitored with color change and UV-Vis spectrum. The color of the reaction mixture started changing to yellowish brown within 1hr and to dark brown after 8 hr. This color is attributed to the excitation of surface plasmon resonance. The absorption spectra of silver nanoparticles solution consists a single sharp surface plasmon resonance band at 405 nm (Figure: 4 and Table: 1). The most characteristic part of silver solution is a narrow plasmon absorption band observable in the 350 – 600 nm regions. The distinct visible peak was observed at 405nm which is an indication of reduction of silver. This indicates that by UV method, silver gets reduced in a faster way than the conventional method. Control silver nitrate solution neither developed the dark brown color nor did they display the characteristic band, indicating that abiotic reduction of silver nitrate did not occur under the used conditions.

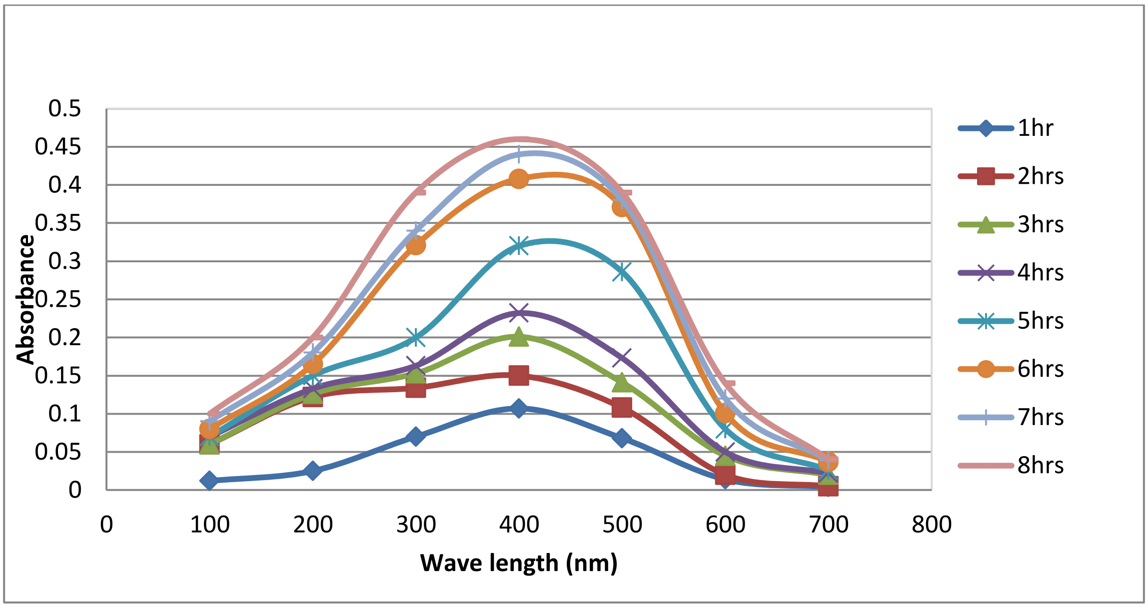

Figure (4): UV-Visible absorption spectra of synthesized silver nanoparticles, showing the surface plasmon resonance peak of silver nanoparticles at 425 nm, showing the color change upon formation of silver nanoparticles at different incubation time.

Table (1): Absorbance values obtained by spectrophotometer at wave length 400nm in different time.

Duration time	Absorbance at 400nm(peak)
A-1 hrs	0.107
B-2 hrs	0.150
C-3 hrs	0.201
D-4 hrs	0.232
E-5 hrs	0.320
F-6 hrs	0.408
G-7 hrs	0.44
H-8 hrs	o.46

Centrifugation:

After the centrifugation of silver nanoparticles solution at different incubation time interval (1-8hrs), the samples were dried and weighing after washed three times with 20 ml of de-ionized water to get rid of the free proteins/ enzymes that are not capping the silver nanoparticles. The changes in pellet weight (gm/time) gave an evidence of correlation between incubation time and the amount of synthesized silver nanoparticles, as in table (2). After 1hs and 2hrs of incubation time, there was a decrease in pellet weight when they were compared with 6, 7 and 8 hrs of incubation time. 1 ml of *C. zeylanicum* plant extract mixing with 50 ml of 1mM $AgNO_3$ solution, gave a deep dark color pellet. (Figure:5).

Table (2): Amount of the silver nanoparticles (SNPs) that synthesized by *Cinnamon zeylanicum* barks powder extract (CBPE).

CBPE(ml)	Time	SNPs(gm)
100ml	1hrs	0.11
100ml	2hrs	0.15
100ml	3hrs	0.18
100ml	4hrs	0.20
100ml	5hrs	0.24
100ml	6hrs	0.28
100ml	7hrs	0.30
100ml	8hrs	0.35

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Figure (5): Appearance of silver nanoparticles after centrifugation.

Electron Microscopy:

Electron Microscopy images of silver nanoparticles solution are shown in (Figure: 6). These observations indicate the adsorption and/or deposition of silver nanoparticles onto the surface of roughly sphere-shaped polydispersed particles. The Ag-nanoparticles that emerged in the images have variety of shapes: spherical, triangle and irregular. As can be seen in (Figure: 6) typical example, presence of rings patterns in the selected area electron diffraction reveals the single face-centered cubic crystalline nature of the spherical nanoparticles with a preferential growth direction along the Ag. The shape evolution of photomorphic silver nanoparticles (SNPs) was noticed in electron microscope images of samples prepared at various times. The average size of the nanoparticles ranged between 8 and 20 nm for *C. zeylanicum* bark extract, with a few larger particles exceeding 60 nm only in the case of *C. zeylanicum* bark extract at the longer reaction time.

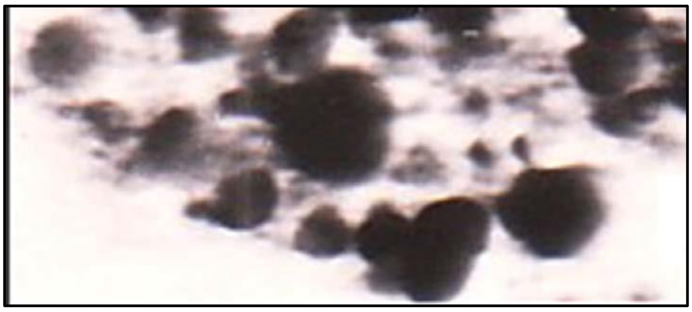

Figure (6): Crystalline clusters of silver nanoparticles.

Discussion:

Synthesis of silver nanoparticles using *Cinnamon zeylanicum* plants barks extract:

The extract was already pale and the color of the solution was immediately intensified by the addition of the aqueous AgNO3 solution. The color intensity increased with time. After 2 hrs, the color intensity was even higher; and giving a darker looks after 8 hrs. The intensity of the color increased with increasing the time of reaction and this in agreement with (32). Due to excitation of surface plasmon vibrations in the metal nanoparticles (39,40). *C. zeylanicum* bark is rich in terpenoids, including linalool, eugenol and methyl chavicol (29,30). In addition; some protein is also present in the bark. Terpenoids are believed to play an important role in silver nanoparticle biosynthesis through the reduction of silver (Ag) ions (31). This result is in agreement with Raut *et al*., (41) which they reported that reduction of silver ion into silver nanoparticles during exposure to the plant extracts could be followed by colour change due to excitation of surface plasmon vibrations in silver nanoparticle. Silver nanoparticles exhibit interesting optical properties directly associated with localized surface plasmon resonance which is highly depends on the morphology of the nanoparticles (16).

Centrifugation:

Centrifugation of silver nanoparticles solution, refers to ability to production of good amounts of SNPs by using plants with low cost, low hazardous and high eco-friendly properties, this in agreement with (32).

Spectrophotometry:

Increase in absorbance with respect to time was noticed in UV–vis spectrum. The peak area at 400 nm was increased with increasing reaction time. The sharp peak at around 400 nm is the absorbance spectra emanating from the Ag nanoparticles, previous studies have shown that the spherical Ag-NPs contribute to the absorption bands at around 400 nm in the UV–visible spectra (42,43). The increase in the absorbance values with increasing reaction time demonstrates the higher production of silver nanoparticles, which is due to the availability of more reducing biomolecules for the reduction of silver ions at long duration. Similar results were reported by Huang *et al*., (37). The silver nanoparticles produced from *Cinnamon zeylanicum* plants barks extract were observed to be very stable in the solution, even 3 months after their synthesis, which validates the application of *Cinnamon zeylanicum* plants barks extract as biomaterials for the synthesis of silver nanoparticles (36). *C. zeylanicum* bark is rich in terpenoids, including linalool, eugenol and methyl chavicol, and in chemicals, including cinnamaldehyde, ethyl cinnamate and caryophyllene [29].In addition; some protein is also present in the bark. Terpenoids are believed to play an important role in silver nanoparticle biosynthesis through the reduction of Ag ions. Shankar *et al*., [31] reported the possibility of terpenoids from geranium leaf in the synthesis of nano-sized silver particles. Polyols such as terpenoids, flavones and polysaccharides in the *Cinnamomum camphora* leaf were reported to be the main cause of the bioreduction of silver ions and proteins are reported to bind to the nanoparticles either through free amine groups or cysteine residues in the proteins [44]. A similar mechanism may have operated in the present case where the proteins extracted from the *C. zeylanicum* bark capped the Ag nanoparticles, thereby stabilizing them. To summarize these results, the water-soluble fractions comprised of complex polyols [45,46] in the biomass were believed to have played a major role in the bioreduction of Ag ions.Optical properties and colour exhibited by silver nanoparticles are due to surface plasmon resonance [39,40].

Shankar *et al*., [31] reported that the peak at 370 nm corresponds to the transverse plasmon vibration in the Ag nanoparticles, whereas the peak at 440 nm was due to the excitation of longitudinal plasmon vibrations also reported that quasi-linear superstructures of nanoparticles when geranium leaf extract was used as the biomaterial. Similar results were reported by Huang *et al.*, [37]. Our results display that quasisymmetric absorption band centered at 400 nm, which indicates that the nanoparticles in the solution are quasispherical approximately 8 nm in diameter, such this structures were observed in all dosages of *C. zeylanicum* bark extract and this in agreement with [47,48,49].

Electron microscopy:

Most of the nanoparticles were roughly spherical or circular in shape with smooth edges were observed in *C. zeylanicum* bark extract dosages.

The particle size distribution varied marginally with variation in the reaction time. The number of particles increased with increasing time due to the variation in the amount of reductive molecules. These results were in agreement with those reported by [50] and [51].

Conclusion:

From the previous results, we can concluded that the approach of green synthesis seems to be cost effective, eco-friendly and easy alternative to conventional methods of silver nanoparticles synthesis.

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