Green Synthesis of Silver Nanoparticles by Kumquat (Fortunella Margaarita) Fruit Extract and Efficacy the Antimicrobial Activity

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Abstract

In the present study, environment friendly and cost effective silver nanoparticless were synthesized using the Kumquat (*Fortunella margarita*) fruit extract as the reducing and capping agent. The nanoparticless were characterized using UV-visble, FT-IR, XRD, and SEM methods. The surface plasmon resonance peaks in absorption spectra for silver colloidal solution showed an absorption peak at 420 nm in a UV-visible spectrum. The functional biomolecules such as carboxyl groups present in the seaweed responsible for the silver nanoparticles formation were characterized by FT-IR. The XRD results suggested that the crystallization of the bio-organic phase occurs on the surface of the silver nanoparticles or vice versa. The broadening of peaks in the XRD patterns was attributed to particle size effects and the average particles size about 27.19 nm.

The results shows that silver nanoparticles synthesised by Kumquat (*Fortunella margarita*) fruit juice has effective antibacterial activities on the test isolates as indicated by the diameter of their zone of inhibition. The inhibition zone was 16 mm for *Enterobacter cloacae,* 18 mm for *Escherichia coli, Proteus mirabilis, Bacillus sp., Staphylococcus aureus* and *Streptococcus spp.* 20 mm for *Klebsiella pneumonia* and 14mm for *Pseudomonas aeruginosa*. The antimicrobial activity of Kumquat (*Fortunella margarita*) fruit juice against test bacteria was lowest efficiency; the inhibition zone was 10 mm for *Enterobacter cloaca*e and *Pseudomonas aeruginosa*. 12 mm for *Escherichia coli* and *Proteus mirabilis*. 11 mm for *Klebsiella pneumonia*. 13 mm for *Bacillus sp.* and *Streptococcus spp.* and 14 mm for *Staphylococcus aureus*. Whereas the test shows the silver nitrate solution has no effect against tested isolates.

The study revealed that the silver nanoparticles synthesis by Kumquat fruit extract could be as a therapeutic agent for human microbial infections.

Key words: Silver nanoparticles, pathogenic bacteria, Kumquat (*Fortunella margarita*), fruit extract, antimicrobial activity.

Introduction

Nanotechnology is a rapidly growing science of producing and utilizing nano-sized particles. A number of approaches are available for the synthesis of silver nanoparticles, such as thermal decomposition (Navaladian, *et al*., 2007), electrochemical (Starowicz, *et al*., 2006) microwave assisted process (Sreeram, *et al*., 2008) and green chemistry (Begum, *et al*., 2009).Many of the nanoparticle synthesis or production methods of nanoparticles involve the use of hazardous chemicals, low material conversions and high energy requirements. So, a growing need to develop an environmentally friendly process for nanoparticle synthesis without using toxic chemicals is gaining importance. Biosynthetic methods employing either microorganisms or plant extracts have emerged as a simple and viable alternative to chemical synthetic procedures and physical methods. Several microorganisms, such as bacteria, fungi and yeasts, have come up as nano factories, synthesizing metal nanoparticles of Ag and Au. However, the use of plants for the fabrication of nanoparticles has drawn attention, because of its rapid, economical, eco-friendly protocol, and it provides a single step technique for the biosynthesis process (Huang, *et* *al*., 2007). Biological approaches using microorganisms and plants or plant extracts for metal nanoparticle synthesis have been suggested as valuable alternatives to chemical methods. An important branch of biosynthesis of nanoparticles is the application of plant extract to the biosynthesis reaction. Synthesis of quasi spherical silver nanoparticles used a purified apiin compound, extracted from henna leaf at ambient conditions (Kasthuri, *et al*., 2009). Using green tea, *C. sinensis* extracts, as a reducing and stabilizing agent, gold nanoparticles and silver nanostructures were produced in aqueous solution at ambient conditions (Nestor, *et al*., 2008). Plant extracts from live alfalfa, the broths of lemongrass, geranium leaves and others have served as green reactants in Ag NP synthesis. The reaction of aqueous AgNO3 with an aqueous extract of leaves of a common ornamental geranium plant, Pelargonium graveolens, gave Ag NPs after 24 h (Shankar, *et al*., 2003). Silver nanoparticles ranging from 55 to 80 nm in size, and triangular or spherical gold nanoparticles, were fabricated using the novel sundried biomass of *Cinnamomum canphora* leaf (Huang, *et al*., 2007). A simple procedure applying *Aloe vera* leaf extract has been used for the synthesis of gold nanotriangle and spherical silver nanoparticles. *Aloe vera* extract showed more spherical silver nanoparticles with increasing the amount of added extract (Chandran, *et al*., 2006). Silver nanoparticles were successfully synthesized using the latex of *Jatropha curcas*. The plant, *Jatropha curcas*, is commercially important, as bio-diesel is extracted from its seeds on an industrial scale. Crude latex was obtained by cutting the green stems of *J. curcas* plants (Bar, *et al*., 2009). In another research work, biosynthesis of silver nanoparticles was also conducted using Cycas leaf extract (Jha and Prasad, 2010). Cycas is rich in flavonoids, broadly belonging to the class of phenolic compounds.

Kumquats (Fortunella spp.) belong to the Citrus genus are fruit, usually eaten raw as a whole fruit together with the peel, excluding the seeds. The peel is sweet and edible with a typical aroma due to the presence of flavonoids and terpenoids (Koyasako and Bernhard, 1983). Kumquats are also an excellent source of nutrients and phytochemicals, including ascorbic acid, carotenoids, flavonoids and essential oils. Some Fortunella plants, such as *Fortunella japonica* Swingle, *Fortunella margarita* (Lour.) Swingle and *Fortunella crassifolia* Swingle are commonly cultivated in the southern region of China. *F. crassifolia*, known as jingdan or meiwa kumquat, has a typical citrus character. As with other citrus fruits, *F. crassifolia* can be candied, prepared as marmalade, added to fruit salad and preserved as a whole in sugar syrup in food industry.

It is well known that *F. crassifolia* is rich in flavonoids. Previously, 3', 5'-di-*C*-β-glucopyranosylphloretin, a characteristic flavonoid, was well-studied in the genus *Fortunella* (Ogawa *et al*., 2001). Recently, the antioxidant activity of *F. crassifolia* has been reported (Kondo *et al*., 2005). However, studies on the chemical composition and biological activities of *F. crassifolia* peel oils are limited. From a dietary viewpoint, essential oils represent added value for kumquat fruit because, in addition to their contribution to the flavor, they play an important role in human health, as with other non-nutritive phytochemicals such as polyphenols and flavonoids (Schirra *et al*., 2008). The *Citrus* essential oils have various functional properties, such as an attractive aroma, a repellant agent against insects and animals, and antioxidant activities. A previous study has reported the antimicrobial action of citrus oils (Subba *et al*., 1967). Meanwhile, citrus oils are not only available to the food industry, but are also generally recognized as safe and have been found to be inhibitory, in both oil and vapor form, against a range of both Gram-positive and Gram-negative bacteria (Fisher, and Phillips, 2008). Moreover, there are a large number of studies on plant essential oils regarding their antimicrobial properties in order to develop a source of antimicrobial ingredients for the food industry (Mkaddem *et al*., 2009, Zeng *et al*., 2011, Salleh *et al*., 2011).

With the emergence and increase of microbial organisms resistant to multiple antibiotics, and the continuing emphasis on health-care costs, many researchers have tried to develop new, effective antimicrobial reagents free of resistance and cost. Such problems and needs have led to the resurgence in the use of Ag-based antiseptics that may be linked to broad-spectrum activity and far lower propensity to induce microbial resistance than antibiotics (Jones, *et al*., 2004). The antibacterial effects of Ag salts have been noticed since antiquity (Silver and Phung, 1996) and Ag is currently used to control bacterial growth in a variety of applications, including dental work, catheters, and burn wounds (Catauro *et al*., 2004, Crabtree *et al*., 2003).

The present work has focused on the development of the easy synthesis of silver nanoparticles by an environmentally friendly procedure. In Iraq the kumquat fruits called Japan oranges which is used for diabetes and high pressure patients; because they thinks this fruit played important roles in regulating glucose and lipid metabolic disorders. Japan oranges extract was used for the silver nanoparticles synthesis, and evaluation of their antibacterial activity against various human multi drug resistant pathogenic bacteria.

Methods and materials

Collection of pathogens

We are collected multiple antibiotic-resistant isolates, which included *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumonia*, *Streptococcus sp*., *Enterobacter cloacae*, *Bacillus sp*., *Pseudomonas aeruginosa*, and *Staphylococcus aureus* used for the antimicrobial activity from microbiology diagnosis laboratory, Al-Numan hospital.

Synthesis of silver nanoparticles

For the synthesis of silver nanoparticles, we used Kumquat (*Fortunella margarita*) fruit extract for reducing and capping agent which is purchased from the local market at Baghdad City, Iraq, and silver nitrate ($AgNO_3$) purchased from Merck limited, India. Kumquat fruit extract was prepared by taking 25g of thoroughly washed and finely crushed Kumquat fruit mixed with 100ml deionized water in 500 ml of Erlenmeyer flask and then boiling the mixture for 10 min before finally decanting it. For the reduction of Ag+ ions, 5ml of Kumquat fruit extract was mixed to 45 ml of 0.002M aqueous of $AgNO_3$ solution drop wise with constant stirring at 50-60ºC and observe the colour change (Ahmad and Sharma, 2012). .

Characterization of silver nanoparticles

1- UV-Vis Spectra analysis:

The reduction of pure Ag+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium after 30 min. UV-Vis spectrophotometer is procured from Shimadzu. A small aliquot of the sample was taken for UV-Vis spectrum analysis (350-750 nm). The maximum absorbance spectrum of As-Ag nanoparticles was observed at 455 nm.

2- Fourier Transform Infra Red Spectroscopy (FT-IR)

FT-IR measurements were carried out using Perkin (8300 FT-IR Shimadzu Spectrophotometer) the range from 4000 cm^{-1} to 400 cm^{-1}. After complete reduction of $AgNO_3$ ions by Naringe leaf extract, the mixture was centrifuged at 10000 rpm for 10 min to remove protein or other bioorganic compounds that were present in the solution. The silver nanoparticles pellet obtained was air dried. The dried nanoparticles were mixed with the potassium bromide (KBr) to made thin pellets and were used for FT-IR analysis in transmittance mode.

3- X-Ray Diffraction (XRD) analysis

Resulting solution of the developed nanoparticles of silver was centrifuged at 10,000 rpm for 30 min. The solid residues of Ag NPs were washed twice with double distilled water and then dried at 80ºC to obtain powder Ag NPs used for X-ray powder diffraction measurements. The powder X-ray diffraction (XRD) patterns were recorded on (Shimadzu XRD-6000) with copper radiation (Cu K_{α}, 1.5406 Å) at 40 kV and 30 mA.

3- SEM Analysis of Silver Nanoparticles

Scanning Electron Microscopic (SEM) analysis was done using (Inspect S 50) SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid.

Determination of antimicrobial activity

Antibacterial activity of the silver nanoparticles by using Kumquat (*Fortunella margarita*) fruit extract was carried out by disc diffusion method (Cruickshank, 1968). Nutrient agar medium plates were prepared, sterilized and solidified. After solidification bacterial cultures were swabbed on these plates. The sterile discs were dipped in silver nanoparticles solution (100 µg/ml) and placed in the nutrient agar plate and kept for incubation at 37ºC for 24 hours. Zones of inhibition for (kumquat fruit extract and silver nitrate solution) as control, SNPs and silver nitrate were measured. The experiments were repeated thrice and mean values of zone diameter were presented.

Results

The kumquat (*Fortunella margarita*) Swingle (FM) belongs to the genus Fortunella Swingle, a close relative of the genus Citrus L, and both genera were grouped into the true citrus group of the family of Rutaceae. FM fruits, when eaten fresh, have a sweet outer peel and a tart, juicy flesh Figure (1). This type of kumquat (*Fortunella margarita*) fruit characterized by elongate oval shape figure (2). The reduction of Ag^{+} into Ag-NPs during exposure to Kumquat (*Fortunella margarita*) fruit extract was able to be followed by the color change. The fresh suspension of Kumquat fruit extract was yellow. However, after the addition of $AgNO_3$ and stirring for one hour at room temperature, the emulsion turned dark brown. The color changes in aqueous solutions are due to the surface-plasmon resonance (SPR) phenomenon (Figure 3B and C). The result obtained in this investigation is interesting because it can serve as a foundation in terms of identification of potential forest plants for synthesizing Ag-NPs.

Figure (1) *Fortunella margarita*, kumquat, is in the *Rutaceae* family.

Figure (2) *Fortunella margarita*, kumquat, is in the *Rutaceae* family.

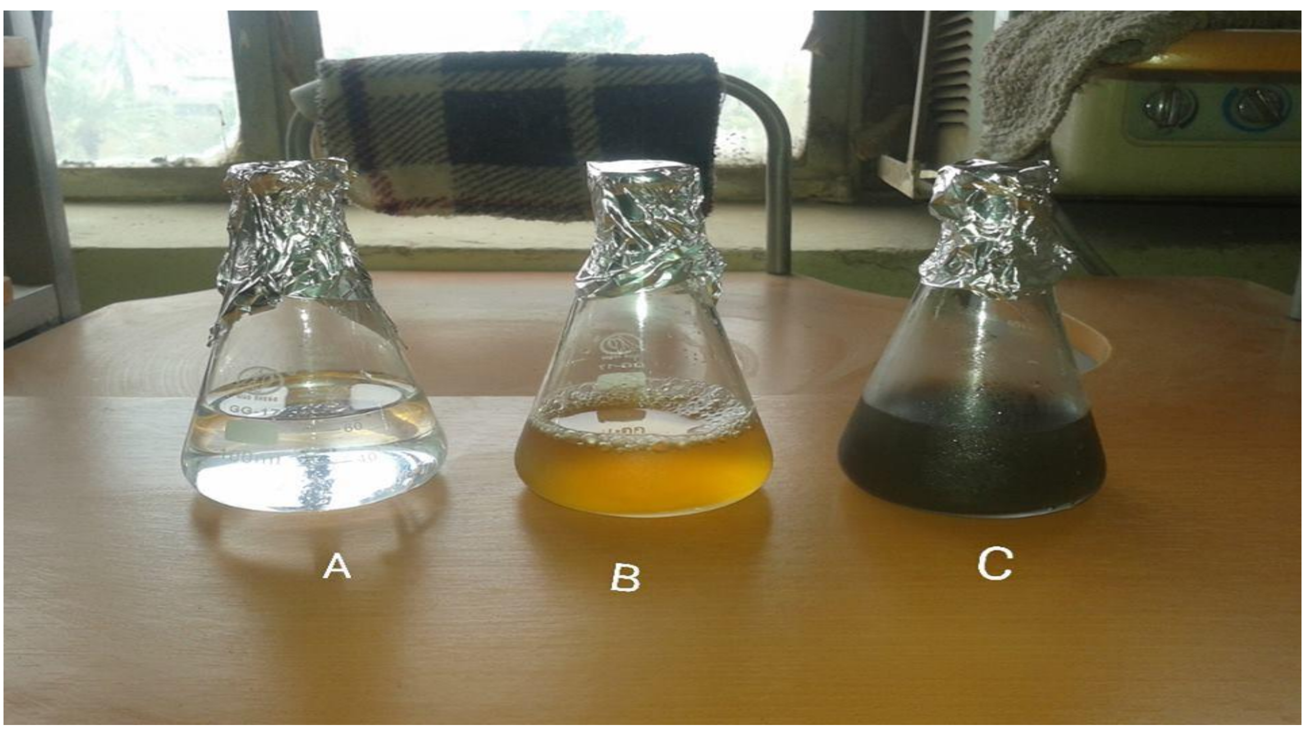

Figure (3). Photograph of Silver nitrate solution (A), Kumquat (*Fortunella margarita*) fruit juice (B) and silver/ Kumquat (*Fortunella margarita*) fruit juice (C) emulsions after one hour of stirring time.

UV-Vis Spectrophotometry

The Formation of metal nanoparticles by reduction of the aqueous metal ions during exposure of Kumquat (*Fortunella margarita*) fruit juice may be easily followed by UV–Vis spectroscopy (UV- shimadzu spectrophotometer). UV-Vis absorption spectrum of silver nanoparticles in the presence of Kumquat (*Fortunella margarita*) fruit juice leaf extract is shown in figure (4). The surface plasmon resonance peaks in absorption spectra for silver colloidal solution showed an absorption peak at 420 nm in a UV-visible spectrum, suggesting that the nanoparticles were dispersed in the aqueous solution with no evidence for aggregation in UV-Vis absorption spectrum Figure (4).

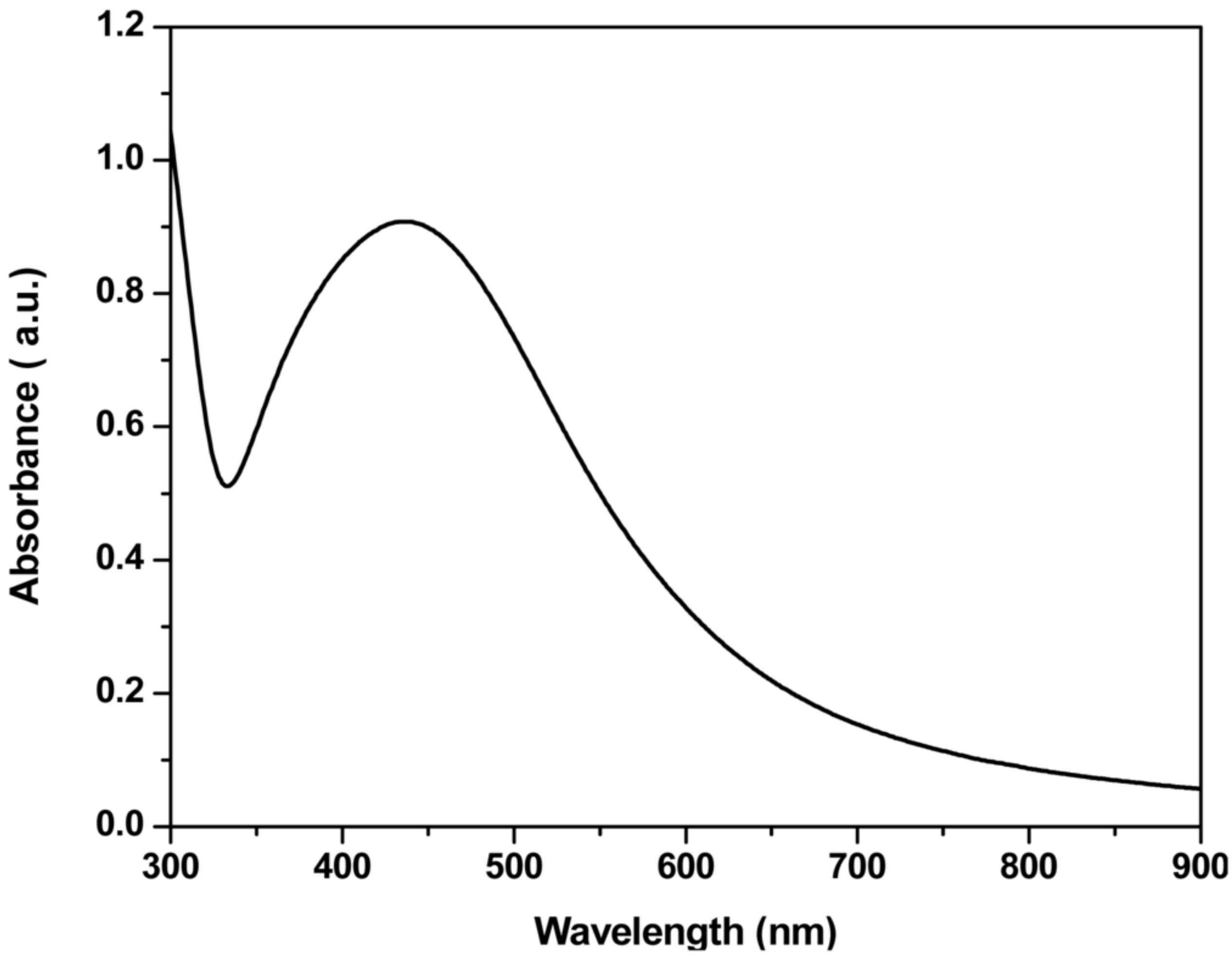

Figure (4). UV-Vis absorption spectra of Silver nanoparticles synthesized by exposure of Kumquat (*Fortunella margarita*) fruit juice with 0.002M silver nitrate.

Fourier Transform Infra Red Spectroscopy

The results of FTIR analysis of this study show different stretches of bonds shown at different peaks; 3327.21—N– H stretch, 1641.42 —C=C, and 1211.30—C=O. Figure 5 shows the peaks near 3440cm^{-1}, and 2968 cm^{-1} assigned to OH stretching and aldehydic C–H stretching, respectively. The weaker band at 1629cm^{-1} corresponds to amide I arising due to carbonyl stretch in proteins. The peak at 1051 cm^{-1} corresponds to C–N stretching vibration of the amine. The peak near 1743 cm^{-1} corresponds to C=C stretching (non conjugated). The peak near 866 cm^{-1} assigned to C=CH2 and the peaks near 678 cm^{-1} and 638 cm^{-1} assigned to CH out of plane bending vibrations are substituted ethylene systems –CH=CH. FTIR spectra of silver nanoparticles exhibited prominent peaks at 1641, and 1382 cm−1. The spectra showed sharp and strong absorption band at 1641 cm−1 assigned to the stretching vibration of (NH) C=O group (Barbara et al., 2004).

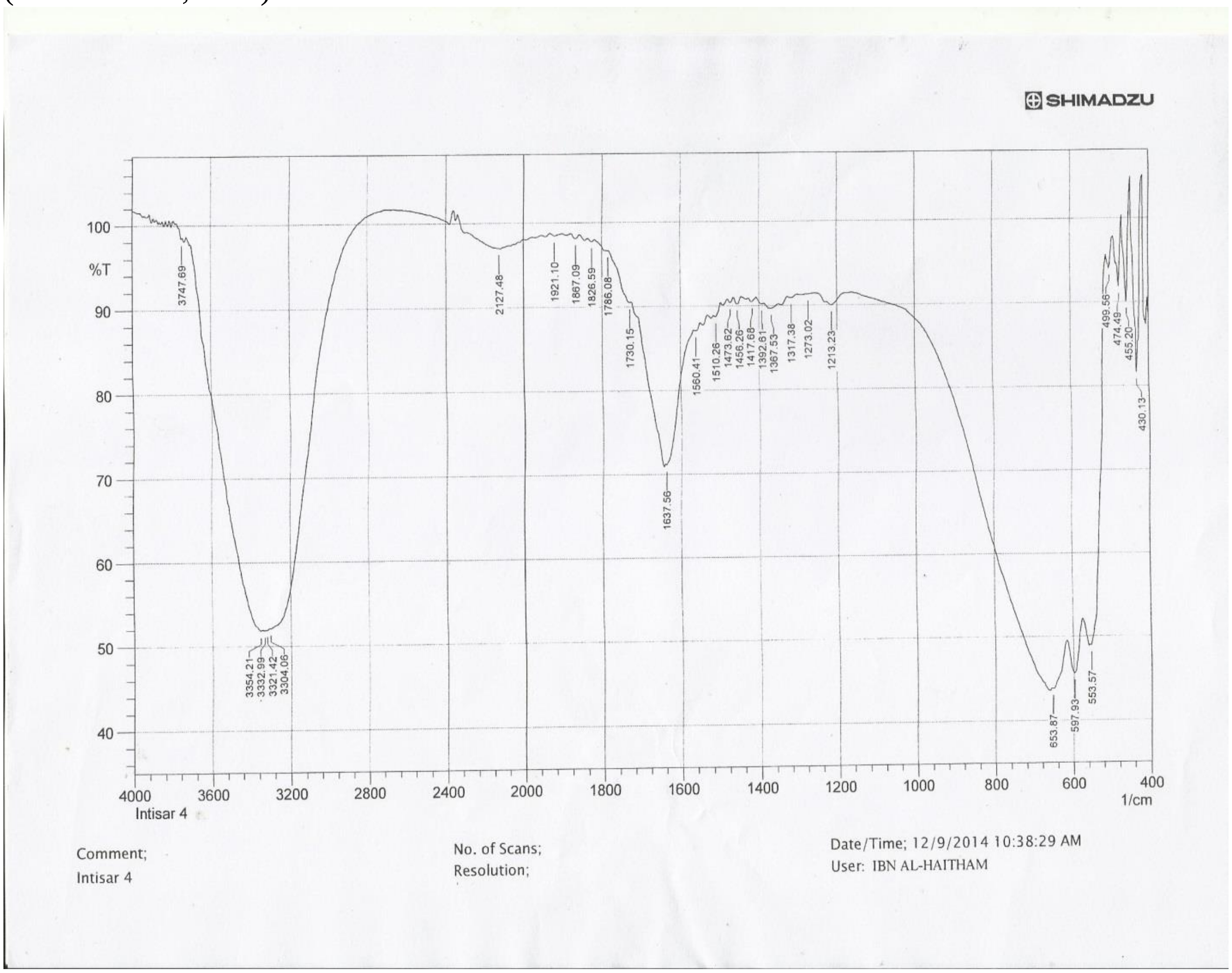

Figure (5). Fourier Transform Infra Red Spectroscopy image of Silver nanoparticles synthesized by exposure of kumquat (*Fortunella margarita*) fruit juice with 0.002M silver nitrate.

Powder X-ray diffraction

Figure (6) shows the XRD patterns of Ag-NPs. The X-ray diffraction patterns of the Ag-NPs synthesized by using $AgNO_3$ and using the Kumquat (*Fortunella margarita*) fruit juice as the reducing and capping agent are shown in Fig. 6. All the reflections correspond to pure silver metal with face centered cubic symmetry .The reflections were indexed as (111), (200) and (220) with the corresponding 2θ values of 38.128, 44.315 and 64.468 respectively (JCPDS 04-0783). The intensity of peaks reflected the high degree of crystallinity of the silver nanoparticles. However, the diffraction peaks were broad indicating that the crystallite size is very small. The average particle size of Ag-NPs can be calculated using the Debye - Scherrer equation (Langford and Wilson, 1978).

$D = K \lambda / \beta \cos\theta$

where K is the Scherrer constant with value from 0.9 to 1) shape factor), λ is the X-ray wavelength (1.5418 Å ,β is the width of the XRD peak at half-height and θ is the Bragg angle and D is the grain size. From the Scherrer equation, the average crystallite size of Ag-NPs is (27.19 nm).

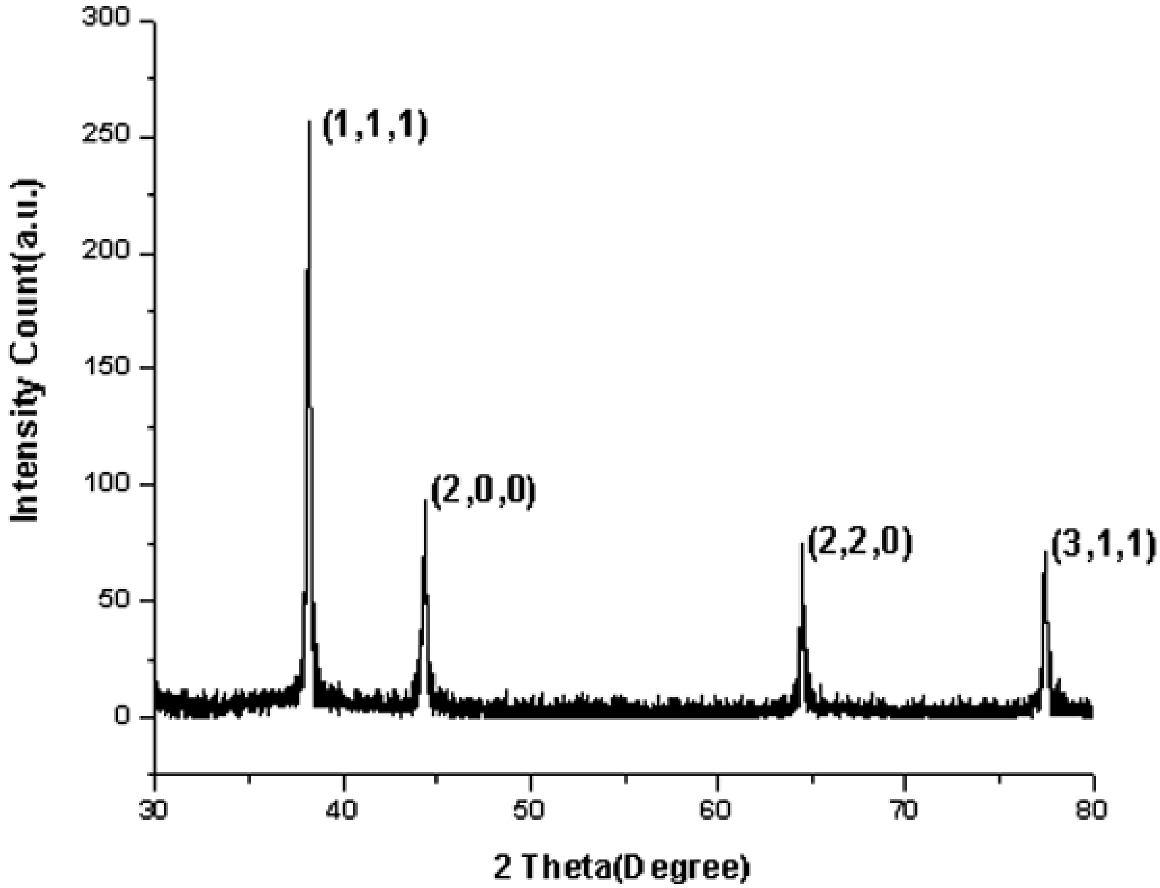

Figure (6). X ray diffraction of Silver nanoparticles synthesized by exposure of Kumquat (*Fortunella margarita*) fruit juice with 0.002M silver nitrate.

SEM analysis of Silver nanoparticles

The silver nanoparticles synthesized by the help of Kumquat (*Fortunella margarita*) fruit juice were scanned by SEM as shown in figure (7). It reveals that silver nanoparticles seem to be spherical in morphology and particles form cluster. It is easy to notice that the examined particles consist of a number of smaller objects of a few micrometers in size. However, we did not manage to examine the structure of the observed nanoparticles because of difficulties connected with getting higher magnification. In Figure (8), a standard EDX spectrum recorded on the examined sample is shown. In the middle part of the presented spectrum a strong peak located at 3 KV. This maxima is directly related to the silver characteristic line L. The maximum located on the left part of the spectrum at 0.2 kV clearly comes from carbon. Quantitative analysis proved high silver contents (100%) in the examined samples the result shown in table (1).

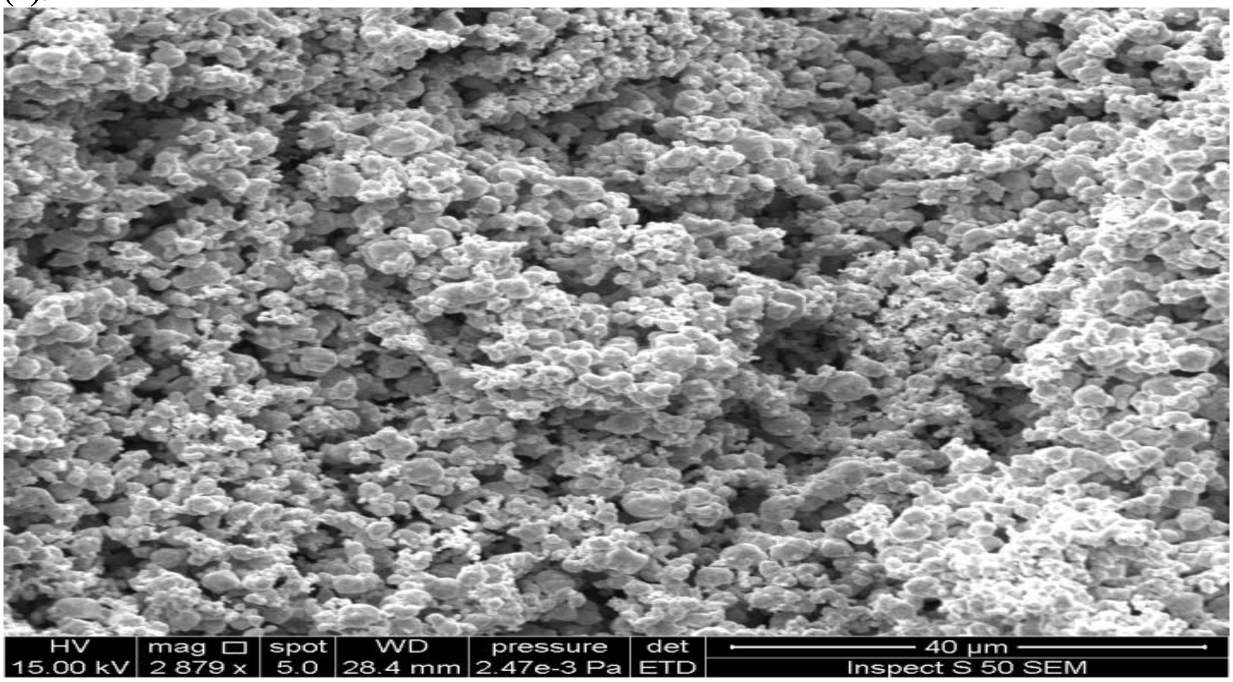

Figure (7). SEM micrographs of silver nanoparticles synthesised by Kumquat (*Fortunella margarita*) fruit juice

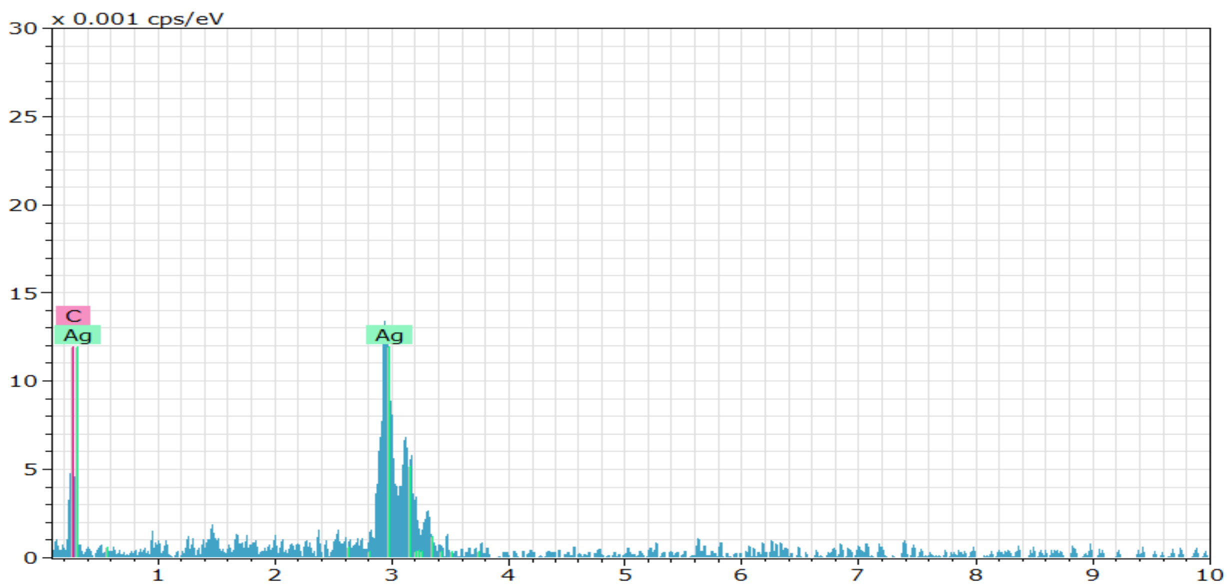

Figure (8).EDX characteristic spectrum obtained for silver powder.

Table (1) shows the elements in silver nanoparticles

Element	AN	series	[wt.%]	[norm. wt.%]	[norm. at.%]
Carbon	6	K-series	0	0	0
Silver	47	L-series	75.13866	100	100
		Sum:	75.13866	100	100

The data in Table (2) and Figure (8-11) shows that silver nanoparticles synthesised by Kumquat (*Fortunella margarita*) fruit juice has effective antibacterial activities on the test isolates as indicated by the diameter of their zone of inhibition. The inhibition zone was 16 mm for *Enterobacter cloacae*, 18 mm for *Escherichia coli*, *Proteus mirabilis*, *Bacillus sp.*, *Staphylococcus aureus* and *Streptococcus spp.* 20 mm for *Klebsiella pneumonia* and 14mm for *Pseudomonas aeruginosa*. The antimicrobial activity of Kumquat (*Fortunella margarita*) fruit juice against test bacteria was lowest efficiency; the inhibition zone was 10 mm for *Enterobacter cloaca*e and *Pseudomonas aeruginosa*. 12 mm for *Escherichia coli* and *Proteus mirabilis*. 11 mm for *Klebsiella pneumonia*. 13 mm for *Bacillus sp.* and *Streptococcus spp.* and 14 mm for *Staphylococcus aureus*. Whereas the test shows the silver nitrate solution has no effect against tested isolates.

Table (2). The inhibitory activity of the Ag-NPs synthesis by Kumquat (*Fortunella margarita*) fruit juice against the tested bacteria as demonstrated by diameters of the inhibition zone (mm)*.

Isolated bacteria	Zone of Inhibition		
	Silver nitrate sol.	Kumquat fruit juice	Kumquat fruit juice / Ag-NPs
*Enterobacter cloaca*e	0	10	16
Escherichia coli	0	12	18
Klebsiella pneumonia	0	11	20
Proteus mirabilis	0	12	18
Pseudomonas aeruginosa	0	10	14
Bacillus sp.	0	13	18
Staphylococcus aureus	0	14	18
Streptococcus spp.	0	13	18

* Zone of inhibition, including the diameter of the cup plate method (8.0 mm) .The recorded value is mean value of 3 replicates.

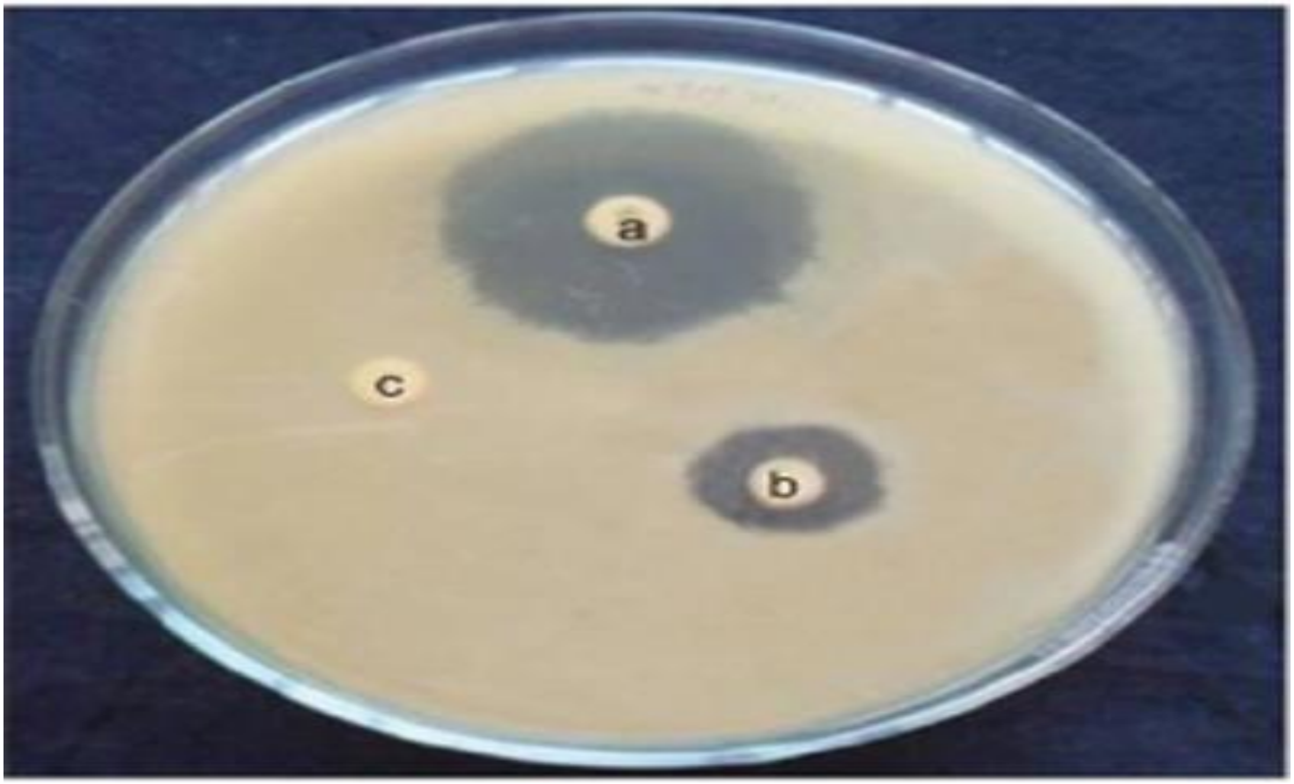

Figure (8). The antibacterial effect of Silver nanoparticles synthesis by Kumquat fruit juice (a), Kumquat fruit juice (b) and Silver nitrate solution (c), using the test bacterium *Klebsiella pneumonia*.

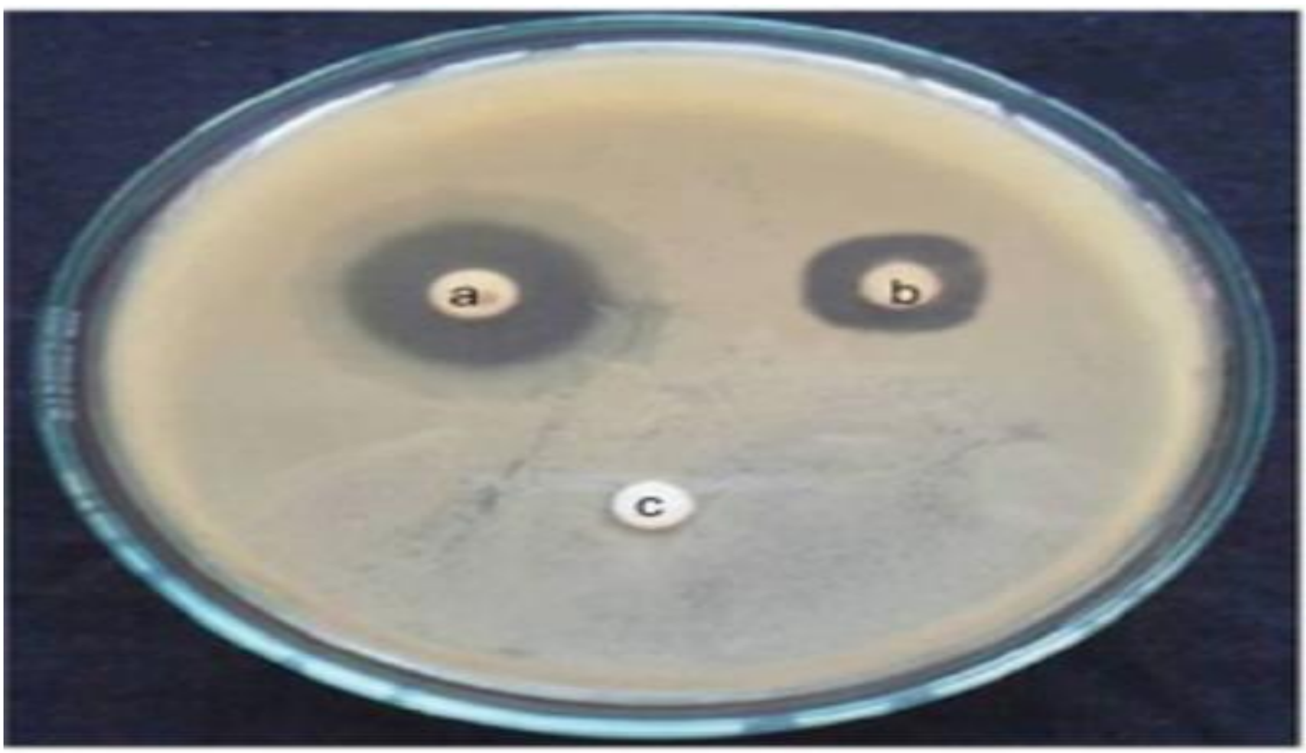

Figure (9). The antibacterial effect of Silver nanoparticles synthesis by Kumquat fruit juice (a), Kumquat fruit juice (b) and Silver nitrate solution (c), using the test bacterium *Staphylococcus aureus*.

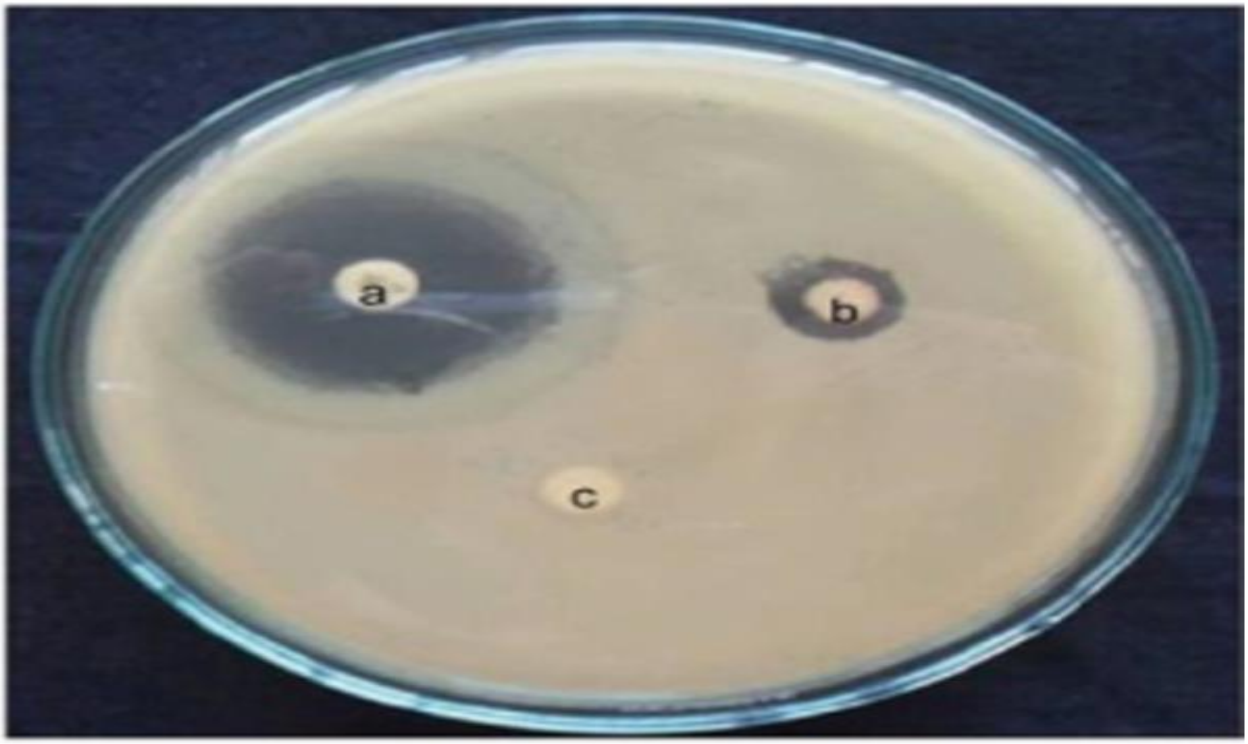

Figure (10). The antibacterial effect of Silver nanoparticles synthesis by Kumquat fruit juice (a), Kumquat fruit juice (b) and Silver nitrate solution (c), using the test bacterium *Escherichia coli*.

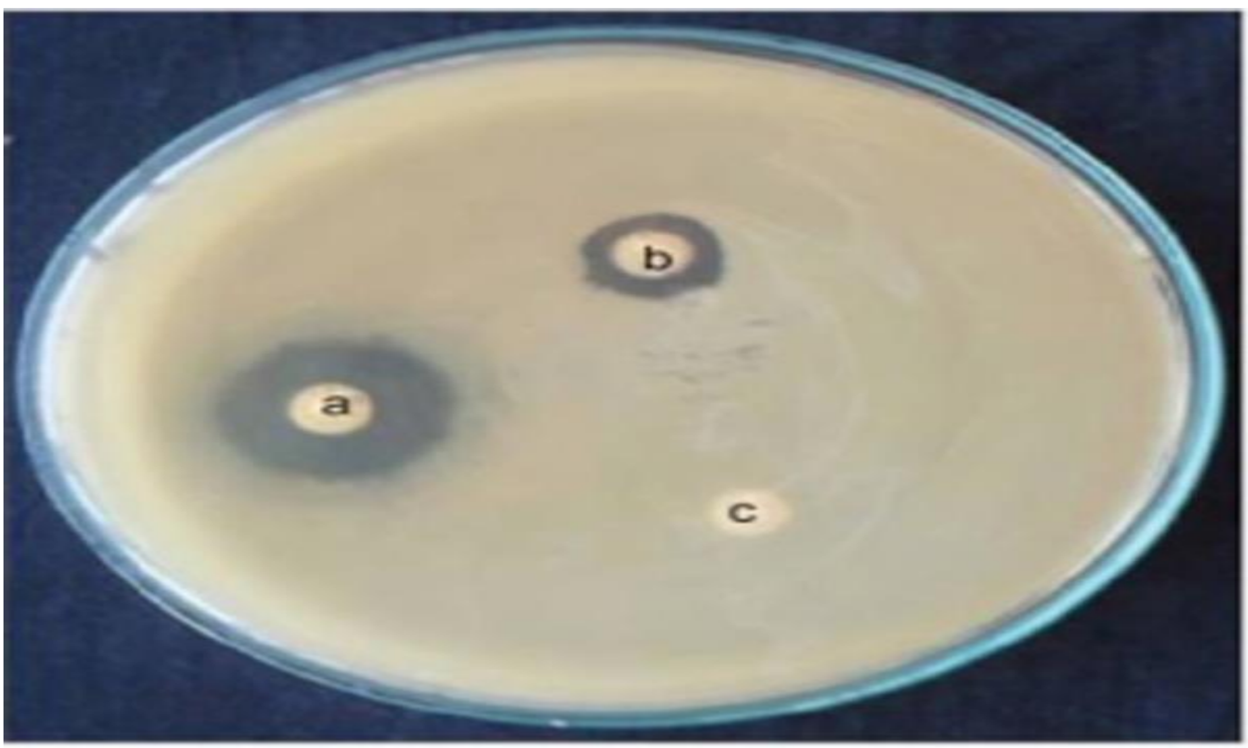

Figure (11). The antibacterial effect of Silver nanoparticles synthesis by Kumquat fruit juice (a), Kumquat fruit juice (b) and Silver nitrate solution (c), using the test bacterium *Pseudomonas aeruginosa*.

Discussion

Silver nanoparticles are nanoparticles of silver which are in the range of 1 and 100 nm in size. Silver nanoparticles have unique properties which help in molecular diagnostics, in therapies, as well as in devices that are used in several medical procedures. The major methods used for silver nanoparticle synthesis are the physical and chemical methods. The problem with the chemical and physical methods is that the synthesis is expensive and can also have toxic substances absorbed onto them. To overcome this, the biological method provides a feasible alternative. The major biological systems involved in this are bacteria, fungi, and plant extracts. The major applications of silver nanoparticles in the medical field include diagnostic applications and therapeutic applications.

The synthesis of metal and semiconductor nanoparticles is an expanding research area due to the potential applications for the development of novel technologies. Generally, nanoparticles are prepared by a variety of chemical methods which are not environmentally friendly. We have reported a fast, convenient and extracellular method for the synthesis of silver nanoparticles by reducing silver nitrate with the help of Kumquat (*Fortunella margarita*) fruit extract. The nanoparticless were characterized using UV-visble, FT-IR, XRD, AFM, and SEM methods. The surface plasmon resonance peaks in absorption spectra for silver colloidal solution showed that the absorption maximum range was at 380-440 nm. The functional biomolecules such as carboxyl groups present in the seaweed responsible for the silver nanoparticles formation were characterized by FT-IR. The XRD results suggested that the crystallization of the bio-organic phase occurs on the surface of the silver nanoparticles or vice versa. The broadening of peaks in the XRD patterns was attributed to particle size effects and the average particles size about 27.19 nm.

The results shows that silver nanoparticles synthesised by Kumquat (*Fortunella margarita*) fruit juice has effective antibacterial activities on the test isolates as indicated by the diameter of their zone of inhibition. The inhibition zone was 16 mm for *Enterobacter cloacae,* 18 mm for *Escherichia coli, Proteus mirabilis, Bacillus sp., Staphylococcus aureus* and *Streptococcus spp.* 20 mm for *Klebsiella pneumonia* and 14mm for *Pseudomonas aeruginosa*. The antimicrobial activity of Kumquat (*Fortunella margarita*) fruit juice against test bacteria was lowest efficiency; the inhibition zone was 10 mm for *Enterobacter cloaca*e and *Pseudomonas aeruginosa*. 12 mm for *Escherichia coli* and *Proteus mirabilis*. 11 mm for *Klebsiella pneumonia*. 13 mm for *Bacillus sp.* and *Streptococcus spp.* and 14 mm for *Staphylococcus aureus*. Whereas the test shows the silver nitrate solution has no effect against tested isolates Table (2) and Figure (8-11). Our interpretation of these results, the silver nanoparticles synthesised by Kumquat (*Fortunella margarita*) fruit extract has another mechanism to kill bacteria not found in Kumquat (*Fortunella margarita*) fruit extract. This finding agreement with the study conducted in the city of Hilla –Iraq, Hindi *et al.*, 2014 reached to the lowest antibacterial activity of Citrus fruits and leaves extract against most of the study bacterial isolates.

The mechanism of the inhibitory effects of Ag ions on microorganisms is partially known. Some studies have reported that the positive charge on the Ag ion is crucial for its antimicrobial activity through the electrostatic attraction between negative charged cell membrane of microorganism and positive charged nanoparticles (Hamouda, *et al*., 2000, Dibrov, *et al*., 2002, Dragieva, *et al*., 1999). In contrast, Sondi and Salopek-Sondi, (2004) reported that the antimicrobial activity of silver nanoparticles on Gram-negative bacteria was dependent on the concentration of Ag nanoparticle, and was closely associated with the formation of ‘pits’ in the cell wall of bacteria. Then, Ag nanoparticles accumulated in the bacterial membrane caused the permeability, resulting in cell death. However, because those studies included both positively charged Ag ions and negatively charged Ag nanoparticles, it is insufficient to explain the antimicrobial mechanism of positively charged Ag nanoparticles. Therefore, we expect that there is another possible mechanism. Amro *et al*. suggested that metal depletion may cause the formation of irregularly shaped pits in the outer membrane and change membrane permeability, which is caused by progressive release of lipopolysaccharide molecules and membrane proteins (Amro, *et al*., 2000). Also, Sondi and Salopek-Sondi speculate that a similar mechanism may cause the degradation of the membrane structure of *E. coli* during treatment with Ag nanoparticles (Sondi and Salopek-Sondi, 2004). Although their inference involved some sort of binding mechanism, still unclear is the mechanism of the interaction between Ag nanoparticles and component(s) of the outer membrane. Recently, Danilczuk and co-workers (2006) reported Ag-generated free radicals through the ESR study of Ag nanoparticles. We suspect that the antimicrobial mechanism of Ag nanoparticles is related to the formation of free radicals and subsequent free radical–induced membrane damage.

Our results support the hypothesis that Ag nanoparticles can be prepared in a simple and cost-effective manner and are suitable for formulation of new types of bactericidal materials.

Conclusions

This study describes a simple environmentally ecofriendly benign method of synthesis of silver nanoparticles from plants which is the best source. This method can be further used for industrial production of nanoparticles at room temperature and with a single step. This investigation provides evidence that plant extract stabilized nanoparticles may be ideal candidates for future studies exploring their use in biomedical and pharmacy applications. This synthesis procedure offers a less cost-effective and green alternative to traditional protocols that may be readily scaled up for industry as a result of the low synthesis temperatures and time required. Since Kumquat (*Fortunella margarita*) fruit is easily available throughout the nation, the active nano compound from this can be prepared and used as effective antibacterial reagents even against multidrug resistant bacteria, and home-available, safe, cheap and with no side effect like the synthetic drugs.

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التخليق الاخضر لمنمنمات الفضة باستخدام مستخلص فاكهة الكمكوات وتقيم الفعالية المضادة للجراثيم.

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الخلاصة

في الدراسة الحالية صنعت منمنمات الفضة بطريقة صديقة للبيئة واقل تكلفة، ودلك باستخدام عصير فاكهة الكمكوات (فوتينيلا ماريكريتا) كعامل مختزل ومثبت. درست خصائص منمنمات الفضة باستخدام المطياف للاشعة فوق البنفسجية – المرئية و الاشعة تحت الحمراء و حيود الاشعة السينية ومجهر القوة الدرية و المجهر الالكتروني الماسح. أثبت الفحص بالمطياف أن ذروة الامتصاص عند مدى 420 نانومتر. اثبتت نتائج الفحص بالاشعة تحت الحمراء وجود مجموعات فعالة عديدة. نتائج الفحص بجهاز حيود الاشعة السينية تشير إلى طبيعة التشتت المتعدد من الجسيمات النانوية المحضرة بالطريقة البايولوجية. وتشير النتائج أن تبلور المرحلة البيولوجية العضوية يحدث على سطح الفضة النانوية أو العكس بالعكس. ويعزى توسيع قمم في أنماط حيود الاشعة السينية إلى تأثيرات حجم الجسيمات. تم مسح منمنمات الفضة المخلقة بمساعدة مستخلص أوراق النارنج بوساطة المجهر الالكتروني الماسح ومنه نستنتج ان معدل متوسط الحجم لمنمنمات الفضة كانت 27.19 نانوميتر.

أظهرت النتائج ان منمنمات الفضة المخلقة بوساطة عصير فاكهة الكمكوات (فوتينيلا ماريكريتا) تمتلك نشاط مضاد للجراثيم فعال والدي استدل عليه من قطر منطقة التثبيط ، حيث كان قطر التثبيط 16 ملم للأمعائية المذرقية، 18 ملم للاشريكية القولونية، المتقلبة الرائعة، العصوية س، المكورات العنقودية الذهبية و العقدية س. 20 ملم لكليبسيلا الرئوية و 14 ملم للزائفة الزنجارية. وكانت فعالية مستخلص فاكهة الكمكوات (فوتينيلا ماريكريتا) تجاه الجراثيم المختبرة أقل فعالية ، حيث كان قطر التثبيط 10 ملم للأمعائية المذرقية والزائفة الزنجارية. 12 ملم للاشريكية القولونية و المتقلبة الرائعة. 11ملم لكليبسيلا الرئوية. 13 ملم العصوية س. و العقدية س. و 14 ملم المكورات العنقودية الذهبية. بينما أظهر الاختبار ان محلول نترات الفضة لم يمتلك تأثير تجاه العزلات المختبرة. وكشفت الدراسة أن تخليق منمنمات الفضة بوساطة مستخلص فاكهة الكمكوات يمكن أن تكون كعامل علاجي للعدوى الميكروبية الإنسان.

الكلمات المفتاحية: منمنمات الفضة، جراثيم مرضية، عصير الفاكهة، الكمكوات (فورتينيلا ماريكريتا)، الفعالية المضادة للجراثيم.