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Green synthesis of silver nanoparticles using oak leaf and fruit extracts (*Quercus*) and its antibacterial activity against plant pathogenic bacteria

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Abstract

Silver Nanoparticles as one of Nanotechnology products has extensive medical, industrial and agricultural applications. This paper describes a rapid and eco-friendly method for green synthesis of silver nanoparticles from aqueous solution of silver nitrate using Oak leaf and fruit extracts (*Quercus infectoria*) in a single-pot process. It was observed that the use of Oak leaf and fruit extracts makes a fast and convenient method for the synthesis of silver nanoparticles and can reduce silver ions into silver nanoparticles within 80 min of reaction time without using any severe conditions. Green synthesis of silver nanoparticles (AgNPs) was characterized by UV-visible (UV–vis) spectroscopy. The UV–vis spectra gave surface Plasmon resonance for synthesized silver nanoparticles peak at 415–445 nm. Further, the AgNPs showed an effective antibacterial activity toward plant pathogenic bacteria.

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Introduction

Nanotechnology is an important enabling technology which has wide variety of potential applications in the biomedical, agricultural, optical, and electronic fields (Albrecht *et al*., 2006). Silver nanoparticles exhibit new or improved properties depending upon their size, morphology, and distribution. Production of nanoparticles can be achieved through different methods. There are various physical and chemical methods employed for the synthesis of metal nanoparticles (Shankar *et al*., 2004; Panacek *et al*., 2006). However, these methods have certain disadvantages due to involvement of hazardous chemicals, high-energy requirements and radiation. Biological methods of nanoparticles synthesis using microorganism like algae (Lengke *et al*., 2006), bacteria (Husseiny *et al*., 2007; Shahverdi *et al*., 2007), fungi (Gajbhiye *et al*., 2009; Govender *et al*., 2009), and actinomycetes (Ahmad *et al*., 2003), and plant or plant extract have been suggested as possible eco-friendly alternatives to chemical and physical methods. Using plant for nanoparticles synthesis can be advantageous over other biological processes by eliminating the elaborate process of maintaining cell cultures (Shankar *et al*., 2004). Synthesis of nanoparticles also can be suitably scaled up for large-scale and cost effective (Parikh *et al*., 2008).Many research papers reported the synthesis of silver nanoparticles using plant extracts such as *Acalypha indica* leaf (Krishnaraj *et al*.,2010) *Trianthema decandra* roots (Geethalakshmi and Sarada, 2010) *Sesuvium portulacastrum* leaves (Nabikhan *et al*.,2010); *Ocimum sanctum* (Tulsi) leaf (Singhal *et al*.,2011); *Garcinia mangostana* (mangosteen) leaf (Veerasamy *et al*.,2011) *Nicotiana tobaccum* leaf (Parasad *et al*.,2011); *Ocimum tenuiflorum*, *Solanum trilobatum*, *Syzygium cumini*, *Centella asiatica*, and *Citrus sinensis* leaves (Logeswari *et al*., 2012); *Ficus benghalensis* leaf (Saxena *et al*., 2012); and *Olea europaea* leaves (Awwad *et al*., 2012).In this present investigation we are going to report a green method for the synthesis of silver nanoparticles using aqueous Oak leaf and fruit extracts and no toxic chemicals are used as reducing and stabilizing agent during the synthesis. *Q. infectoria* is a tree of significant economic importance.

Oak is one of the most important and abundant species in west of country specifically in Zagros zone. Zagros mountain range is the widest and major growth place of different species of oak in Iran, so this zone is very important. South Zagros is the growth place of special species, Q. Brantii, that it includes wide part of Iran like as Ilam. Every year more amount of produced oak is destroyed without using. Antimicrobial properties are one of the most important and applicable properties of silver nanoparticles that is used in medical, industry and agriculture fields. Most studies showed effective activity of silver nanoparticles in human photogene bacteria (Quang *et al*., 2013). However, their effects on plant's pathogen bacteria are not studied completely. Silver nanoparticles can be used in prevention and controlling damages of plants photogenes infection in future.

Materials and methods

Materials

All analytical reagents and media components were purchased from Sigma Chemicals (St. Louis, MO, USA). Deionized water was used throughout the reactions. All glass wares were washed with dilute nitric acid HNO 3 and distilled water, then dried in hot air oven.

Preparation of oak leaf and fruit extracts

Fresh leaf and fruit of *Q. infectoria*, Fig. 1, were collected from local source, Iran, Ilam. *Q. infectoria* leaf and fruit were washed several times with water to remove the dust particles and then sun dried for two days to remove the residual moisture. The dried *Q. infectoria* leaf and fruit were cut into small pieces and were milled using a mortar. 3 g of ground fruit were boiled in a 250-ml glass beaker along with 100 ml of sterile distilled water for 10 min in 60 ˚C, separately.Then the extract was cooled to room temperature and filtered with Whatman No. 1 filter paper, once for leaf and twice for fruit extract. Filtrate was collected and stored in the dark at 4 ˚C to be used within one week.

Methods

Synthesis of AgNPs

In a typical reaction procedure, 1 ml of *Q. infectoria* leaf and fruit was added to 30 ml of 1 × 10 −3 M aqueous $AgNO_3$ solution, at room temperature. The transparent color of the mixture of silver nitrate and *Q. infectoria* fruit extract at 0 min of reaction time changed very fast at room temperature after 10 min and become to a deep brown suspended mixture after 80 min. Changing in color of the mixture of silver nitrate and *Q. infectoria* leaf extract lasted more time and become to a brown suspended mixture after 80 min. This indicated that *Q. infectoria* fruit speeds up the biosynthesis of silver nanoparticles more than leaf extract. The ratio of fruit and leaf extract and $AgNO_3$ solution were 1 to 30, 2 to 30 and 3 to 30 by volume, respectively. In addition of room temperature the study was done in 30, 60 and 90 ˚C and different pH values for investigation of temperature and pH effect in speed of silver nanoparticles formation. The pH of the solutions was adjusted using 0.1 N H 3 PO 4 or 0.1 N NaOH solutions.UV-visible (UV–vis) spectra showed strong surface plasmon resonance (SPR) peak at 415–445 nm and thus indicating the formation of silver nanoparticles. The AgNPs obtained by *Q. infectoria* leaf and fruit were centrifuged at 15,000 rpm for 5 min and subsequently dispersed in sterile distilled water to get rid of any uncoordinated biological materials.

Antimicrobial activity assay

Bacterial strains and isolates

Three identified isolates namely *Pectobacterium carotovorum*, *Ralstonia solanacearum* , *Erwinia amylovora* and *Xanthomonas citri* were supplied from plant pathology department, Ilam University, Ilam, Iran. All bacterial were maintained routinely on nutrient agar at 4 ˚C. A twenty-four hour nutrient broth culture of tested bacteria was grown in an orbital shaking incubator, centrifuged, washed twice with PBS and suspended in sterile distilled water. The disc diffusion method was employed to determine the rate of growth inhibition of bacteria by the nanoparticles solution. Plates (9-cm diameter) were prepared with 25 ml pre warmed (40- 50 °C) nutrient agar (NA) inoculated with 100 µl of suspension containing 10^8 CFU/ml of each microbes. The 50 µl of the sample of nanoparticles solution were loaded on 6 mm sterile paper discs. The loaded disc was placed on the surface of the NA plate and were stored at 4 °C for 2 h and then incubated at 37 °C for 16 h. A disc (6 mm diameter) of standard antibiotic Gentamycin (10 µg/disc) was used as a positive control and 50 µl of deionized water was used as a negative control. Antimicrobial activity was evaluated by measuring the diameter of the inhibition zone around the discs (mm). All tests were performed in triplicate and the mean of the diameters of the inhibition zones was calculated, data sets were subjected to analysis of variance(ANOVA) and the Duncan's multiple range test using SPSS software (SPSS 16.0.2). In all cases, a value of $p < 0.05$ was considered significant. The data were expressed as mean ± S.D.

Results and discussion

UV–visible of Ag nanoparticles formed at room temperature

In order to monitor the formation and stability of silver nanoparticles, the absorption spectra of the synthesized silver nanoparticles were recorded against water. Fig. 2 shows the UV visible spectra of silver nanoparticle formation using constant AgNO 3 concentration (1×10^{-3} M) with different leaf and fruit extract concentrations at room temperature after 80 min. The color of the solutions changed from transparent to pale yellow to brown for leaf extract, and from pale to brown to deep brown for fruit extract depending on the extract concentration due to the production of silver nanoparticles (Fig. 3). It was observed that the surface plasmon resonance (SPR) of AgNPs peak is 415–445 nm, indicating the reduction of silver nitrate into silver nanoparticles.

As the concentration of the oak leaf and fruit extract increases, the absorption peak gets more sharpness and shift was observed from 445 to 420 nm that indicates a reduction in the mean diameter of the silver nanoparticles. The shifted and sharp narrow shape SPR band indicating the formation of spherical and homogeneous distribution of silver nanoparticles

was observed. This indicates that low quantities of the extract can reduce silver ions, but do not protect most of the quasi-spherical nanoparticles from aggregating because of the deficiency of biomolecules to act as protecting agents. On the other hand, at higher extract concentrations the biomolecules act as reducing agent and cap the nanoparticle surfaces protecting them from aggregation. Similar studies showed that a comparatively higher extract ratio is responsible for the synthesis of symmetrical nanoparticles (Sosa *et al.*, 2003).

Table 1. Zone of inhibition of silver nanoparticles synthesized by Oak leaf and Fruit Extrads against plant Pathogenic bacteria.

Silver nanopartide samples	Zone of in hibition (mm) against patho genic bacteria															
	Erwinia amylovora				*Pectobacterium carotov orum*				*Xanthomonas citri*				*Ralstonia solanacearum*			
	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
40 min	17	12	17	0	17	11	13	0	16	10	14	0	16	11	15	0
80 min	17	16	22	0	17	14	19	0	16	13	18	0	16	14	18	0

Effect of temperature

The effect of different temperature on nanoparticle formation presented by UV–visible spectra in Fig. 4. It was observed that the absorbance increases with increasing temperature due to the production of silver nanoparticles. This experiment suggests that the slow reduction rate of the Ag ions for the formation of AgNPs at room temperature can be accelerated by increasing temperature of the reaction mixture.

Fig. 1. picture of Oak leaf and Fruit.

Effect of pH

Figure 5 shows the effect of pH on formation of silver nanoparticles by fruit extract. It can be seen that absorbance increases with increasing pH from 2 to 8 and then decreases. Furthermore, it is observed that the brown color of the nanoparticles appeared shortly after mixing the $AgNO_3$ with the extract. 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. 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. The particle size is expected to be larger in acidic medium than in basic medium.

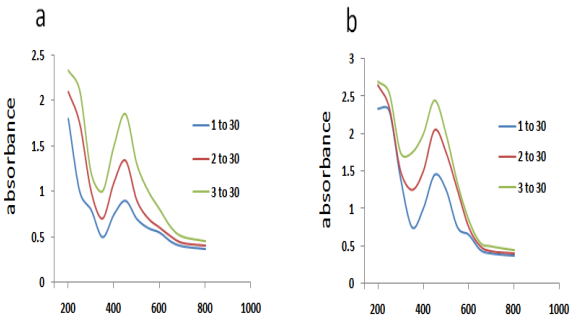

Fig. 2. UV–vis spectra of silver nanoparticles at different concentrations of leaf (a) and fruit extract(b).

Antimicrobial assay

The antimicrobial activity of silver nanoparticles synthesized by oak leaf and fruit extract was investigated against various plant pathogenic bacteria using disc diffusion method. Although many study described antibacterial activity of Ag Nps against

human pathogenic bacteria, however to date no any study investigated activity of Ag Nps against plant pathogenic bacteria (Quang *et al.*, 2013). The antibacterial activity of different solutions containing Ag Nps demonstrated that all Gram bacteria were inhibited by Ag Np solutions with different extents. The results of the antibacterial assay are presented in Table 1. Comparison of data shows that for all plant pathogen the Ag Nps solution from 80 min after start, have greater activity than Ag Nps solution from 40 min after start for Ag Nps that synthesized by both oak leaf and fruit extract. The activity of these solutions was mainly due to the different amounts of Ag-Nps formed upon lapse of time that process kept on. This was confirmed by the changing in color of the mixture as well as UV results. UV visible spectra of the two solutions revealed that conversion values of Ag+ions to Ag0 were increased for 40 to 80 min after process start. The activity of Ag-Nps synthesized by leaf extract has less activity than Ag Nps synthesized by fruit extract that may be due to the different amounts of Ag-Nps formed in two reaction or different character of Ag Nps such size, morphology or other character that affected apply antibacterial activity. For Ag-Nps of leaf extract only the 80 min showed a similar growth inhibition effect against *E. amylovora*.But Ag-Nps of leaf extract, the 80 min showed significant growth inhibition against all bacteria. The maximum growth inhibition was observed in fruit 80 min Ag Nps(22 mm) against *E. amylovora* and the minimum growth inhibition was observed in leaf 40 min Ag Nps (10 mm) against *X. citri* .

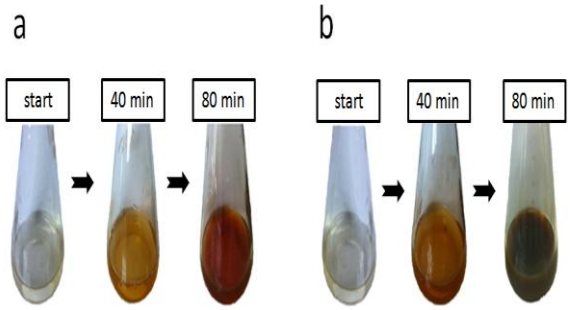

Fig. 3. Change in the color of the solution in before, 40 min and 80 min after the process of reduction changed from transparent to pale yellow to brown for leaf extract(a) , and from pale to brown to deep brown for fruit extract(b) in The ratio of 3 to 30.

Some researchers investigated the antimicrobial activity of silver nanoparticles against various plant pathogenic fungi. Silver nanoparticles were effective against against *viz. Botrytis cinerea*, *Rhizoctonia solani*, *Colletotrichum gloeosporioides*, *Magnaporthe grisea* and *Pythium ultimum*, *Raffaelea sp.* (Kim *et al.*, 2009) *B. sorokiniana* and *M. grisea* (Jo *et al.*, 2009), Fusarium and Phoma fungi (Gajbhiye *et al.*, 2009).

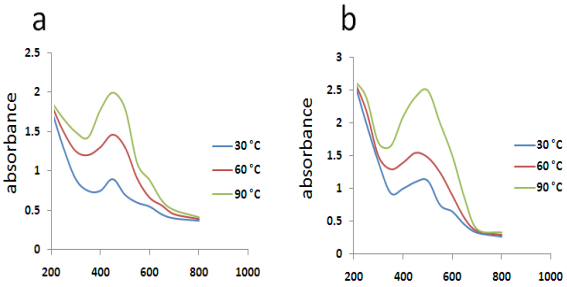

Fig. 4. UV–vis spectra of Ag NPs as a function of temperature (10^{-3} M $AgNO_3$ and 1 ml leaf (a) and fruit (b) extract).

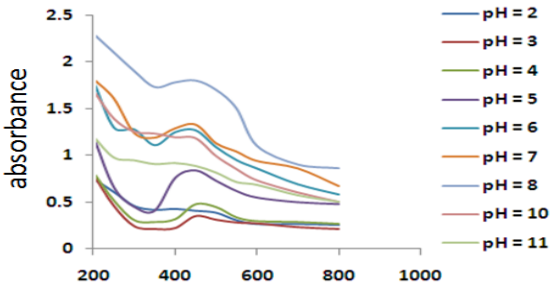

Fig. 5. Effect of pH on the formation of Ag NPs at room temperature.

In this study silver nanoparticle was effective against plant pathogenic bacteria.The species are a plant pathogen with a diverse host range, including many agriculturally and scientifically important plant species. They are a very economically important pathogen due to its lethality, persistence, contagious, wide host range and broad geographic distribution. Commercial chemicals have generally proven to be ineffective in controlling the pathogen and are not recommended as a means of control (Rodriguez *et al.*, 2012; Gnanamanickam, 2007), in addition, the use of some antibiotics for prevent new infections has led to resistant bacteria in some areas (Iank *et al.*, 2003). Investigation of antibacterial activity against plant pathogenic bacteria can be utilized as a new and

effective approach for prevention and control of plant pathogen infection in future.

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