Biosynthesis of Gold Nanoparticles Using Extract of Grape (Vitis Vinifera) Leaves and Seeds

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Abstract- A novel synthesis gold nanoparticles (GNPs) using a water extract of grape leaves and seeds as a natural source of both a reducing and stabilizing agents were reported. The gold nanoparticles obtained were characterized by UV–visible spectra, transmission electron microscopy (TEM) and X-ray diffraction (XRD). TEM studies showed the particles to be of various shapes and sizes. The XRD patterns of the GNPs showed a (1 1 1) preferential orientation of the gold nanoparticles. Fourier transform infra-red spectroscopy (FTIR) measurements showed the GNPs having a coating of the extract compounds indicating a possible role of phenolic groups for capping and stabilization of the gold nanoparticles. The photoluminescence measurements revealed the oxidation of the extract compounds during the process of Au³⁺ reduction. As we used green and eco-friendly synthetic reagents used in this method, the gold nanoparticles could have potential for application in bio-molecular imaging and therapy.

Keywords- Gold Nanoparticles; Grape Leaves; Grape Seeds Extract; Antioxidant

I. INTRODUCTION

Recently, a great attention was given to green synthesis of gold nanoparticles with uniform structures and interesting optical properties by using several types of plants that contain different antioxidant compounds. The using plant extract in AuNPs synthesis is more effective and faster than which is synthesized by micro-organisms. The reduction rate using plant's extract has been found to be much faster and stabler as compared to micro-organisms [1-4].

Vitis vinifera L. (common grape) belonging to Vitaceae family has been heralded for their medicinal and nutritional value for thousands of years. Egyptians ate grapes more than 6,000 years ago, and several ancient Greek philosophers praised the healing power of grapes. Phenolic compounds present in nearly all parts of grape are increasingly believed to exhibit antioxidants and antimicrobial activities. The leaves are composed of wide range of polyphenols including anthocyanins, flavonoids and also organic acids, mainly malic, oxalic acid and tartaric acid; citric, fumaric and succinic acid can be detected in the leaves only in traces [5, 6]. Phenolic compounds that found in grape leaves are myricetin, ellagic acid, kaempferol, quercetin and gallic acid [7]. Major phenolics in leaves are myricetin, ellagic acid, kaempferol, quercetin, and gallic acid, with average concentrations of 157.6, 66.7, 8.9, 9.8, and 8.6, respectively. Myricetin is specific antioxidant agent and responsible for the reduction which plays a major role in preparation of GNP’s. Myricetin also a naturally occurring flavonoid found in berries, fruits, vegetables, herbs, tea and wine. The leaves are used to treat hypertension [8, 9], diarrhea, hemorrhage and varicose veins [10], inflammatory disorders [5, 11] and to reduce blood glucose levels in diabetics [12]. Leaves of V. vinifera have also shown hepatoprotective effect on acetaminophen induced hepatic DNA damage [13]. The structures of some of grape leaves and seeds constituents are shown below with respect:
On the other hand, grape seeds are rich sources of monomeric phenolic compounds, such as (+)-catechins, (-)-epicatechin and (-)-epicatechin-3-O-gallate and dimeric, trimeric and tetrameric procyanidins and these compounds act as antimutagenic and antiviral agents [14]. Grape seeds proanthocyanidins (GSP), a combination of biologically active bioflavonoids including oligomeric proanthocyanidins, have been shown to exert a novel spectrum of biological, therapeutic, and chemopreventive properties [15-17]. Oligomeric proanthocyanidins were provided to be highly bioavailable and provide significantly greater protection against damage of oxidative stress than vitamins C, E and β-carotene [18, 19]. Total phenolic compounds concentration was about 2178.8, 374.6, 23.8, and 351.6 mg/g GAE (gallic acid equivalent) in grape seed, skin, flesh, and leaf, respectively [20].

In this context, we have designed a simple, rapid and green synthetic route for the production of gold nanoparticles employing a renewable, biodegradable, non-toxic natural extract from the leaves and seeds of V. vinifera L. The synthesis was carried out in environmentally, biologically benign aqueous medium at ambient temperature and pressure; without using any form of external chemical reducing and stabilizing agents.

II. EXPERIMENTAL

A. Materials

HAuCl4·3H2O 99.9% was purchased from Aldrich. Fresh leaves of V. vinifera L. were collected and washed. The leaves extract was prepared by boiling 2.0 g of grape vine leaves broth for 15 min, filtered and completed to 100 ml. The seeds were collected and washed with deionized water, air dried and crashed to give powder. 2.0 gram of the seed powdered was boiled for 15 min, filtered and completed to 100 ml with deionized water. The prepared extract of both leaves and seeds used as bioreducing agent was stored in the dark at 4°C to be used within one week. A stock solution of 2.5 x 10⁻² M HAuCl₄ was prepared by dissolving 1.0 g HAuCl₄·3H₂O in 100 ml deionized water.

B. Instrumentation

The UV-vis spectra were recorded at room temperature using θ-Helios SP Pye-Unicam spectrophotometer with samples in quartz cuvette. Photoluminescence spectra were recorded on a Perkin Elmer LS 50B luminescence spectrophotometer. X-ray diffraction (XRD) studies were recorded on a JCPDS No. 03-0921 X-ray diffraction. Transmission electron microscopy (TEM) studies were performed using a JEOL-JEM 1200 electron microscope operating at an accelerating voltage of 90 KV. For the TEM measurements, a drop of a solution containing the particles was deposited on a copper grid covered with amorphous carbon. After allowing the film to stand for 2 minutes, the extra solution was removed by means of blotting paper and the grid allowed drying before the measurement. Fourier transform infrared (FTIR) spectra were recorded at room temperature on a Nicolet 6700 FTIR spectrometer. For FTIR measurements of capped gold nanoparticles, a small amount of gold nanoparticles (0.01g) dried at 60°C for 4 h was mixed with KBr to form a round disk suitable for FTIR measurement. To obtain the FTIR spectrum of the extract, an appropriate amount of the extract was mixed with KBr. Thermogravimetric analyses were carried out with a heating rate of 10°C/min using a Schimadzu DT-50 thermal analyzer.

C. Synthesis of Gold Nanoparticles

For the synthesis of the gold nanoparticles, a certain volume of the grape leaves extract (0.1-5) ml of 2% solution was added to the 0.05 ml HAuCl₄·3H₂O solution and the volume was adjusted to 10 ml with de-ionized water. The final concentration of Au⁺ was 1.25 x 10⁻³ M. The reduction process of Au³⁺ to Au nanoparticles was followed by the change of the color of the solution from yellow to violet to dark pink and green depending on the extract concentration. The effect of HAuCl₄·3H₂O concentration on the nanoparticles formation was studied by adding different HAuCl₄·3H₂O concentrations (0.01-0.1) ml of HAuCl₄·3H₂O to 2 ml extract 2% solution and the volume was adjusted to 10 ml (2.5x10⁻⁵-2.5x10⁻⁴ M). The effect of reaction time was studied using (1.3x10⁻⁴ M AuCl₄⁻ and 2 ml extract in 10 ml flask) and the reaction was followed by using spectrophotometer. GNPs which are extracted from grape leaves or seeds solution are centrifuged, washed by ethyl alcohol and dried.

For the nanoparticles prepared at different pH values, the pH of the solutions (1.3x10⁻⁴ M AuCl₄⁻ and 2 ml extract in 10 ml flask) was adjusted using 0.1 N HCl or 0.1 N NaOH solutions.

III. RESULTS AND DISCUSSION

A. UV–visible and TEM Analysis of Gold Nanoparticles Using Grape Leaves Extract

The primary variable in the reaction condition was the concentration of the grape leaves and extract. The formation and stability of gold nanoparticles was followed by Uv-visible spectrophotometry. Figure 1 shows the Uv-visible spectra of gold nanoparticles formation using constant HAuCl₄ concentration (1.25x 10⁻⁴ M) with different concentrations of extract from 0.1 to 5 ml (0.02% to 0.1%). The inset shows photos of the color change of gold nanoparticles with changing the grape leaves extract concentration from 0.1 to 1 ml. As is clear from the inset in Figure 1, the color changed from pale yellow to a vivid ruby red after 1 h of standing. Such a color transition indicated the change in the metal oxidation and formation of gold.
nanoparticles. As shown in the Figure 1, UV-vis spectra showed the appearance of strong SPR band absorption peak centered at about 545 nm. Addition of the extract from 2 to 5 ml leads to slightly increase in the absorption as shown in Figure 2, indicating complete reduction of Au^{3+}. It can be noticed that the gradual increase of the absorbance spectra is accompanied with a shift in the $\lambda_{\text{max}}$ from 564 to 531 nm. This blue shift suggests that by increasing the extract concentration, the particle size decreases.

![UV-vis spectra](image)

**Fig. 2** The concentration effect of the grape leaves extract on the AuNPs formation at $\lambda_{\text{max}}$ 545 nm

The possibility of controlling the particle size and shape by changing the composition of the reaction mixture is investigated. Figure 3 shows the TEM images of the gold nanoparticles synthesized using different grape leaves broth concentrations (A: 0.1%, B: 1%) with 1.25 mM HAuCl$_4$ at room temperature. As can be seen, a mixture of plate (triangles and pentagons) and spheres was obtained at low grape leaves concentration while only spherical shapes were obtained at high concentration, 1%, of the grape leaves extract. Also, Figure 3 shows that the particle size decreases with an increase in the leaves broth concentration. Similar changes in the shapes and size were observed on phyllanthin assisted gold nanoparticle biosynthesis [21] and for the gold prepared using olive leaves extract [22]. The use of a low concentration of the plant extract reacting with HAuCl$_4$ led to the formation of hexagonal or triangular gold nanoparticles, while the shape of the nanoparticles changed to spherical on increasing the concentration of the extract. This could be explained as the low quantities of the extract can reduce the AuCl$_4^-$ ions, but do not stabilize most of the quasi-spherical nanoparticles from aggregating because of the deficiency of biomolecules to act as stabilizing agents. Similar studies showed that the comparatively higher extract ratio is responsible for the synthesis of symmetrical nanoparticles [23-25].

![TEM images](image)

**Fig. 3** TEM images of the gold nanoparticles formed by the reaction of 1.25 mM HAuCl$_4$ and various concentrations of the grape leaves broth at room temperature: (A) 0.1%, (B) 1%
A variety of synthetic routes have been used to generate nanoprisms, nanotriangles, nanoplates, or nanodisks. In particular, these prisms have SPRs that are tunable throughout the visible and near-IR (NIR) regions of the spectrum by controlling nanoprism edge length, thickness, and tip morphology [26].

To obtain the optimum ratio of AuCl$_4^-$ extract for the better quantity of the prepared nanogold, the experiment was designed to add different metal concentrations to 2 ml of the extract. With increase in the concentration of the metal ion from $2.5 \times 10^{-5}$ to $2.5 \times 10^{-4}$ M, the band at 550 nm shows shift to longer wavelength suggesting increase in particle size and only at 0.1 ml ($2.5 \times 10^{-4}$ M) AuCl$_4^-$, a significant peak was found at 750 nm characteristic for the longitudinal plasmon resonance of either Au nanorods or triangular or hexagonal shaped AuNPs (34) with the shift of the band at 550 nm to shorter wavelength. The spectra are shown in Figure 4.

![Fig. 4 Effect of addition of Au$^{3+}$ to 2 ml of the grape leaves extract](image1)

Figure 5 shows the effect of time on the formation of the AuNPs. It can be seen that the absorption spectra of the solution increased suddenly after 3 min and slow increase upon increasing time to 30 min. This indicates that the time needed for the formation of gold nanoparticles using grape leaves extract is very short. It is also important to mention that after 24 h, the spectrum of the nanoparticles reveals an increase in the absorption about 20% and a broadening is observed suggesting the formation of different structures upon increasing exposure time of the gold ions to the extract.

![Fig. 5 UV–visible spectra of Ag nanoparticles as function of The effect of time on the formation of gold nanoparticles with 2 ml of grape leaves extract, [Au$^{3+}$] = 1.25x10$^{-4}$ M at $\lambda_{max}$=545 nm](image2)

**B. XRD for Gold Nanoparticles**

XRD analysis of the nanoparticles obtained at high grape leaves extract concentration showed intense peaks corresponding to (111), (200), (220) and (311) Bragg's reflection based on the Cubic Closest Packed Structure Face Centered Cubic (ccpsfcc) of gold nanoparticles (Fig. 6). The broadening of Bragg's peaks indicates the formation of nanoparticles. The mean size of gold nanoparticles was calculated using the Debye–Scherrer's equation by determining the width of the (111) Bragg's reflection. The size of the nanoparticles was thus determined to be about 13.1 nm for gold nanoparticles. In TEM micrograph, gold nanoparticles in the size range of 18–25 nm were observed (Fig. 3).

**C. Photoluminescence of AuNPs**

The fluorescence spectra of the nanoparticles formed as a function of GLE concentration are shown in Figure 7. The fluorescence spectra ($\lambda$ excitation = 328 nm) exhibit different behavior dependent on the extract concentration range. Addition of low concentration of the extract, emission spectra showed band at 370 nm and at 430 nm. The intensity of the band at 370 nm is decreased, while the band intensity at 430 nm is slightly increased. After addition of 1.5 ml of the extract (corresponding to 0.3%), the fluorescence band reached the maximum intensity with a shift to 445 nm. In order to explain these phenomena,
one has to recall Figures (1-3) up on addition of low concentration of the GLE while by addition up to 1.5 ml, the absorption increased monotonically, and any extra increased of the extract led to a very small increase in absorption indicating that 1.5 ml (0.3%) was enough to reduce most of the gold ions to nanogold. This result with the fact that low concentration of the extract gives different nano-structures could explain the decrease in the band at 370 nm and the increase in the band at 430 nm. Addition of more extracts more than 1.5 ml led to disappearance in the bands at 370 and 430 nm and a new band with 445 nm appeared which is quenched by addition of extract more than 2 ml. The analysis of the fluorescence intensity at 430 and 445 nm are depicted in Figure 8.

![Fig. 6 XRD patterns recorded for gold nanoparticles synthesized by treating grape leaves extract with AuCl₄⁻ aqueous solutions. The Bragg reflections are indexed on the basis of the fcc gold structure.](image)

![Fig. 7 Photoluminescence of AuNPs as a function of grape leaves extract (λₑₓ = 328 nm)](image)

![Fig. 8 Analysis of the fluorescence intensity of AuNPs as a function of extract concentration](image)

It can be observed that at low concentration up to 1 ml extract the fluorescence intensity at 340 nm shows a slight increase in the intensity and the highest intensity is observed at 1.5 ml extract where the ratio HAuCl₄: extract is about 1:30 as observed from absorption spectra.

In order to investigate the origin of the peaks at 370, 340 and 445 nm, if it originates from the extract or from the nanoparticles, the fluorescence spectra of the leaves extract alone were measured (Figure 9). The leaves broth itself is found to
be luminescent with an emission at 445 nm when it is excited with 330 nm. It can be seen that increasing the extract concentration increases the fluorescence intensity reaching a maximum after the addition of 1.5 ml of the extract. This is consistent with the increase in the absorption spectra in Figure 1. On the other hand, a further increase of the extract concentration leads to fluorescence quenching. So, it can be concluded that at low extract concentration, upon addition of the leaves extract to the Au³⁺, the blue shift from 445 to 430 nm could be due to the oxidized form of the antioxidants present in the extract resulting from reduction of Au³⁺ to Au nanoparticles, and the extract is just enough to reduce the Au³⁺ to Au nanoparticles. Recall the TEM measurement, Figure 3, the band at 370 nm could result from the triangle shapes formed at low extract concentration. At higher concentration, the nanoparticles are capped with the extract constituents and only spherical particles exist and the fluorescence intensity suddenly increased with increasing extract concentration. This increase in the intensity with shifting the wavelength back to 445 nm (of pure extract) indicates that the extract concentration is enough for reduction and capping of the produced nanoparticles. Further increase in the extract concentration leads to fluorescence quenching due to self quenching of the extract.

In order to inspect if there are more different reducing agents present in the solution, emission spectra of the extract and the AuNPs solutions at high and low extract concentrations were measured in Figures 10 and 11 respectively.

Figure 10 shows that the excitation at 300-320 nm at high extract concentration (1 ml extract) gives maximum emission peak observed at 445 nm, while the excitation at 330-360 nm gives another peak at 450 shifted with excitation wavelength to longer wavelength as observed as red shift. This could indicate the presence of another emitting species in the extract layer about the gold NPs which is more pronounced with excitation at 360 nm and which is responsible to get on gold nanoparticles in spherical shape.

On the other hand, the emission spectra of the AuNPs solution with low extract concentration (0.05 ml Au³⁺, 0.3 ml extract) at different excitation wavelength are depicted in Figure 11.
At this concentration the nanoparticles with triangle structure are formed and the extract was not enough to reduce and to cap the nanoparticles. It can be seen that, beside the band at 430 nm due to the oxidized form of the extract, a new sharp band changing its position with the excitation wavelength is observed. This sharp peak could be due to the presence of the triangle and hexagonal shapes present at low extract solution. This explanation is supported by the disappearance of this peak; as shown in Figure 12, upon increasing the extract concentration to have more spherical particles.

**D. Effect of pH on the Formation and Stability of AuNPs**

Figure 13 shows the effect of pH on the formation of gold nanoparticles. It can be seen that the absorbance increases with increasing pH from 4.3 to pH 6 with blue shift in the spectra and from pH 7 to 9.5 the absorbance decreased with the spectrum becomes wider. The size of the particles was followed by measuring TEM at pH 4.3 and pH 8.6, Figure 14. The size of the particles at pH 4.3 was larger with formation of hexagonal structures. Furthermore, the particles formed in acidic medium were unstable and precipitated within 12 h while the particles prepared at pH 9.5 were stable for one week.
E. FTIR Spectra

Various vibrational frequencies, in the range of 4000–400 cm\(^{-1}\) of the extract and AuNPs loaded samples were shown in Figure 15. The IR spectra of the dried leaves exhibit a strong and wide band at 3421 cm\(^{-1}\) due to presence of the OH stretching frequency of the hydrogen donating substituents (hydroxyl groups), attached to the aromatic ring structures of flavonoids compounds in the leaves. This band shows high shift to 3282 cm\(^{-1}\) on the gold nanoparticles. The IR bands (Fig. 3.15 (a)) observed at 1315 and 1730 cm\(^{-1}\) in dried grape leaves are characteristic of the C–O and C=O stretching modes of the carboxylic acid group possibly of gallic acid and malic acids present in the grape leaves. The amide I band appears as very strong band at 1627 cm\(^{-1}\) and amide II band as a medium broad shoulder at 1650 cm\(^{-1}\) in the leaves. These amide I and II bands arise due to carboxyl stretch and N–H deformation vibrations in the amide linkages of the proteins [27–28] present in it. The medium broad band at 1315-1441 cm\(^{-1}\) is the C–N stretching mode of aromatic amine group [29]. The C–O–C and C–OH vibrations [30] of the protein in the leaves appear as a very strong IR band at (1072 cm\(^{-1}\)). The medium intense band at (1729 cm\(^{-1}\)) is observed for the C=O stretching mode in the IR spectrum of gold nanoparticles (Fig. 15(b)) indicates the presence of –COOH group in the material bound to Au nanoparticles and the C=O groups do not attach to the gold nanoparticles. The amide II band has become more prominent in the spectrum of gold and amide I band is shifted to higher frequency (1655 cm\(^{-1}\)) compared to that of plain leaves (1627 cm\(^{-1}\)). It is well-known that proteins can bind to Au nanoparticles through the free amine groups or carboxylate ion of amino acid residue in it [31]. The presence of the IR bands due to C=O stretching at (1731 cm\(^{-1}\)) and the prominent appearance of the amide I and amide II bands with large shift from that of the plain leaves indicate the possibility that gold nanoparticles are bound to proteins through free amine groups. However, signals for different functional groups found in the IR spectrum of AuNPs indicated that some phenolic compounds were bound to the surfaces of GNPs that remained despite repeated washing. Through free amino (–NH\(_2\)) or carboxylic (–COOH) groups, these compounds might have interacted with gold surface making AuNPs highly stable.

F. Thermogravimetric Analysis

The TGA plot of the capped gold nanoparticles prepared using high grape leaves extract (Fig. 16) showed a steady weight loss in the temperature range of 50°C–500°C. The weight loss of the nano powder due to desorption of bioorganic compounds in the AuNPs was 73.35%. This high value indicates that the bioorganic compounds could crystallize with the gold nanoparticles.
G. Formation of AuNPs Using Grape Seeds Extract

Grape seeds are waste products of the winery and grape juice industry. These seeds contain lipid, protein, carbohydrates, and 5%–8% polyphenols, depending on the variety. The grape seed extract is considered as a powerful antioxidant that prevents premature ageing and disease [32]. The majority of studies on grape phenolics have been conducted using proanthocyanidin-rich seeds extracts. The phenolic content and composition of grape seed extract (GSE) depends on the solvent used for extraction. [33] obtained the highest yield of extract using ethylacetate-water (17:3, v:v). Extraction with methanol gave maximum yield of the extract but with a lower content of flavanols.

H. Effect of Grape Seeds Concentration

The Uv-visible spectra of gold nanoparticles formation using constant HAuCl4 concentration (1.3x 10-4 M) with different concentrations of extract from 0.1 to 1.6 ml (equivalent to 0.02 to 0.32%) are shown in Figure 17. It can be seen that addition of the extract from 0.1 to 0.8 ml leads to increase in the absorption, while addition of the extract from 0.9 to 1.6 ml leads to a slight decrease in the absorption indicating the attainment of the saturation in the bio-reduction of Au3+. Comparing the spectrum in Figs. 1 and 14, it can be noticed that upon addition of 0.8 ml the absorption value in the case of using seeds extract is higher than the leaves extract. This suggests that the amount of antioxidants in the seeds is more than that in the leaves extract, which agrees with literature survey [20]. As a result, we found that GNPs are smaller in size by using grape seeds than grape leaves extract as using same concentrations of these extracts.

Fig. 16 TGA of the capped AuNPs prepared using grape leaves extract

Fig. 17 Plasmon resonances of gold nanoparticles reduced by different grape seeds extract concentrations

Fig. 18 TEM images of the gold nanoparticles synthesized using different grape seeds concentrations (a: 0.1%; 0.5 ml, b: 1%; 5 ml) with 1.25 mM HAuCl4 at room temperature
Figure 18 shows the TEM images of the gold nanoparticles synthesized using different grape seeds concentrations (A: 0.1%, B: 1%) with 1.25 mM HAuCl₄ at room temperature.

As can be seen, a mixture of plate (triangles and pentagons) and spheres was obtained at low grape seeds concentration while only spherical shapes were obtained at high concentration, 1% of the grape seeds extract similar to those obtained for the grape leaves water extract.

I. Effect of pH on the Formation of AuNPs Using GSE

The effect of changing pH of the solution on the AuNPs formation is presented in Figure 19.

![Fig. 19 Effect of pH on the formation of AuNPs using GSE](image)

It can be seen that increasing the pH from 3 to 9.8 leads to increasing in the absorbance and as increase in absorbance peak sharpens indicates that there is increase in homogeneity of the nanoparticles shape.

J. XRD of AuNPs Prepared Using GSE

The phase of the prepared nanoparticles was investigated by X-ray diffraction technique. The Au NPs synthesized by GSE showed clear peaks at 38.21 (1 1 1), 44.34 (2 0 0), 64.78 (2 2 0), 77.67 (3 1 1) (Fig. 20).

![Fig. 20 XRD patterns recorded for gold nanoparticles synthesized by treating grape seed extract with AuCl₄⁻ aqueous solutions](image)

The Bragg reflections are indexed on the basis of the fcc gold structure in TEM micrograph, gold nanoparticles in the size range of 10–17 nm were observed (Fig. 18).

K. FTIR Spectra

The FTIR spectra of untreated and treated grape seed extract samples containing gold nanoparticles are depicted in Fig. 21.

The untreated seed extract sample shows medium or strong absorption bands about at 3313, 2926, 1745, 1654, 1620, 1520, 1390, 1290 and 1110 cm⁻¹. The absorption band at 1745 cm⁻¹ can be assigned carbonyl peak (C=O stretching) of carboxylate content in plant-based extract which may be from gallic acid and proanthothianidin gallate present in the aqueous seed extract.
Bands originating from hydroxy group (from phenolic compounds) at 3313 and 1110 cm\(^{-1}\), as well as medium band at 1520 cm\(^{-1}\) indicating presence of amide/amine groups, would be expected due to plant-origin of these samples.

Infrared spectra of the gold nanoparticles revealed the medium bands at 1082, 1380 and strong bands at 1614 and 3413 cm\(^{-1}\) (Fig. 21(b)). The band at 1380 cm\(^{-1}\) corresponds to C–N stretching vibrations of aromatic amines. The band at 1614 cm\(^{-1}\) corresponds to C=O stretching of amide I band and 3413 cm\(^{-1}\) corresponds to –NH stretching in amide (II) and 1082 cm\(^{-1}\) is characteristic of C–OH stretching of secondary alcohols. The weaker band at 2929 cm\(^{-1}\) corresponds to asymmetric stretching of C–H groups. The absorption band corresponding to C=O group at 1745 cm\(^{-1}\) in untreated seeds is shifted to 1730 cm\(^{-1}\) appeared as shoulder. [35] reported that terpenoids and reducing sugars present in the neem extract were responsible for the stabilization and capping of gold nanoparticles. [36] demonstrated the synthesis of gold nanoparticles using geranium leaves extract and showed the oxidation of alcohol groups of terpenoids to carbonyl groups and the presence of amide (III) of proteins during the formation of gold nanoparticles. In the present study, FT-IR spectrum confirmed the presence of aromatic amine, amide (II) groups and secondary alcohols as capping and reducing agents of gold nanoparticles. It was difficult using FT-IR to determine the presence of the antioxidants present in the grape seeds (mentioned above) and their role in the reduction of the gold ions to AuNps.

**IV. CONCLUSION**

- As the extract concentration increases, particles size decreases.
- Several shapes were obtained at low grape leaves concentration while only spherical shapes were obtained at high concentration, the grape leaves or seeds extract.
- With increasing the metal ion concentration from 2.5x10\(^{-5}\) to 2.5x10\(^{-4}\) M, in particle size increases which is clear from the shift of the maximum wavelength to longer wavelength.
- The time needed for the formation of gold nanoparticles using grape extracts is very short 30 min.
- Gold nanoparticles (GNPs) formed in acidic medium were unstable and precipitated within 12 h while GNPs prepared at pH = 9.5 were stable for one week.
- As compare XRD for (GNPs) size formed by grapes leaves and grape seeds extract, we found that GNPs are smaller in size by using grape seeds than grape leaves extract.
- In future, we will study the effect of our prepared GNPs on liver tumor cells inhibition as a natural treatments.

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