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RESEARCH ARTICLE

Green synthesis and antibacterial application of silver nanoparticles using Oak Peel extract

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ABSTRACT

The use of various plant materials for the synthesis of nanoparticles is considered as green synthesis, which does not involve any harmful chemicals. In this study, silver nanoparticles were synthesized by a fast, one-step method and a green biosynthesis method by regenerating a silver nitrate solution using *Oak peel* extract containing biomolecule which as a reducing and stabilizing element Suitable to play a role. Effective parameters such as pH of solution, silver nitrate concentration, temperature and contact time were studied and optimized The structure and properties of nanoparticles were determined by spectroscopic absorption analyzes UV-Visible, Scanning electron microscopy, X-ray diffraction and Fourier transform infrared spectroscopy. According to the Scherer-Debye equation, the size of the nanoparticles was measured 29 nm. Then the antibacterial effect of nanoparticles produced against two strains of pathogenic bacteria, gram positive *Staphylococcus aureus* and gram negative *Escherichia coli* was investigated by disk diffusion and determination of minimum inhibitory concentration (MIC) methods. The results of the antibacterial activities test showed that the nanoparticles produced from Oak Peel had a good effect on both bacteria. The purpose of this research is to synthesis and develop a new method for the preparation of silver nanoparticles using environmental methods.

How to cite this article

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INTRODUCTION

Considering the excessive use of antibiotics and increased resistance to bacteria, finding suitable alternatives for antibiotics is essential. For this reason, extensive studies have been done about the potential for using antimicrobial compounds in plants and the use of nanoparticles to

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control and treat pathogens [1]. Nanotechnology refers to the design, characterization, production, and use of structures and tools, with a nanometric scale and size control (1-100 nanometer) [2]. In general, chemical, physical and biological methods can be used as common methods for the production of nanoparticles [3-7]. In recent years, the use of biological methods such as the use of fungi [8], bacteria [9] and plant extracts [10] because of their simple, low cost, high efficiency, non-toxic and environmentally friendly has paid special attention to other methods [11]. Application of plant extracts as biological substances proves its suitability for the synthesis of nanoparticles. Plants or their extracts provide a biological synthesis pathway for the synthesis of several more biocompatible metal nanoparticles, allowing for synthesis in a given size and shape. The use of plant products for the synthesis of nanoparticles adds new dimensions to modern Nano biotechnology. Among nanomaterials and nanoparticles, silver nanoparticles have attracted particular attention due to their size-dependent properties, including optical, chemical, electrical, catalytic and antimicrobial properties [12, 13]. Bio-produced silver nanoparticles have beneficial properties such as high levels, small size and high dispersion, which have resulted in an increase in the antimicrobial effects of silver nanoparticles compared with silver [14]. With the increase in nanoscale silver antimicrobial effects, silver nanoparticles can be used to combat various pathogens, so today, with the development of nanotechnology and the production of silver nanoparticles, these nanoparticles are widely used in many sciences such as medicine, pharmacy, Cosmetics have found [15]. The use of silver and its nanoparticles has been boosted as a powerful bactericidal agent [16, 17].

The antibacterial activity of silver nanoparticles and their beneficial use in biotechnology and the specific containment of germs have been studied in various studies, so that silver nanoparticles can be effective in inhibiting the respiratory system of bacteria on metabolism and reproduction processes of microorganisms [18] and cause damage to the cell membrane of the bacteria [19]. Several studies have demonstrated the synthesis of silver nanoparticles using plants. Gardea-Torresdey et al., (2003) reported for the first time the production of silver nanoparticles by plants [20]. Also, plants such as Chenopodium album, Rhus coriaria and Sinensis reduced silver ions in sizes below 50 nm [21-23]. Silver nanoparticles synthesized from Pinus eldarica and Garlic with sizes of 20-30 and 10-40 nm exhibited good antimicrobial properties against some bacteria [24, 25]. Recently, the synthesis of silver nanoparticles from oak fruits and its relatively good antimicrobial activity against hospital infections has been reported [26]. In this present study, green synthesis silver nanoparticles by Peel Oak extract and evaluation of its antibacterial activities, had reported.

EXPERIMENTAL SECTION

Chemicals and microorganisms

All the chemicals used in this high purity test were prepared. Sodium Hydroxide (NaOH,40gr/mol), Hydrochloric Acid (HCL,37.5%) from Merck-Germany, and the microorganisms used include the Gram-positive bacteria, Staphylococcus aureus (ATCC: 25923) and the gram-negative, Escherchia coli bacterium (ATCC: 25922) From the Iranian Institute for Scientific and Industrial Research (IROST) and Peel Oak were collected from Oshtorankooh mountain, Lorestan, Iran. During the experiment, two distilled water was used for washing and solubilizing.

Preparation of Peel Oak extract

20 grams of Peel Oak with 200 mL of two distilled water had mixed in an erlenmeyer flask and placed on a heater at 80 °C for 15 minutes. After

separating with Whatman No. 1 filter paper, sub filtration solution was collected for subsequent experiments and placed in the refrigerator at 6 ° C for proper storage of the extract [27].

Initial biosynthesis of silver nanoparticles by Peel Oak extract

In order to synthesize silver nanoparticles, 10 ml of the aqueous extract of the Peel Oak was added to 90 ml of silver nitrate of 1 mM in an erlenmeyer flask at room temperature. The color of the solution changed to brown, which indicates formation of silver nanoparticles (Fig.1). Spectroscopy was detected by UV-Vis spectroscopy [27]. Then, effective parameters such as pH of the solution, concentration of silver nitrate, temperature and contact time were obtained to prepared more uniform and smaller size nanoparticles.

Optimization of pH of solution for the synthesis of silver nanoparticles

According to the shape of the six erlenmeyers flask, 32 ml solution of Peel Oak extract and 2 ml of silver nitrate solution 0.001 M was poured and their pH was adjusted on 2, 4, 6, 8, 10 and 12, respectively. Then, the erlenmeyers flask were placed on a shaker at 150 rpm for 30 minutes at ambient temperature. UV-Vis spectra were taken from 300 to 800 nm. In this way, the optimum pH was determined for silver nanoparticles synthesis and in all subsequent experiments, pH of the solutions was adjusted to optimum pH (Fig.2). In all experiments, sodium hydroxide 0.1M and hydrochloric acid 0.1M were used to adjust pH.

Optimization of silver nitrate concentration for synthesis of silver nanoparticles

To study the effect of the concentration of metal ion in the synthesis of nanoparticles, in 4 erlenmeyer's flask, 2ml of Peel Oak extract to was added to 32 ml various concentrations (0.001, 0.003, 0.005 and 0.01 M). Then, the pH of all of them was adjusted to optimized pH, and the solutions were placed on a shaker at 150 rpm for 30 minutes at ambient temperature. UV-Vis spectra were taken from 300 to 800 nm (Fig.3). In this way, the silver nitrate concentration was determined for synthesis of silver nanoparticles. In all subsequent experiments, optimized concentration of silver nitrate was used.

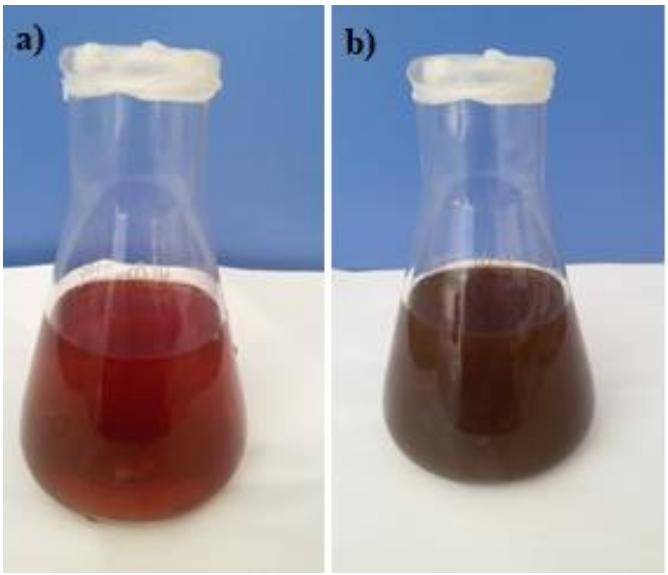


Fig 1. a) Peel Oak pure extract; b) Colloidal solution of silver nanoparticles after reduction of silver nitrate by Peel Oak extract

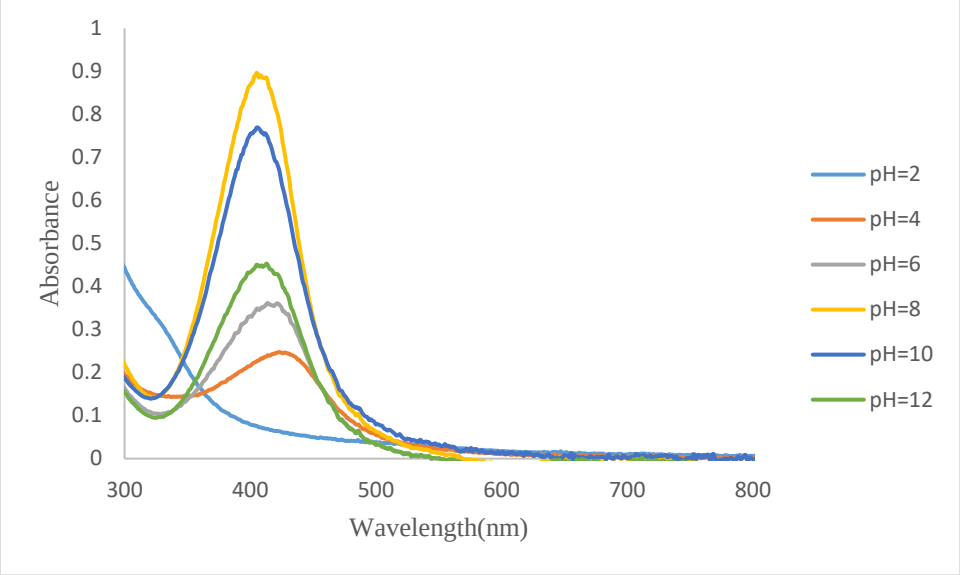


Fig.2. Effect of pH on silver nanoparticles synthesis by Peel Oak extract (32ml Agno3 0.001M, 2ml extract solution, stirring rate 150rpm, time: 30 min)

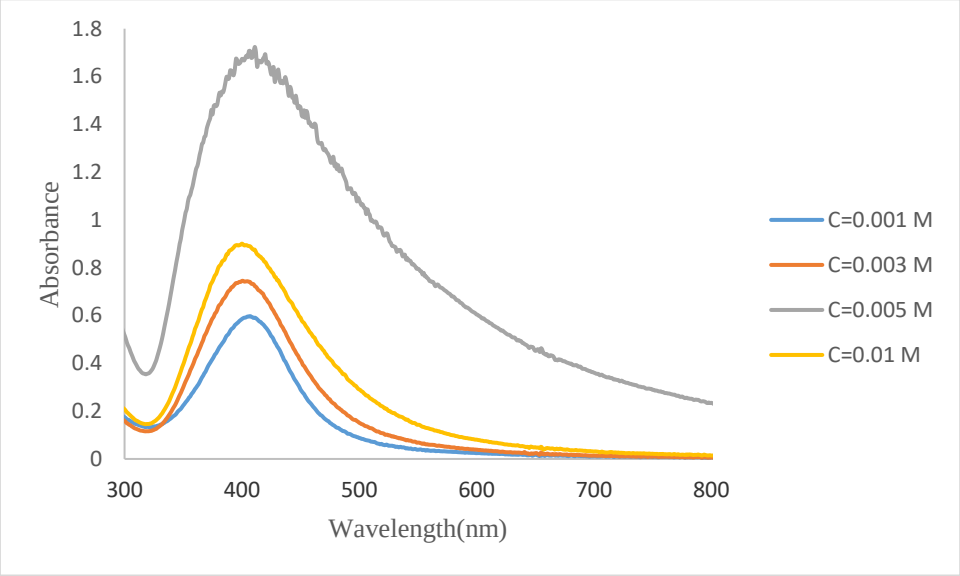


Fig.3. Effect of silver nitrate concentration on silver nanoparticles synthesis by Peel Oak extract (2ml extract solution, pH=8, stirring rate 150rpm, time: 30 min)

Optimization of temperature for synthesis of silver nanoparticles

Determining the best temperature, according to optimized parameters obtained from the previous steps, in 6 erlenmeyer flasks, 2ml Peel Oak extract and 32 ml of metal salt solution with optimized molarity added and the pH adjusted to optimized pH, then the erlenmeyer flasks inserted at temperatures 25, 40, 70, 90, 100 and 110°C respectively. The absorptions of the solutions were read by UV-vis spectrophotometer in range 300 to 800 nm (Fig.4). According to the results, the best temperature was determined.

Optimization of contact time for synthesis of silver nanoparticles

Determining appropriate contact time, in the 9 erlenmeyer flasks, after adding 2ml Peel Oak extract and 32 ml of metal salt solution with optimized concentration and pH adjustment, the erlenmeyer flasks were placed in the oven at optimum temperature. After 10 minutes, one of the erlenmeyer flasks exited and the absorbance of the solution was measured by spectrophotometric method, and the subsequent erlenmeyer flasks out of the oven and adsorbed all the solutions with The UV-vis spectrophotometer read in the range of 300 to 800 nm after 20, 35, 60, 90, 120, 140 and 160 minutes, respectively(Fig.5). According to the results, the appropriate contact time was determined for the synthesis of silver nanoparticles.

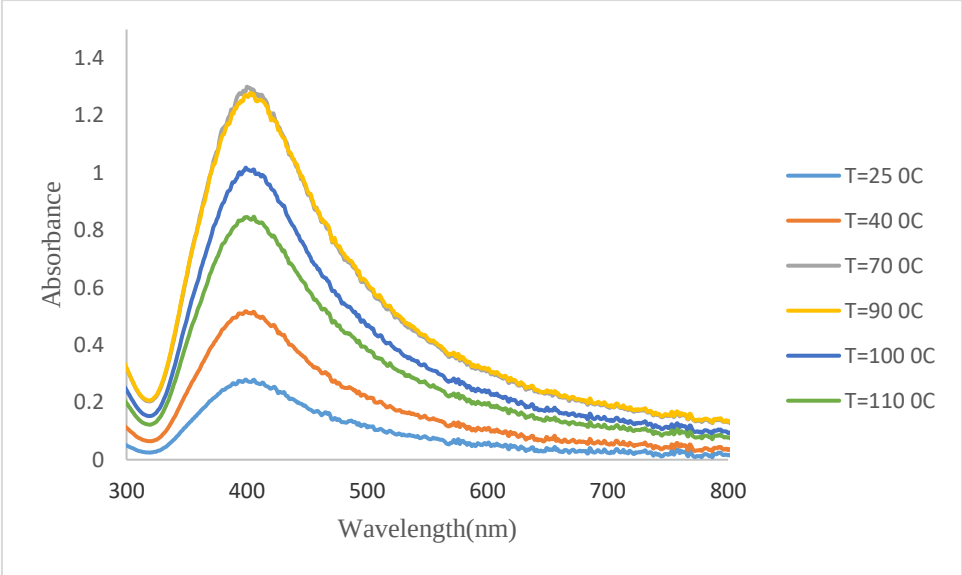


Fig.4. Effect of temperature on silver nanoparticles synthesis by Peel Oak extract (2ml extract solution, 32 ml silver nitrate concentration 0.005M, pH=8, time:30 min)

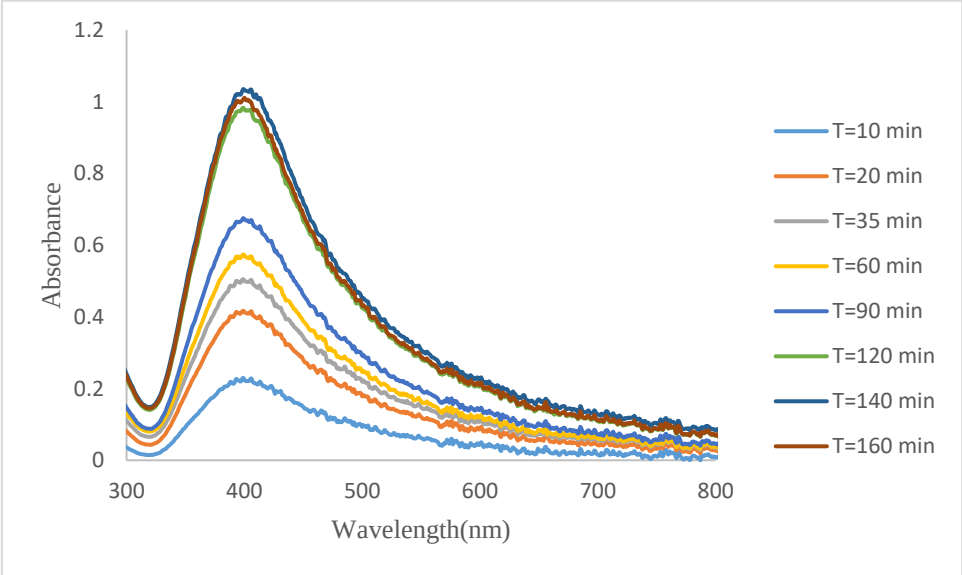


Fig.5. Effect of contact time on silver nanoparticles synthesis by Peel Oak extract (2ml extract solution, 32 ml silver nitrate concentration 0.005M, pH=8, temperature:90 ^{0}C)

Antibacterial activities

Disk diffusion method

First, 20 grams of nutrient agar powder were added in 1000 ml of distilled water, heat it to dissolve until the color solution had yellow clear. Then, solution placed in autoclave at 121 ° C for 15 minutes. Then the medium poured into sterilized plates and after cooling, used for antibacterial test.

In this study, the antibacterial activity of silver nanoparticles on Staphylococcus aureus and Escherchia Coli was investigated. The bacterial strains were purchased from the Center for Scientific and Industrial Research of Iran and the study was carried out according to the instructions of the mentioned center. First, the bacteria were cultured in nutrient broth culture medium and culture medium was used to cultivate the microorganisms in the plate using Muller Hinton Agar culture media. To begin the culture, suspension of bacteria with a concentration of 0.5 Macfarland was given by swab in three directions in each plate. Inside the wells were poured 0.01ml of silver nanoparticles, silver nitrate and extract of the plant into a temperature of 37 ° C for 24 hours and finally the diameter of the inhibition zone of the bacteria was measured by ruler and the mean size The halo was calculated.

MIC

0.21 g of powdered Moeller Hinton Broth medium was added in 20 ml of distilled water and placed in an autoclave for one hour and used for microbial testing. For this purpose, the tested strains mentioned in the above experiment were used. On the other hand, different concentrations of silver nanoparticles were prepared. 70 µl of each culture medium was prepared from each culture medium, and 70 µl of different nanoparticles of extracts were added to 96 µl plates. Then, 70 µl of a bacterial suspension of 0.5 McFarland was added to each plate. In total, this test was performed at 210 µl per well. The first plate was considered as a negative control (culture medium and bacteria) and the last plate was considered as positive control (silver nanoparticles and bacteria). Samples were kept in incubator for 24 hours at 37 ° C. The first well without turbidity was reported as the minimum inhibitory concentration in milliliters per ml.

RESULTS AND DISCUSSION

In this study, pH is one of the most important effecting parameters on synthesis of silver nanoparticles. In the past, reports have also been reported on the effect of pH on the formation of nanoparticles. Reports indicate that pH does not have a significant effect on the shape of nanoparticles, and only effects on the size of the nanoparticles [27]. Fig. 2 represents the nanoparticle formation was confirmed by the appearance of surface plasmon resonance (SPR) with broader peaks (400-460 nm). In examining this effective parameter, the results show that absorbance increases with increasing pH from 2 to 8 and then decreases at pH = 10 and 12. Also, the UV–Vis spectra obtained by varying the pH shows broad peaks at pH 4 and sharp peaks at pH 8, and then we observed a decrease in absorptions and absorption peaks with the increase in pH, that due to the reduction in the size of the silver nanoparticles at elevated pH a blue shift in the wavelength of SPR band was observed in UV–Vis spectra. The blue shift of absorption spectra of nanoparticles can be correlated to the formation of small size NPs at alkaline pH [28], which could be argued that silver ions were hydrolyzed to give rise to stable species of silver ion hydroxides and eventually, the entry of these ions into the biological recovery process is prevented [29]. Also, it is observed that acidic condition suppresses the formation of silver nanoparticles, this is because extremely acidic conditions render the biomolecules present in the plant extracts to be inactive. Andreescu et al. 2007

and Chahardooli et al 2014 reported a similar pH effect at elevated pH. [30-32] Therefore, pH =8 was selected as the optimum pH.

Studies have shown that by increasing the silver nitrate concentration, the observed absorption increases as well, due to with the increase in metal ion, ion of reducing and reducing plant extracts [33]. In the Peel Oak extract according to Fig.3 this increase continued to 0.005M, but at a higher concentration it was observed a significant decrease in the absorbance value associated with the nanoparticles. Reducing nanoparticle absorption by increasing the amount of metal ion concentration due to the bonding of nanoparticles and the synthesis of larger sized nanoparticles was reported earlier [34]. Therefore, silver nitrate concentration=0.005 M was selected as the optimum silver nitrate concentration.

The reaction temperature plays a significant role in determining the size and distribution of particle size. Proper reaction temperatures produce nanocrystals with a narrow size distribution. At such a temperature, the nucleation and growth stages occur individually and can even delay the start of the growth stage, so that occur after the formation of the nuclei. In general, the increase in reaction temperature, increased the reduction reaction speed. Andreescu et al., 2007 was reported A rapid synthesis rate of silver nanoparticles at higher temperatures [30]. Moreover, reports show that spherical nanoparticles were synthesized more frequently at high temperatures when compared with the nanoparticles formed at low temperature [32]. However, there is a contradiction in the effects of temperature on particle size and morphology, and it seems that the optimum temperature for the production of silver nanoparticles by green synthesis method should be empirically obtained for different production conditions. The attention to the figure 4, with increasing temperature, the absorption has also increased, which can be explained by the increase in nucleation with increasing temperature. Therefore, we considered the temperature of 90 ° C as the optimum temperature.

Given the surface plasmon resonance of nanoparticles synthesized over time, the peaks obtained for silver nanoparticles clearly show the dramatic effect of contact time on the formation of silver nanoparticles. According to Figure 5, in the contact time effect parameter, absorption has been increasing, which is due to the increase in the size of nanoparticles synthesized by the reaction time, so that after 120 minutes, a peak with a very long width was observed. So the contact time of 120 minutes was chosen as the optimal contact time. Very reports are available on the effect of contact time on the synthesis of silver nanoparticles. Dubey et al., 2010 reported [35] the formation of silver within 10 min of the reaction in Tansy fruit mediated synthesis and Vilchis-Nestor et al., 2008 reported reduction and formation of stable silver nanoparticles within 4 h of reaction with Camellia sinensis extract[36].

Characterization of silver nanoparticles produced from Oak Peel extract

Spectrophotometry (UV-Vis)

Effecting factors on green synthesis, such as pH, concentration of $AgNO_3$, temperature and contact time has been investigated. According to obtained result from optimal parameters (Figures 2-5) silver nanoparticles were synthesized again at optimum conditions: pH=8, concentration of AgNO3 =0.005 M, temperature=90 0C and contact time=120 minutes and characterized by UV-Vis, SEM, TEM, XRD and FTIR devices after drying and washing.

UV-Visible analysis

According Figures (2-5), UV-Vis spectrum absorbance pattern was observed at 413 nm and ranged from 400 to 480 nm that indicate the

presence of silver, which corresponded to result obtained by Muhammad Amin and et.al.,[37]. Using the spectroscopic method, due to the surface Plasmon resonance of particles, it is possible to track the production of silver nanoparticles in the environment. Surface Plasmon resonance is a very important optical phenomenon in metallic nanoparticles, which has exclusive properties in metallic nanoparticles. In fact, Surface Plasmon is the flux of free electrons at the interface between the nanoparticles and the air, and this phenomenon is highly dependent on the size and morphology of the nanoparticles of metal. In fact, frequency and width of the surface Plasmon depends on size of the nanoparticles that smaller nanoparticles will be more intense the surface Plasmon than the big nanoparticles.

SEM and TEM analysis

Electron microscopes such as SEM and TEM are super-capable devices that can convert signals from collisions of electrons to specimens into images with diverse applications. In the scanning electron microscopy, the surface of the object is scanned by an ultra-thin electron beam. On the specific interactions of the electron beam with the sample, create different signals that are used to study morphology such as the shape and size of the crystals. According to Figures 6 and 7, the average size of silver nanoparticles produced from Peel Oak extract is 40 nm and ~10nm respectively, that similar to results of the other researchers. As seen, the silver nanoparticles are completely synthesized, and their spherical and cubic shape has also been identified.

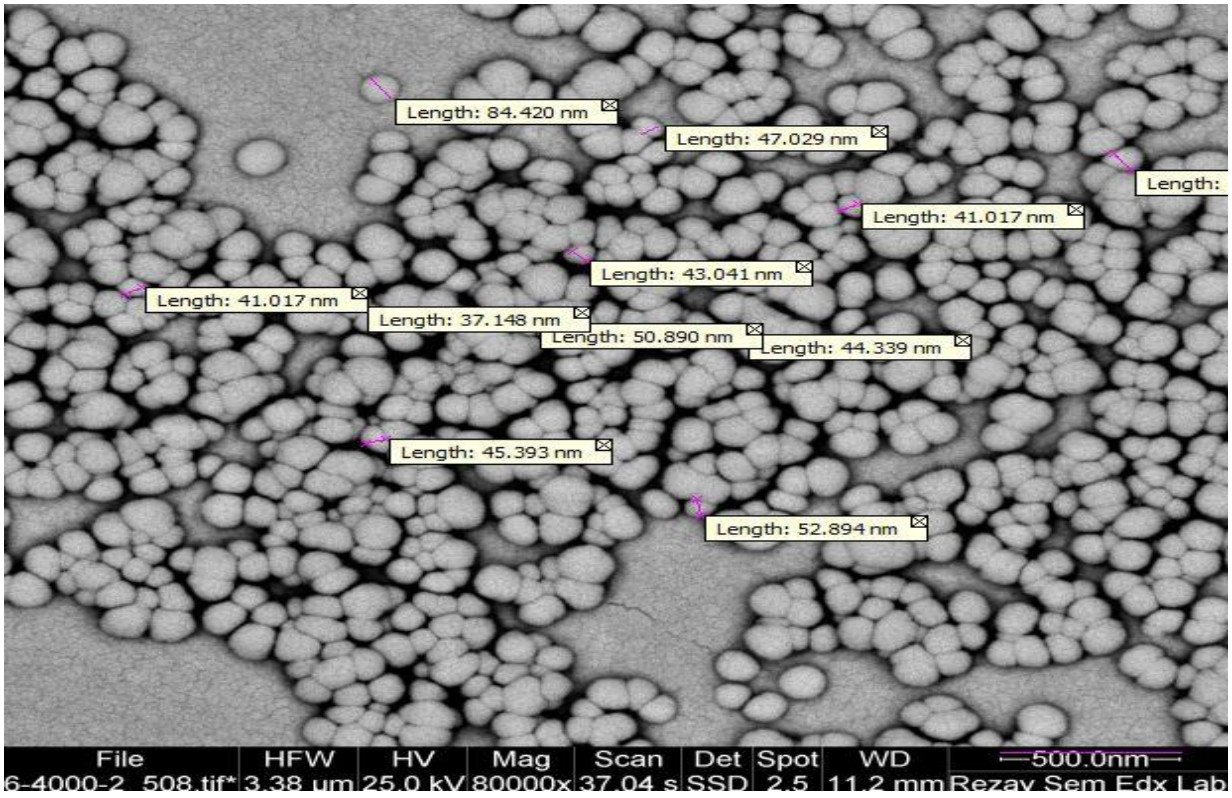


Fig.6. SEM image of silver nanoparticles synthesized from Peel Oak extract

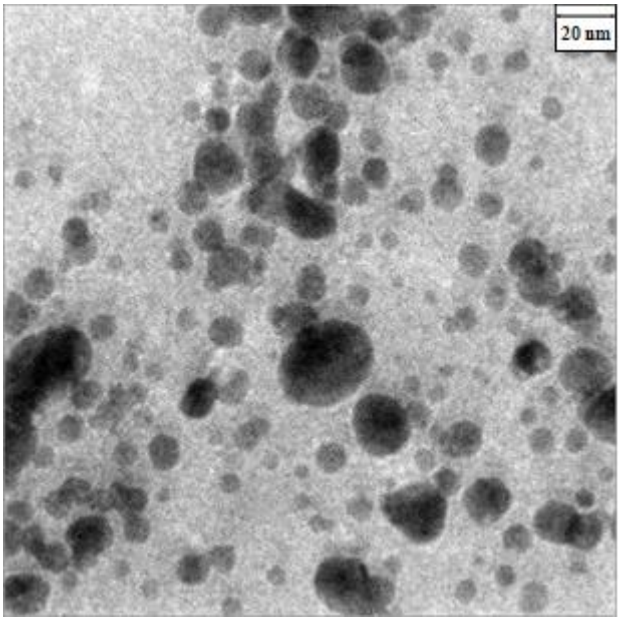


Fig. 7. TEM image of synthesized silver NPs using *Peel Oak* extract

XRD analysis

The crystalline nature of Ag nanoparticles was confirmed from x-ray diffraction (XRD) analysis (Fig. 8). The XRD pattern of the dried Ag nanoparticles synthesized using peel oak extract. The four diffraction peaks were observed at 39.1914, 44.5625, 63.7313 and 77.7787 in the range of 10-80^0.The bright ring arise due to reflection from (111), (200), (220), and (311) planes of silver which is supported by XRD results (JCPDS File No. 87-0717). The results show cubic structure and average size about ~29 nanometer using Debye-Scherrer (equation 1):

$D= K \lambda / \beta \cos \theta$ (1)

Where, D: the size of crystals, λ: X-ray wavelength equal to 1.54046, β: the peak width at half maximum height and θ is the angel between the beam and reflection. (Table 1).

Fourier Transform Infrared (FTIR) analysis

Antioxidant activities of the leaves, peel and fruit components of Oak species have been studied in the literature. Tannin is the major abundant compound in the Oak fruits, Based on the previous reports. Secondary metabolites of the Oak such as tannins are alkaloids, flavonoids and several other aromatic compounds [38-40]. The largest group of antioxidant compounds are flavonoid and phenolic compounds [41]. This indicates that the extract of Oak can reduce silver ions and cap the nanoparticle surfaces protecting them from aggregation.

Effective reducing agents in biosynthesis of nanoparticles include diverse group of water soluble plant metabolites (such as alkaloids, phenolic compounds, and terpenoses) and coenzymes. The organic compounds of the extract can play both reducing and stabilizing effects. According to Figure 9 a, b the band of 1618 cm^{-1} related to the stretching vibration frequency C = O for the alkenes, the 1735 cm^{-1} band refers to the stretching vibration frequency of the carbonyl aldehyde groups, ketones and sterilized bands, 2651 and 2869 cm^{-1} bands are related to the CH-vibration frequency in alkane compounds and 3414 cm^{-1} bands related to the OH-vibration frequency of hydroxyl groups such as phenolic, alcoholic and carboxylic acids compounds. These probabilistic groups are related to the aliphatic, aromatic and protein compounds that are found in the tissue of the

Oak Peel and are absorbed during the synthesis process to the surface of the nanoparticles, and due to their high stability, its absorption is likely to be due to chemical absorption. By comparing different spectra in the form, it seems that certain compounds of the plant play a protective role that encapsulates nanoparticles during the synthesis process and makes them stable.

$(C=O)NH_2$ group in the secondary metabolites of the peel Oak extract causes about shift in the absorption band (1638.77 - 1616 cm^{-1}). The sifting of the weak band 1061.12 cm^{-1} is due to the presence of C−O stretching vibrations of phenolic or C−N stretching of aliphatic amines compounds.

Table 1. Data details for calculating silver nanoparticles size

No.	Position [°2Theta]	FWHM [°2Theta]	h k l	size (nm)
1	39.1914	0.191911	1 1 1	45.87
2	44.5625	0.383813	2 0 0	23.35
3	63.7313	0.255817	2 2 0	38.18
4	77.7787	1.2480	3 1 1	8.54 Ave =28.985

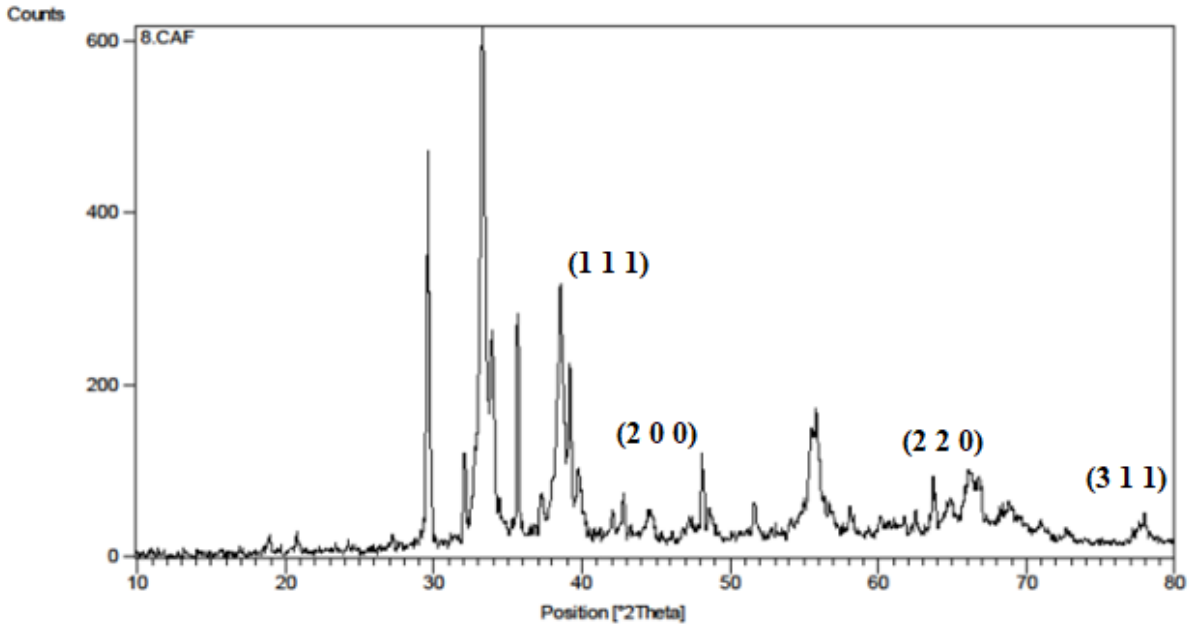
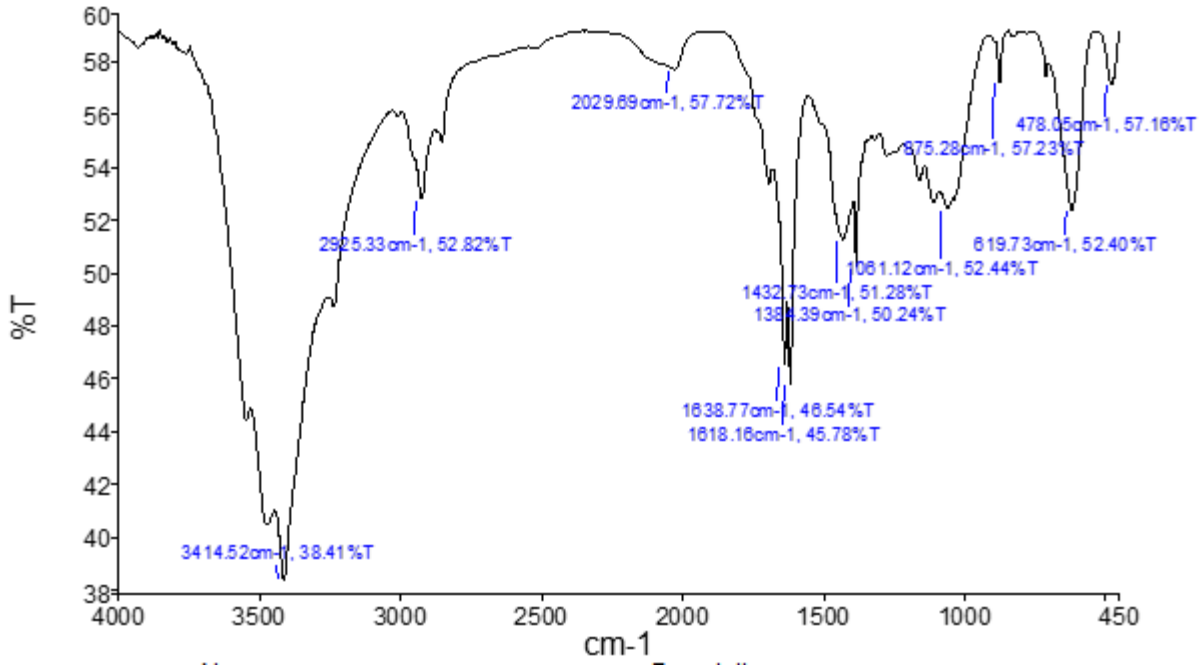
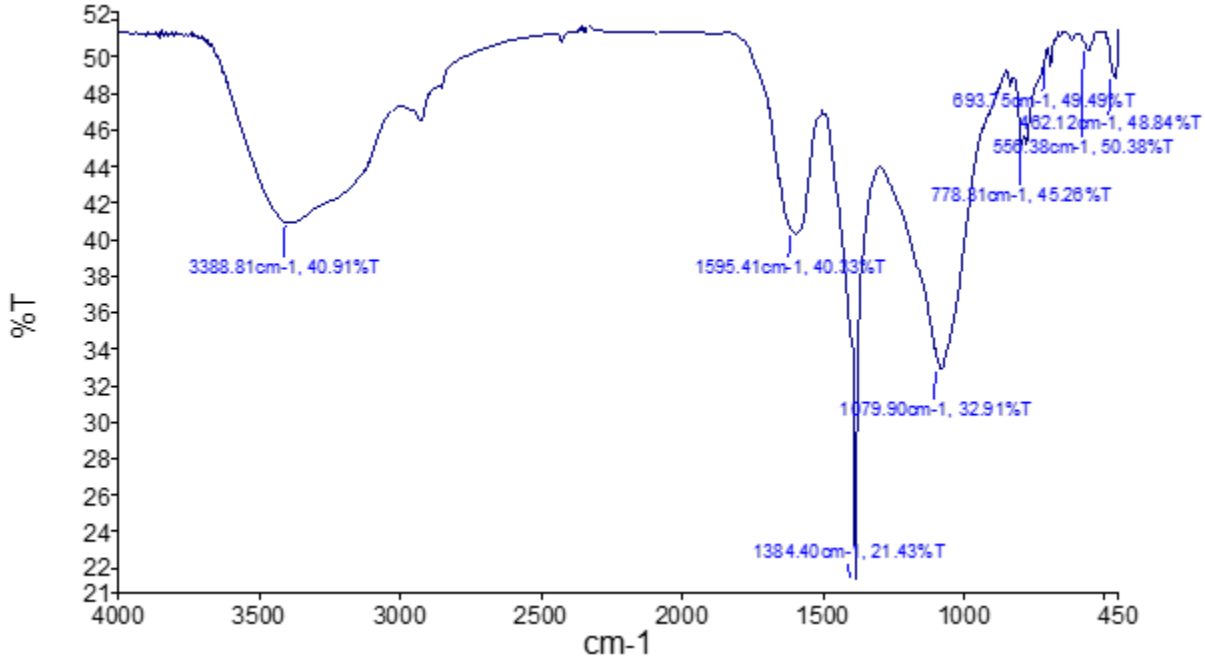


Fig. 8. XRD pattern of synthesized silver NPs using *Peel Oak* extract



a)



b)

Fig. 9. FTIR spectra of a) *Peel Oak extract.* and b) silver NPs synthesized

Antibacterial activities

The nanoparticles produced from plant extracts had appropriate antimicrobial effect on both bacteria (Fig. 10). The results were different for gram positive and gram negative bacteria. Silver nanoparticles penetrate into the bacteria by binding

to the membrane and destroying it, and disrupting its activity. The growth inhibition pattern in Staphylococcus aureus is greater than that of Escherichia coli, which can be explained by the difference in the structure and composition of the membrane of gram positive and negative bacteria. As shown in table 2, the diameter of the inhibition zone in the nanoparticles produced from Peel Oak extract (Staphylococcus aureus 23 mm and Escherichia coli 20.33 mm) was measured. The MIC on Staphylococcus aureus and Escherichia coli for AgNPs with a mean diameter about ~29 nm was 3.12 and 6.25 µg / ml, respectively(Table 3), however, with increasing concentrations (above 6.25 µg / µL), growth in both strains was inhibited. Results obtained in our approach is in good agreement with other researcher's findings. Silver nanoparticles were reported to be synthesized by using Banana peel extract having antibacterial activity against Shrigella sp, Escherichia coli, Klebsiella and Enterobacter aerogens [42]. Silver nanoparticles with diameter 20–30 nm synthesized from Acalypha indica leaf extract have great antimicrobial activity within 30 min against water borne pathogens Escherichia coli and Vibrio cholera ((MIC) = 10 mg/ml [43]. Sathishkumar et al. [44] Synthesized silver nanoparticles and tested against Escherichia coli strain with different concentrations, 2, 5, 10, 25 and 50 mg/L and reported the zone of inhibition of 10.9, 32.4, 55.8, 82 and 98.8% for respective concentrations of silver nanoparticles. Spherical silver nanoparticles with 40–50 nm were produced using leaf extract of Euphorbia hirta which had potential and effective antibacterial property against Bacillus cereus and Staphylococcus aureus [45]. In general, it can be said that the biological synthesis of silver nanoparticles by the extracts is a clean, inexpensive and safe method that does not use any toxic substance in the synthesis of these particles and thus has no side effects. It also has a strong antimicrobial effect that can replace antibiotics.

Table 2. Comparison of Antimicrobial activity of samples

Experimental solution	Zone of inhibition (mm) *Escherichia coli*				Zone of inhibition (mm) *Staphylococcus aureus*			
Extract	6	6	6	6	6	6	6	6
AgNPs	20	20	20.5	20.1	24	24	23	23.66
$AgNO_3$	13	13	13	13	14	14	14	14

Table 3. Minimum inhibitory concentration (MIC) of AgNPs against standard strains of two bacteria

Strain	AgNPs concentration (µg/µL)									
	0.39	0.78	1.56	3.12	6.25	12.5	25	50	100	200
Escherichia coli	+	+	+	+	+	-	-	-	-	-
Staphylococcus aureus	+	+	+	+	-	-	-	-	-	-

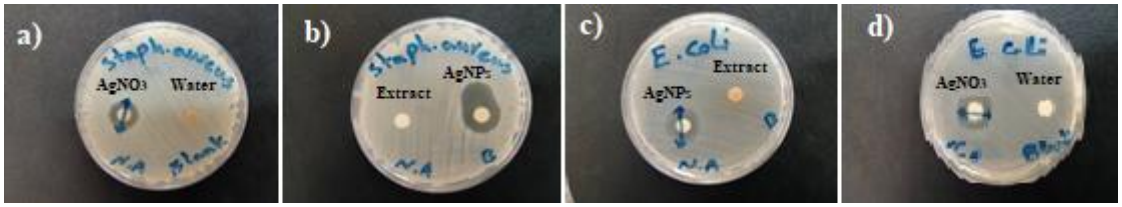


Fig. 10. Antibacterial activities a) Positive and negative control ($AgNO_3$ and water), b) AgNPs and extract against *Staphylococcus aureus*, c) Positive and negative control ($AgNO_3$ and water), d) AgNPs and extract against *Escherichia coli*

CONCLUSION

Ag nanoparticles were successfully synthesized using eco-friendly, simple, rapid, and low cost approach through reduction of $AgNO_3$ solution using Oak peels extract. The usually discarded waste Oak peels were therefore used effectively in this alternative method of synthesizing Ag nanoparticles.

Synthesized Ag nanoparticles were studied using UV-Vis, XRD, SEM, TEM, and FTIR techniques. The reaction mixture of $AgNO_3$ and Oak peels extract displayed vivid colours confirming the synthesis of Ag nanoparticles. UV–Vis spectra showing an absorption band at 413 nm characteristic of the Ag nanoparticles further affirmed the formation of the nanoparticles. SEM images of the biosynthesized Ag nanoparticles at different magnifications showed that the particles were nearly round in shape with smooth surfaces. The TEM images revealed the nanoparticles were granular in nature with sizes in the range of 20-50 nm. X-ray diffraction investigations of the Ag nanoparticles synthesized revealed that the nanoparticles were amorphous in nature with crystal planes at (110), (200) and (112) characteristic for Ag nanoparticles. FTIR spectroscopy designated the contribution of Oak peels extract‘s phenols, nitriles and carboxylic groups in the synthetic process.

The antibacterial properties of the biosynthesized nanoparticles were also studied, as it showed activity on *Staphylococcus aureus* and *Escherichia coli* with MIC values of 3.12 µg/mL and 6.25 µg/mL respectively.

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