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RESEARCH ARTICLE

New Approaches for Preparation of Silver nanoparticles using Quince Plant Extract as Antibacterial Agents

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Abstract

Nanoparticles have been considered as one of the emerging and promising platforms to solve the problem of antibacterial resistance. The uses of plants in synthesis of nanoparticles are new method as its cheap, environmental friendly and easily synthesis. In this paper silver nanoparticles were prepared using the Quince extract to inhibit the viability of some species of bacteria, because of their unique properties and conspicuous therapeutic potential in treating a variety of diseases. UV/visible, SEM and IR spectroscopy have been used to identify the presence of $AgNP_s$. The in *vitro* test which was used to evaluate the potency of nanoparticles as antibacterial agents, two different types of bacteria *(E coli and Staphylococcus)* has been tested against the Quince extract and $AgNP_s$ produced from the extract. The overall results shown that the more than 80-90% of both types of bacteria are non-resistant and eliminated compared with untreated bacteria. Thus quince extract is a good biomaterial for the synthesis of AgNPs as antibacterial agents.

Introduction

Quince is a member of the genus *Cydonia* in the family Rosaceae which includes pears, apples and many other fruits. It is shedding its leaves annually, similar in appearance to a pear and bright golden-yellow when it's mature. Generally, the tree is grown for its attractive pale pink flowers and other functional qualities, while the cooked fruit has been used as food [1].Quince is used as an important medicinal plant all over the world. It is having a long history of ethnobotanical and medical use in Mediterranean region and Central Asia.

The plant is known due to its activity as antidiabetic, antioxidant, antihemolytic, antimicrobial, antiallergic and UV protect ant. Additionally, it is a cheap as a natural source of metabolites with remarkable biological properties; the metabolic process and biological activity of this species have been performed previously in a lot of publications. Particularly, quince leaves used to create a promising natural source of bioactive phytochemicals and are appropriate for application in pharmaceutical and nutritional fields [1, 2].In one hand, the quince extract has been used in co-ordination with chlorogenic acid to increase the efficiency of antibacterial agent [3].Phenolic compounds which consist of quince plant was found to have the best activity as antioxidant agents compared to the crude methanolic extract.

It was reported that the phenolic fraction has ability to provide high depiction for the radicle scavenging of quince fruit and jam [4].The activity of quince as antioxidant agent was evaluated by Folin-Ciocalteu reducing capacity assay, by 2, 2′-diphenyl-1-picrylhydrazyl assay and by the ability to inhibit the 2, 2′-azobis (2-amidinopropane) dihydrochloride induced oxidative hemolysis of human erythrocytes in comparison with green tea. The main phenolic compound 5-O-caffeoylquinic acid in quince leaf extract

produced much higher reduction power than green tea. The leaf extract acts as therapeutic or defensive agent against free radicles [5].On the other hand; several forms of organic and inorganic nanoparticles have been developed as antibacterial agents, especially, metal and metal oxide nanoparticles because they have various modes of action. AgNPs are the greatest investigated nanoparticle and proficient to kill both Gram-positive and Gram-negative bacteria, having even showed to be most effective against drug-resistant species [6].

The toxicity of metal and metal oxide nanoparticles against bacterial cells has been related with the membrane disruption and the generation of reactive oxygen species (ROS) [7].It was informed that the main reason behind the antimicrobial properties of antibacterial nanoparticles is that the release of ions which is designated as the driving force for these properties. In this study, biosynthesis of silver nanoparticles has been used as the fundamental component which acts in co-ordination with the components of the quince extract to enhance the antibacterial activity.

Experimental Session

- Preparation of silver nanoparticles

45 ml of (0.17 mg, 1mM) $AgNO_3$ in 250 ml distilled water was added to 5 ml of plant extract, the mixture was heated at 80°C for 20 minutes for reduction of Ag^+ ions. The reduction of pure silver ions is observed using the UV/visible measurement, which were recorded as a function of time and concentration of reaction mixtures on a UV-spectrophotometer.

- The preparation of plant extraction

Plant leafs were dried after washing many times with distilled water. Dried leafs were cut to small pieces and 0.02 mg was boiled in 100 ml distilled water at 100°C for half hour. The final extract was filtered to be ready for using.

- Determination of Minimum Inhibitory Concentration (MIC)

(100 µl) of various concentrations of test sample was added into sterile micro titre plates. Bacterial cell suspension (100 µl) corresponding to 1 × 108 CFU/ml was added in to each well except control wells. The control well consisted of distilled water and Mueller Hinton (MH) broth to check sterility while those in negative control well were filled with MH broth and bacterial suspension to check the ability of the broth to support the growth of bacteria. The plates incubated at 37°C for 24 hrs. To point out the growth of bacteria, 60µl of 0.2 mg/ml P-Iodonitroterazolium chloride (Hi Media) was added to each well and incubated for another 35 min. The biological active organism is the reason behind the reduction of tetrazolium salt to a red-coloured formazan.

The growth inhibition of bacteria was noticeable as a colourless well whereas, the presence of growth was indicated by the presence of pink-red color. The lowest concentration showing no colour change was considered as the MIC [8].

Results and Discussion

The bio-molecules from a lot of plant components have been used as potential agents for the synthesis of silver nanoparticles (AgNPs) due to their unique properties and obvious therapeutic potential in treating many of diseases [9].In this work, aqueous leaf extract of quince plant has been used as potential agents to synthesize of silver nanoparticles (AgNPs) due to it is a rapid and simple alternative to chemical synthesis.

When the silver nitrate solution was contacted with plant leaf extract, the reduction of the silver ions was observed [10]. The reduction of pure Ag^+ ions has been first controlled using the UV/visible spectra measurement of the extract before and after producing of (AgNPs) using a range of concentrations of $AgNO_3$ started with 0.1-1 mM Figure 1.

It was found that the best concentration to produce (AgNPs) was 1mM of $AgNO_3$ and 10 mM of plant extract .The use of UV/vis spectroscopy as a monitor to identify the characteristic peaks of the formation of various nanoparticles from their different salts. For example, the formations of silver nanoparticles from silver ions show an absorption peak around 450 nm. A clear increase in the characteristic peak with increase in reaction concentration of plant extracts with $AgNO_3$ ions is a clear indicator of nanoparticle formation [11].

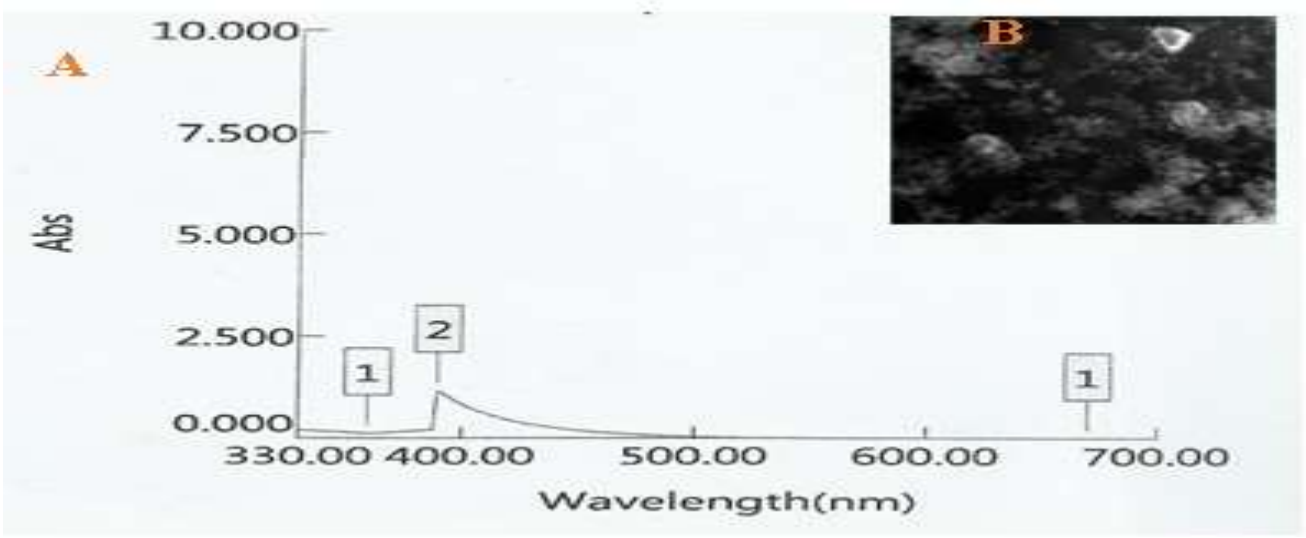

Figure 1 A: the UV/visible spectrum of (Ag NPs), B: the scan electron microscopy (SEM) image of (Ag NPs)

The formations of silver nanoparticles from silver ions show an absorption peak around 450 nm. While, the most indicated absorption of the plant extract was illustrated at 250 nm.

The SEM scan shows that the silver nanoparticles are ranged in a size from 5-8 nm with average of 6.52 ± 0.32 nm **Figure 1B**.

IR spectroscopy was used to determine the nature of associated of plants extract with nanoparticles. This technique has been used in the characterization of silver which was associated molecules from plant extracts **Figure2.**

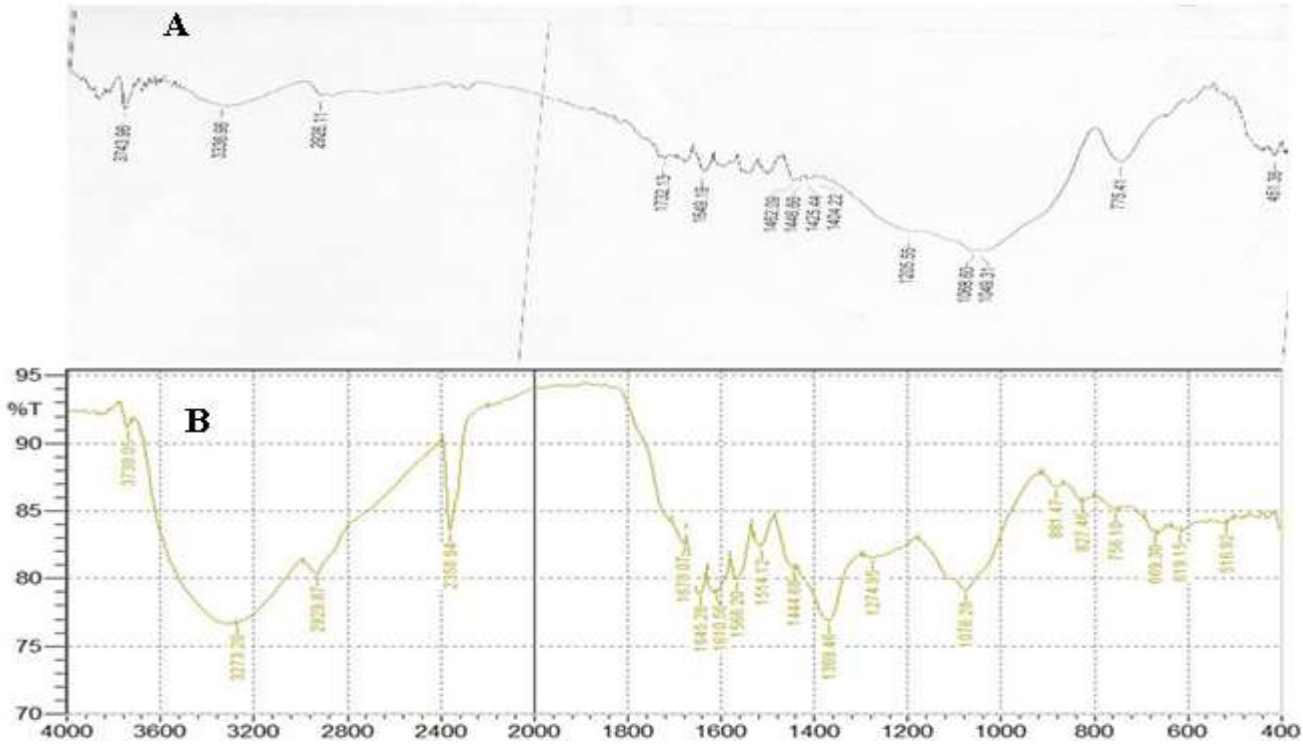

Figure 2: IR spectroscopy of A. quince plant extract and B. AgNPs produced by quince plant extract

FT-IR characterization of the extract alone **Figure 2A** and the AgNPs synthesized with the extract **Figure 2B** showed that both samples contained functional groups that have been expected. Some of the characteristic peaks observed in both samples include **Figure 2A and 2B** a clear peak at 3,738-3,743 and 3,273-3,336 cm^{-1} due to the stretching of O-H in alcohols and phenols; one peak at 1,646 and 1,645 cm^{-1} corresponding to the stretching of C=C in aromatic compounds ; two peaks at 2,926 and 2,929 cm^{-1} due to the stretching of CH_3 and CH_2 in aliphatic compounds; another peak displayed a slight shift after conjugation with silver nanoparticles and extra peaks have been observed **Figure 2B** at 2,358 and 1,369 cm^{-1}.

In addition, the peaks observed are associated with the different components that the extract may be contained, like phenols, which may be carrying out the process of reduction in the Ag^+ ions to produce AgNPs. The characteristic data has clearly shown the role of the plant extract as a stabilizing agent of the AgNPs. Moreover, the results suggest the presence of the components of the extract in the surface of the AgNPs.

In the case of *vitro* study, the lowest concentration of testing samples which were used to inhibit the visible growth of bacterial species after overnight incubation has been evaluated using (MICs). 1:50 serial dilution was used based on 10 and 1 mM as a stock solution from each sample tested (quince extract, AgNO3 and the aqueous-AgNPs), started with10 fold dilution as the largest concentration to evaluate the availability of two types of bacteria (*E. coli* and *Staphylococcus*).Both plant extract and the aqueous-AgNPs were found toxic against both types of bacteria. MIC value of plant extract components has been shown a good inhibition against both types of bacteria at the range of 10- 0.01mM.

Whereas, silver nanoparticles with the aqueous plant extract were shown the most interesting result to kill both Gram-positive and Gram-negative bacteria in a range of 1-0.001 mM having even shown to be effective against the bacterial cells **Table 1.** The mechanisms action of AgNPs against bacterial proliferation are not yet fully understood, the three most common mechanisms of toxicity proposed up to now are: formation of reactive oxygen species (ROS), uptake of free Ag^+ followed by disruption of ATP production and DNA replication and direct damage to cell membranes [12].

It was reported that the antibacterial polymeric nanoparticles have ability to kill microorganisms upon their contact with the cells of bacteria due to the robust interaction of their cationic surfaces with the bacterial cells. Additionally, the antibacterial activity of silver nanoparticles was size dependent. AgNPs commonly in the range of 1 -10 nm attach to the surface of cell membrane and drastically disturb its proper function like permeability and breathing [9, 13].

Table 1: **The activity of plant extract and AgNPs produced from plant extract against the activity of Gram-negative and Gram-positive bacteria**

Tested samples	Concentrations mM	Gram positive bacteria (inhibition)	Gram negative bacteria (inhibition)	Broth (control) No testing samples
The quince extract	10	++++	+++	(98%) growth of (*E.coli*)
	$10x10^{-1}$	+++	+++	
	$10x10^{-2}$	++	++	
	$10x10^{-3}$	++	++	
The quince extract+ (AgNPs)	1	++++	++++	(96%) growth of (*E.coli*) *(Staphylococcus*).
	$1x10^{-1}$	++++	++++	
	$1x10^{-2}$	+++	+++	
	$1x10^{-3}$	++	++	

++ means (50%) inhibition, +++ means (60-70) % inhibition and ++++ means (80-90%) inhibition

At MIC, the treatments show antibacterial activity for both types of bacteria, the MIC using AgNPs produced from plant extract was found at ten times the MIC after using the plant extract only compared with the MIC from control. Thus, the AgNPs produced by quince extract show anti bactericidal activity in both (*E.coli* and *Staphylococcus*) species as illustrated in Table2.

Table2: Comparison of minimum inhibitory concentration (MIC) of quince plant extract and AgNPs for two different types of bacterial

Tested samples	MIC (mM) of Gram positive bacteria	MIC (mM) of Gram negative bacteria
The quince extract	$1x10^{-2}$ mM	$1x10^{-2}$mM
none	10 mM	10 mM
The quince extract+ (AgNPs)	$1x10^{-4}$ mM	$1x10^{-4}$ mM

It can be easily noticed that the aqueous-AgNPs active more than the plant extract as antibacterial agent. Means the green synthesis is a rapid and simple alternative way to chemical synthesis.

Conclusion

Plants have emerged as an efficient candidate for the synthesis of nanoparticles. These biogenic nanoparticles are cost efficient, simpler to synthesize, and focus

toward a greener approach. Silver nano materials exhibit broad spectrum biocidal activity toward bacteria, fungi, viruses, and algae. This motivates its use in medical and agriculture applications. Our study, investigated the ability of quince extract plant to produce AgNPs and increase its stability inside aqueous solution. Moreover, the activity of each testing sample has been evaluated using MIC method to recognize the best one to inhibit the growth of bacteria in its culture media.

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