Green synthesis of gold nanoparticles using extracted, purified flavonoid from *Myrtus communis* Leaves.

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Received: 01/06/2024
Accepted: 19/08/2024
Online: 30/12/2025

ABSTRACT

Background: The parts of *Myrtus communis* (known as Al-Yas) were collected in Oct. 2023 from several areas in Baghdad, Iraq. **Methodology:** An aqueous extract was prepared using the traditional method, and gold nanoparticles were subsequently synthesized from the plant extract. Four different concentrations of AuNPs were prepared. Various methods are used to characterize green-manufactured gold nanoparticles and to determine phenol content. The Folin Ciocalteu assay method and the Aluminum chloride method were used for flavonoid determination. **Results:** The total phenolic content, expressed as gallic acid equivalents, was determined using the following equation, where the phenolic concentration was 33.4 mg/g. According to the straight-line equation, the total flavonoids were 17.66 mg / g of the extracted total flavonoids. A change in the extract color due to a reduction reaction serves as the first indicator for the green synthesis of gold nanoparticles. Additionally, results showed that the diameter of the extract molecules was 253.0 nm, while it was 65.27 nm for green-synthesized AuNPs from crude extract and 44.99 nm for AuNPs from flavonoid. UV-Visible spectroscopy of aqueous extract was absorbed at 285 nm, and it was 550 nm for green synthesized AuNPs from crude extract, while it was 555 nm for AuNPs for flavonoid. Also, FE-SEM images revealed differences in the characteristics of crude extracts and AuNPs derived from crude extracts or from purified flavonoids. **Conclusion:** These results were promising for obtaining nanoparticles with unique properties via a green method that is environmentally safe.

Keywords: Gold nanoparticles, *Myrtus communis*, Green Nano-biotechnology, Gallic acid, Total flavonoids.

https://doi:10.24126/jobrc.2025.19.3.864

INTRODUCTION

The oldest form of treatment is medicinal herbs, which have been utilized in traditional medicine for thousands of years across many nations. Over time, empirical knowledge regarding the advantageous impacts of these substances has been transmitted across human communities (1). Natural products serve as a significant reservoir of pharmacologically active molecules, and numerous current drugs are derived from traditional herbal medicine and used in contemporary pharmacotherapy (2,3). In addition, the unique reverse pharmacognosy approach enabled the discovery of several bioactive phytochemicals (4). Plant primary metabolites, which include simple lipids, carbohydrates, and amino acids linked to photosynthesis, yield products that are then converted into bioactive principles, also known as plant secondary metabolites. Though they are often not involved in the development and metabolism of plants, bioactive principles are essential to their survival because they protect plants from harmful organisms and herbivorous predators (5). Plant secondary metabolites, like flavonoids, are a significant group of compounds characterized by their polyphenolic structure. These compounds are widely found in fruits, vegetables, and some beverages. They possess a diverse range of advantageous biochemical and antioxidant properties associated with several ailments, such as atherosclerosis, cancer, and Alzheimer's disease (AD). Flavonoids play a

crucial role in various nutraceuticals, pharmacological, medicinal, and cosmetic applications and are associated with numerous health-enhancing advantages. These traits result from their capacity to alter crucial cellular enzyme activity, as well as their ability to combat oxidative stress, inflammation, mutations, and carcinogenesis. Furthermore, their strong inhibitory effects on a wide range of enzymes, including cyclo-oxygenase, lipoxygenase, xanthine oxidase (XO), and phosphoinositide 3-kinase (6).

In conventional folk medicine, *M. communis* L. is a commonly used medicinal herb. This plant has been used to extract and isolate several bioactive substances, including polyphenols, semi-myrtucommulone (S-MC), myrtucommulone (MC), 1,8-cineole, α-pinene, myrtenyl acetate, linalool, limonene, and α-terpinolene. It may have a broader range of pharmacological and therapeutic effects, including the treatment of peptic ulcers, hemorrhoids, diarrhea, inflammation, and skin and lung issues, according to experimental and clinical research. Its antifungal, antibacterial, antiviral, anticancer, antidiabetic, antioxidant, neuroprotective, and hepatoprotective properties can also be exploited. Among *M. communis*, the principal chemical constituents are α-Pinene (17.8%), geranyl acetate (6.3%), linalool (17.55%), and 8-cineole (28.62%). Additionally, it has trace amounts of eugenol (1.3%), α-Humulene (1.5%), methyl chavicol (0.5%), isobutyl-isobutyrate (0.8%), and geraniol (1.6%) (7).

Any member of the non-nitrogenous biological pigments that are widely found in plants is referred to as a flavonoid, or flavone. Plant cells contain water-soluble phenolic chemicals called flavonoids, which have an aromatic ring with a -OH group attached. There have been descriptions of over 3,000 distinct flavonoids. A large number of this group's members, most notably the anthoxanthins, give flowers' petals their characteristic yellow hue. The purple and purple-red hues of fall leaves, as well as the red coloring of buds and young shoots, are mostly caused by a second main group called anthocyanins. Typically, flavonoids and flavones are pigments with an ivory or yellow tint (8). While the physiological roles of flavonoids remain undetermined, it is possible that they act as antioxidants and shield the body from UV radiation harm. Their ability to add color to flowers is a major factor in drawing pollinator-carrying insects like butterflies and bees, who aid in plant fertilization. In a similar vein, colorful fruits have a better probability of having their seeds spread by animals attracted to them for food. Additionally, flavonoids influence how plants communicate with the microorganisms in their roots that fix nitrogen. In animals, the flavonoids are comparatively few and small, obtaining their pigments from plants (8).

The synthesis of nanoscale metals is currently a significant concern due to their extensive utilization in various fields such as engineering, medicine, and the environment. Presently, the predominant method for manufacturing metals at the nanoscale involves chemical processes, which give rise to unanticipated outcomes such as environmental pollution, substantial energy consumption, and potential health risks. The concept of green synthesis was developed as a solution to these challenges by utilizing plant extracts instead of synthetic chemical agents to reduce metal ions. Green synthesis is more cost-effective, generates less pollution, and improves environmental and human health safety, making it a superior alternative to conventional chemical synthesis. Recent progress in the eco-friendly production of gold nanoparticles (Au NPs) (9).

METHODOLOGY

Plant collection and pretreatment

The leaves of *M. communis* L., Myrtaceae (called Al-Yas locally), were collected in Oct 2023 from several areas in Baghdad, Iraq, taking into account environmental conditions, such as avoiding plants treated with pesticides. Based on the morphological description, the plant was botanically identified as *Myrtus communis* by the Department of Biotechnology, College of Science, Baghdad University. This plant grows and develops throughout the year.

Preparation of the aqueous extract

The standard method is used to prepare the aqueous plant extract of *M. communis* L. (10), by thoroughly washing the plant parts (leaves) with water to remove surface impurities, followed by a three-day drying period under dry air. 50 g of the substance is pulverized and transferred to a glass beaker with a volume of 500 mL. Includes 250 mL of deionized water. The mixture was thereafter agitated and stirred at a temperature of 45°C for a duration of 24 hours. After that, the mixture was filtered through Whatman No. 1 filter paper and kept at 4°C for later use.

Preparing AuCl3 Solution

A Prepared concentration (1mM) of gold chloride solution was used according to the following molarity law:

W: Weight

M.wt: Molecular weight

V: Volume/ Liters

$$M = W/(M.wt) \times 1000/V$$

$$(1 miliMolar. = 1000 mM)$$

$$M.wt\ AuCl3 = 303.33$$

$$V = 60\ ml$$

$$0.001 = W/303.33 \times 1000/60$$

$$W = 0.0181$$

• A 1mM gold chloride solution was prepared by dissolving 0.0181998g in 60 mL of deionized water.

Biosynthesis of Gold nanoparticles Au NPs (green synthesis)

Following the conventional method (11), 10 mL of the aqueous extract of *M. communis* L. was combined with 60 mL of a gold chloride solution with a concentration of 1 mM. The mixture was heated on a magnetic stirrer plate at 45°C for 30 minutes, and the color change of the mixture was used as preliminary evidence of gold nanoparticle formation. To use as a control in certain tests, 100 mL of the 1 mM gold chloride solution made above was also saved.

Preparation of various quantities of plant extract and gold nanoparticles

The aqueous extract was prepared at four distinct concentrations of *M. communis* L. powder and the gold nanoparticles synthesized from it, as follows: 0.5, 1.0, 1.5, and 2.0 mg/mL, in succession, in addition to the control. The nanoparticles were dried after preparation, and the material's weight was measured. The dry samples were prepared by adding sterile distilled water to the desired concentration, which was maintained till they were used in the different tests.

Characterization of the plant-based green-synthesized gold nanoparticles

The subsequent methods were used to characterize the plant-based green-synthesized alumina nanoparticles. Every test is administered in the Chemistry Department Laboratory at Baghdad University and in the Department of Physics and Chemistry Laboratory at Al-Nahrain University.

1. Atomic force microscope

To determine the size and diameter of the gold nanoparticles, a small volume of the sample solution was placed on a glass slide measuring 1x1 cm. The solution was then left to dry at room temperature before being analyzed using a Dualscope TM DS (12).

2. Ultraviolet-Visible spectrometer

A sample was collected within 48 hours after the creation of the green nanoparticles. It was analyzed using a UV-Visible spectrometer, which measured wavelengths between 200 and 1000 nm. Deionized distilled water served as a reference, and the product's optical characteristics were studied at room temperature (13).

3. Field Emission Scanning Electron Microscopy (FE-SEM)

The shape and particle size of the prepared samples were determined using an FE-SEM-mapping electronic scanner. Five microliters of ready-to-examine solution were placed on a combined gold-and-carbon electron microscope holder clip to prepare the samples. The solutions were dried at room temperature and tested under various magnifying forces (14).

4. Energy-dispersive X-ray spectroscopy (EDS)

The sample must possess stability in a vacuum environment, as the sample chamber is emptied of air to prevent any interference from the atmosphere with the electron beam or X-rays. It is recommended that the surface be as clean as possible. X-ray spectroscopy is a technique that primarily analyzes the required material near the surface. For

quantitative measures, it is necessary to ensure that the sample is thinly shaved to the maximum extent possible (15).

Quantitative determination of total phenol content

To determine phenol content , the Folin-Ciocalteu assay was used (16).

Plant extracts include polyphenols that, when combined with certain redox reagents, generate a blue complex that can be identified by visible-light spectrophotometry (Folin-Ciocalteu reagents). Many pharmacopeias mention the Folin-Ciocalteu technique. Blue chromophore is the product of the FCR reaction. The maximal absorption of chromophores is determined by the concentration of the phenolic component in the solution and the alkaline solution (17). For sample preparation, 50 mg of plant extract was dissolved in 5mL of distilled water to obtain a concentration of 10 mg/mL; for the standard preparation, gallic acid, a phenolic compound, served as the standard reagent. A stock solution was created by dissolving 1 mg in mL of distilled water. Gallic acid standard solutions were prepared by serial dilutions to achieve concentrations of 0.1, 0.25, 0.50, and 1.0 mg/mL. The Folin-Ciocalteu assay was employed to estimate the total phenolic content of the extract. Gallic acid was used as a standard to quantify the concentration of phenolic compounds. 0.5 mL of plant extract at a concentration of 10mg/mL was mixed with 0.5mL of Folin-Ciocalteu reagent (FCR). After 5 minutes of incubation at room temperature, 5 mL of a 7% sodium carbonate (Na_2CO_3) solution was introduced to the mixture. This was followed by the addition of 6.5mL of deionized distilled water and thorough mixing. The solution was incubated in a light-free environment for a duration of 90 minutes at an ambient temperature. A spectrophotometer was used to measure the sample's absorbance at 750 nm relative to the blank. For each concentration, a standard curve was plotted against absorbance. The straight-line equation derived from the plot was used to determine the total quantity of phenolic compounds.

Purification of total flavonoids (18)

The concentrated *M. communis* L. extract was dissolved in 15 mL of methanol and added to the silica gel-packed 2.5*40 cm column. The extract is then filtered using an eluent. The eluent used is ethyl acetate: methanol, 5:5 (100 mL). The Fractions are collected in a vial. The Eluate in the vial is monitored, and the eluate is collected and dried with a rotary evaporator.

Quantitative determination of total flavonoids

For the determination of flavonoid content, the Aluminum chloride method was used (19). Among the analytical methods used to determine the total flavonoid content, the aluminum chloride method is commonly employed for regular screening. This technique is based on the spectrophotometric detection of colored complexes formed between Al(III) and the hydroxyl and carbonyl groups of flavonoids in an alkaline medium, on the one hand, and the hydroxyl and carbonyl groups of flavonoids on the other. For sample preparation, 50 mg of the plant extract was dissolved in 5mL of distilled water to get 10 mg/mL concentration. For standard preparation, a stock solution of rutin (1mg/mL in 50% ethanol) was prepared and serially diluted to get rutin standard solutions with concentrations of 0.12, 0.25, 0.5, and 1.0 mg/mL in 50% ethanol. 1 mL of each concentration of standard Rutin solution and the extracted flavonoids were poured into glass tubes. Then, 0.75 mL of a 5% sodium nitrite solution was added. The mixture was thoroughly mixed and left at room temperature for 5 minutes. Subsequently, 1.5 mL of a solution containing 10% $AlCl_3$ in 50% ethanol was introduced into each tube. The tubes were vigorously shaken and left undisturbed at room temperature for an extra 5 minutes. Ultimately, 5mL of a 1N NaOH solution was added to each tube. The absorbance was measured at 340 nm using a spectrophotometer. The total amount of flavonoids was determined by quantifying quercetin concentration using a linear equation derived from a plotted curve. This curve was obtained by plotting absorbance against different concentrations of quercetin.

RESULTS

Quantitative determination of total phenolic content: Folin-Ciocalteu assay

The quantitative measurement of phenolic components in a plant extract was performed using the Folin-Ciocalteu assay, with gallic acid as a standard. Absorbance was measured at 750 nm to obtain the straight-line equation from the plot, as shown in Figure (1) the phenolic concentration was 33.4 mg/g.

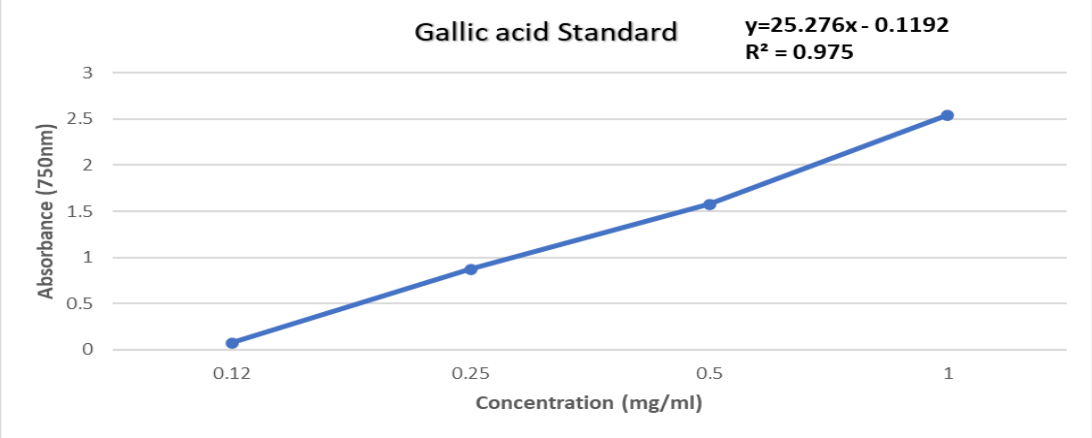

Figure (1) Standard curve for Gallic acid as determined spectrophotometrically at 750 nm.

Quantitative determination of total flavonoids

As shown in Figure (2) the straight-line equation obtained from measuring rutin, which serves as a standard, at 340 nm, was used to calculate total flavonoids, which was 17.66 mg/g of extracted total flavonoids.

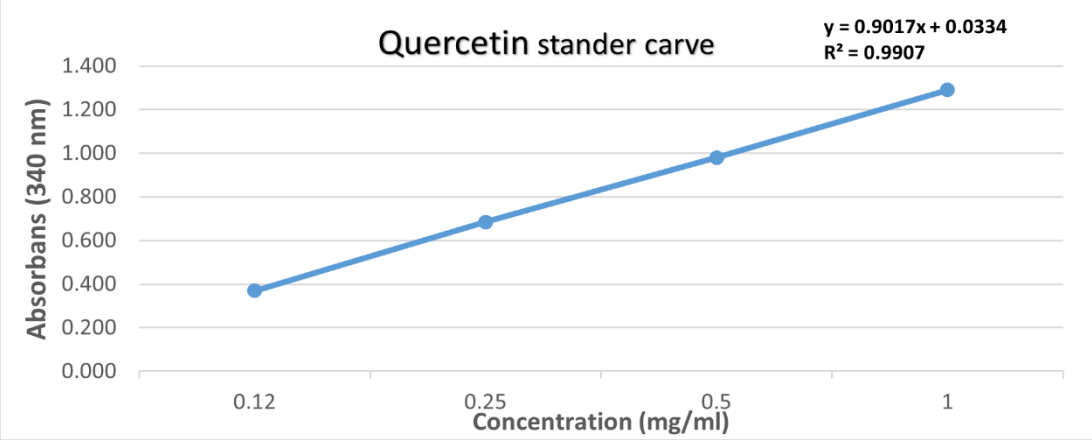

Figure (2) Standard curve for rutin as determined spectrophotometrically at 340 nm.

Characterization of the plant-based green-synthesized gold nanoparticles

Results in Figure (3) show that the color of the *M. communis* L. extract changes from yellow to reddish brown after a 20-minute reduction reaction with gold chloride under stirring. It is considered the first indicator for green synthesis of gold nanoparticles from the aqueous extract of *M. communis* L.

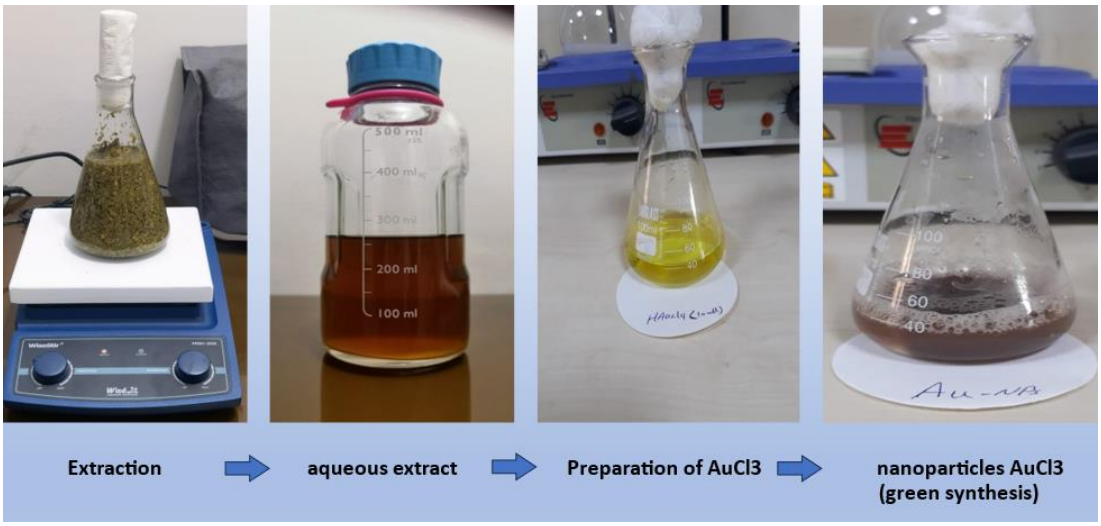

Figure (3) Steps and color profile change of the aqueous extract of *M. communis* L. plant after the addition of gold chloride.

Atomic Force Microscope (AFM)

Figure (4) displayed the 3-dimensional image of the surface morphology of the aqueous extract using Atomic Force Microscopy (AFM). It also characterized the size, surface morphology, and diameter of the extracted molecules, recording a molecular size of 253.0 nm with surface roughness (RMs) of about 8.32 nm.

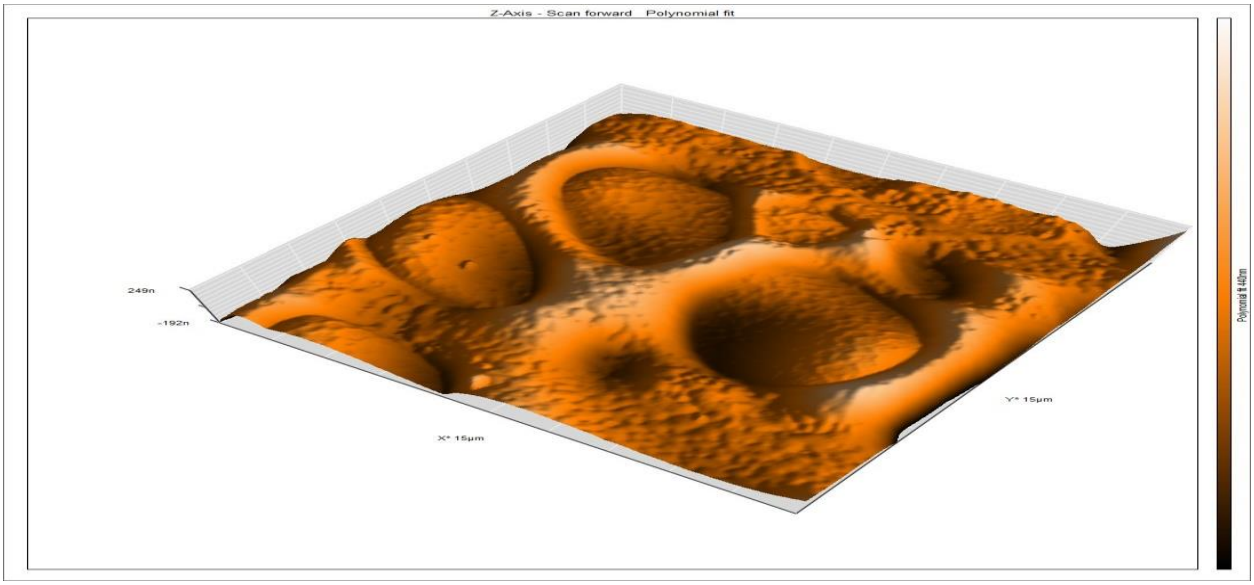

Figure (4) The 3-dimensional image of the surface morphology for the aqueous extract using Atomic Force Microscopy (AFM).

Results in Figure (5) exhibited the presence of green synthesized AuNPs from crude extract, recording 65.27 nm particle size with surface roughness (RMs) about 7.12 nm.

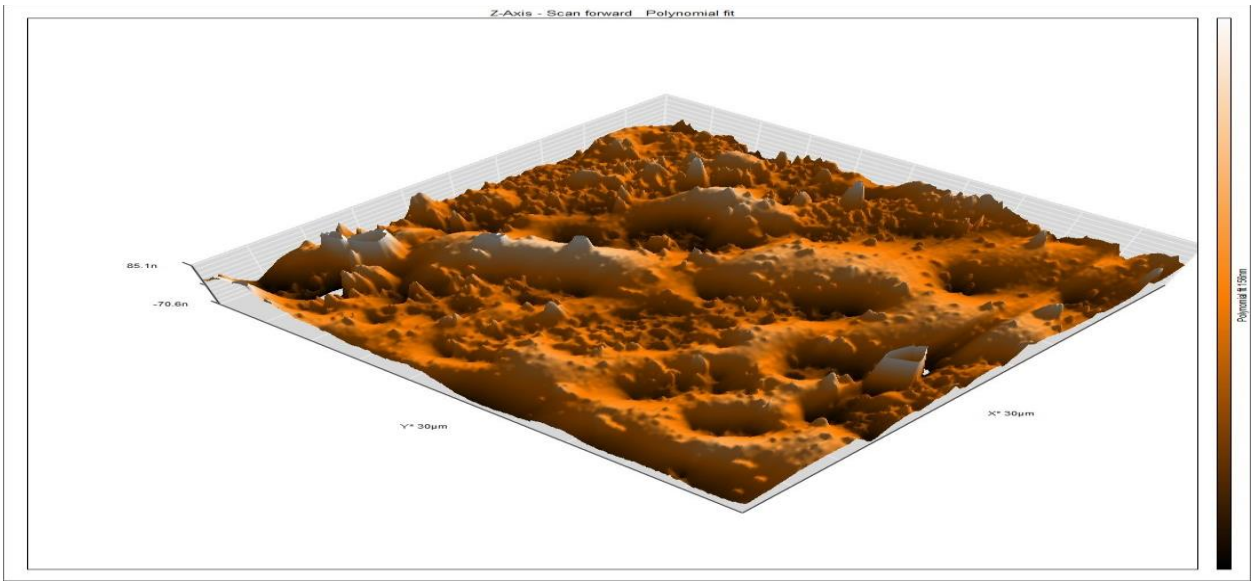

Figure (5) The 3-dimensional image of the surface morphology for the green-synthesized AuNPs from crude extract using Atomic Force Microscopy (AFM).

While results in Figure (6) exhibited the presence of purified flavonoid green synthesized AuNPs, recording 44.99 nm particle size with surface roughness (RMs) about 1.74 nm.

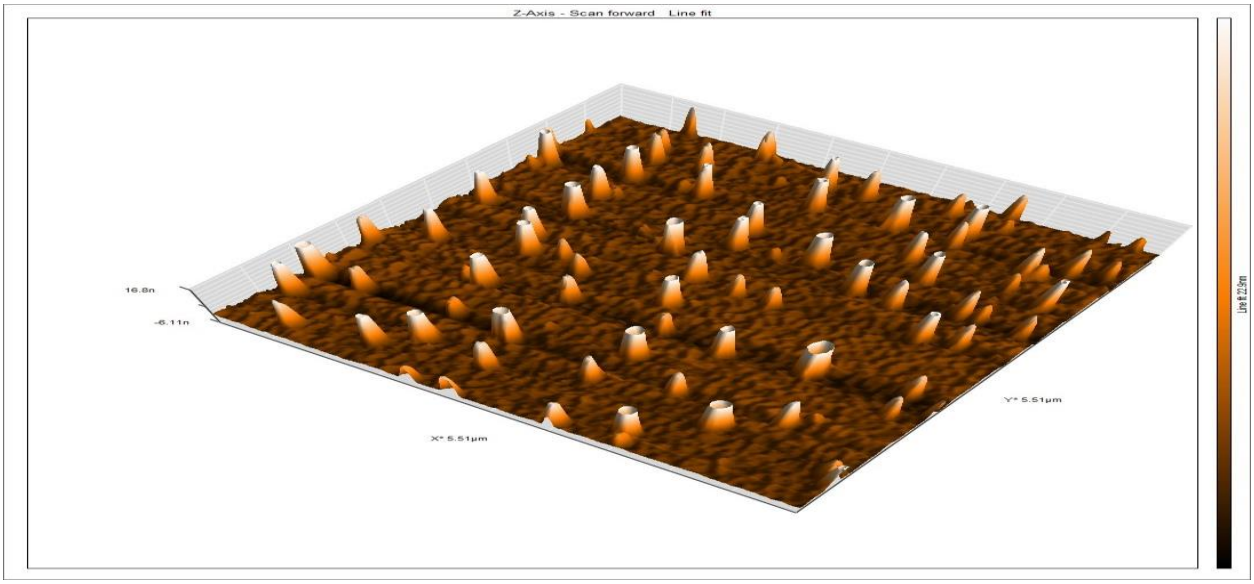

Figure (6) The 3-dimensional image of the surface morphology for the *M. communis* L. purified flavonoid green-synthesized AuNPs using Atomic Force Microscopy (AFM).

UV-Visible absorption analysis

Figure (7) shows that the UV-Visible spectroscopy of the aqueous extract exhibits an absorption at 285 nm. In contrast, the green-synthesized AuNPs from the crude extract exhibit an absorption at 550 nm, as shown in Figure (8). On the other hand, the absorbance of the purified flavonoid green-synthesized AuNPs was 555 nm as shown in Figure (9).

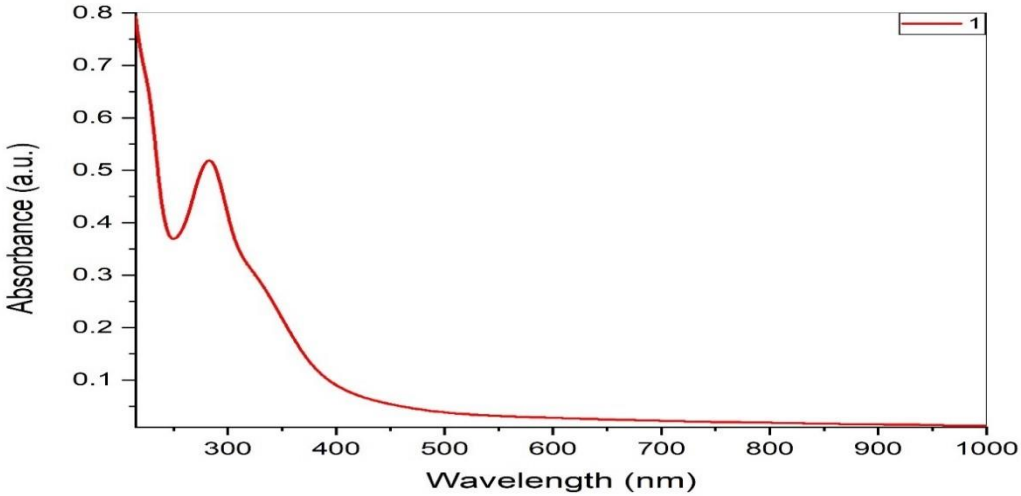

Figure (7) UV-Visible spectrum of aqueous extract

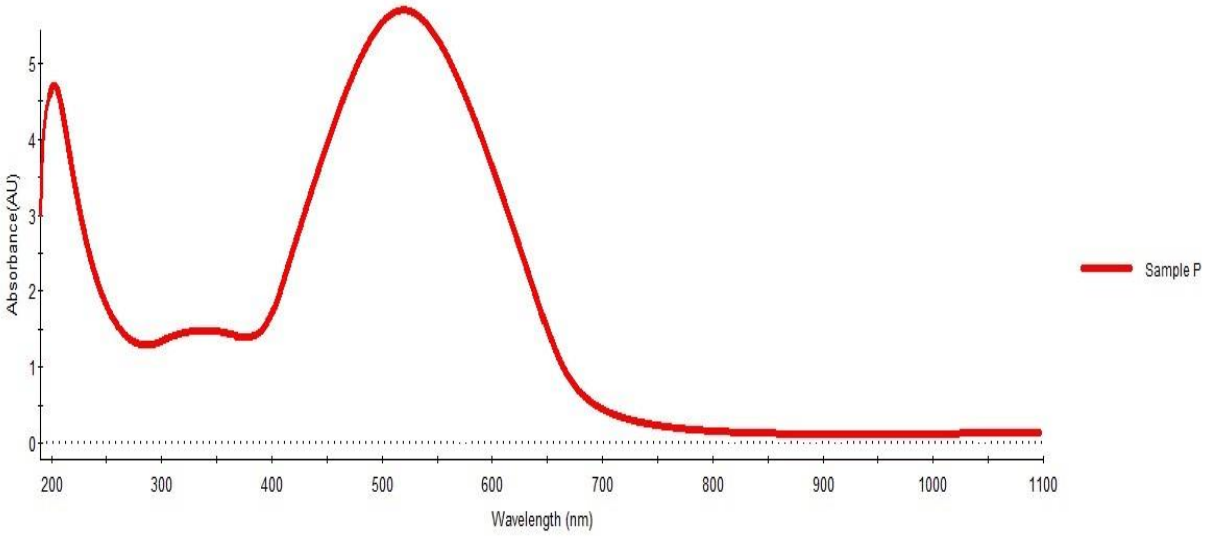

Figure (8) UV-Visible spectrum of biosynthesized AuCl3 green synthesized AuNPs from crude extract

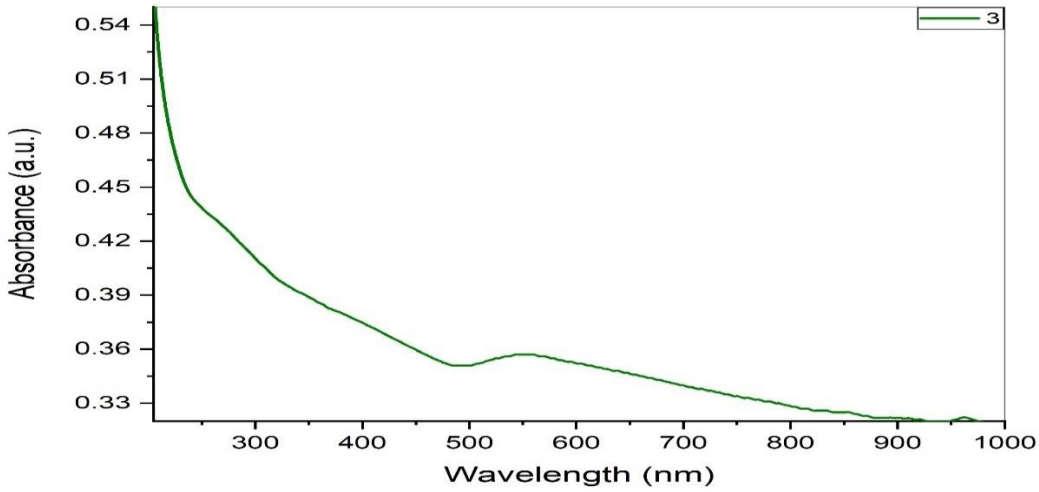

Figure (9) UV-Visible spectrum of purified flavonoid green-synthesized AuNPs

3.3.3. FE-SEM- EDS mapping nanoparticle analysis

The data in Figure (10- A) shows the FE-SEM images of aqueous extract at 10 nm scan area, which determined the surface features, particle size about 78~156nm, structural form with composition, and the result in Figure (10- B) shows the SEM mapping showing the distribution of the compound and attachment in aqueous extract.

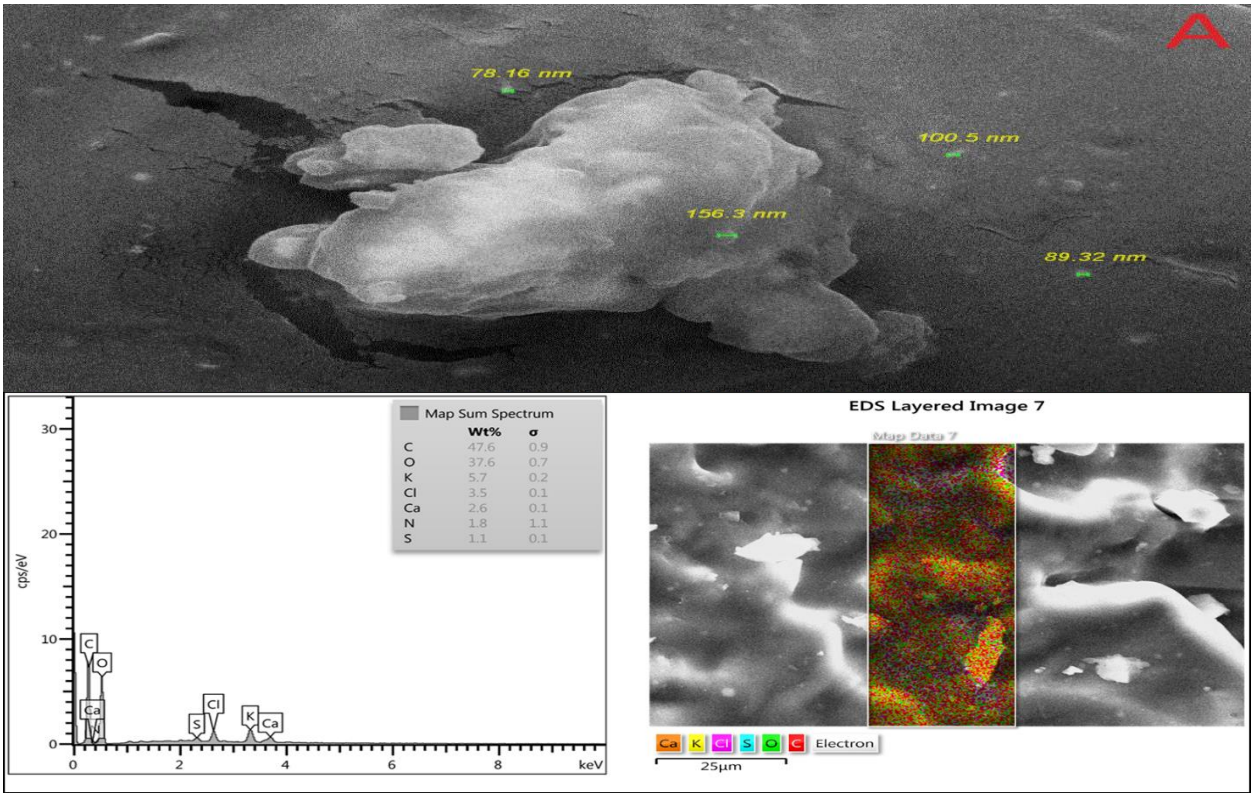

Figure (10) FE-SEM/ EDS mapping image of aqueous extract

The data in Figure (11- A) shows the FE-SEM images of green-synthesized AuNPs from crude extract at 10 nm scan area. It determined the surface features, particle size about 39.08~72.58nm, structural form with composition, while the result in Figure (10-B) of the SEM mapping shows the distribution of the compound and attachment.

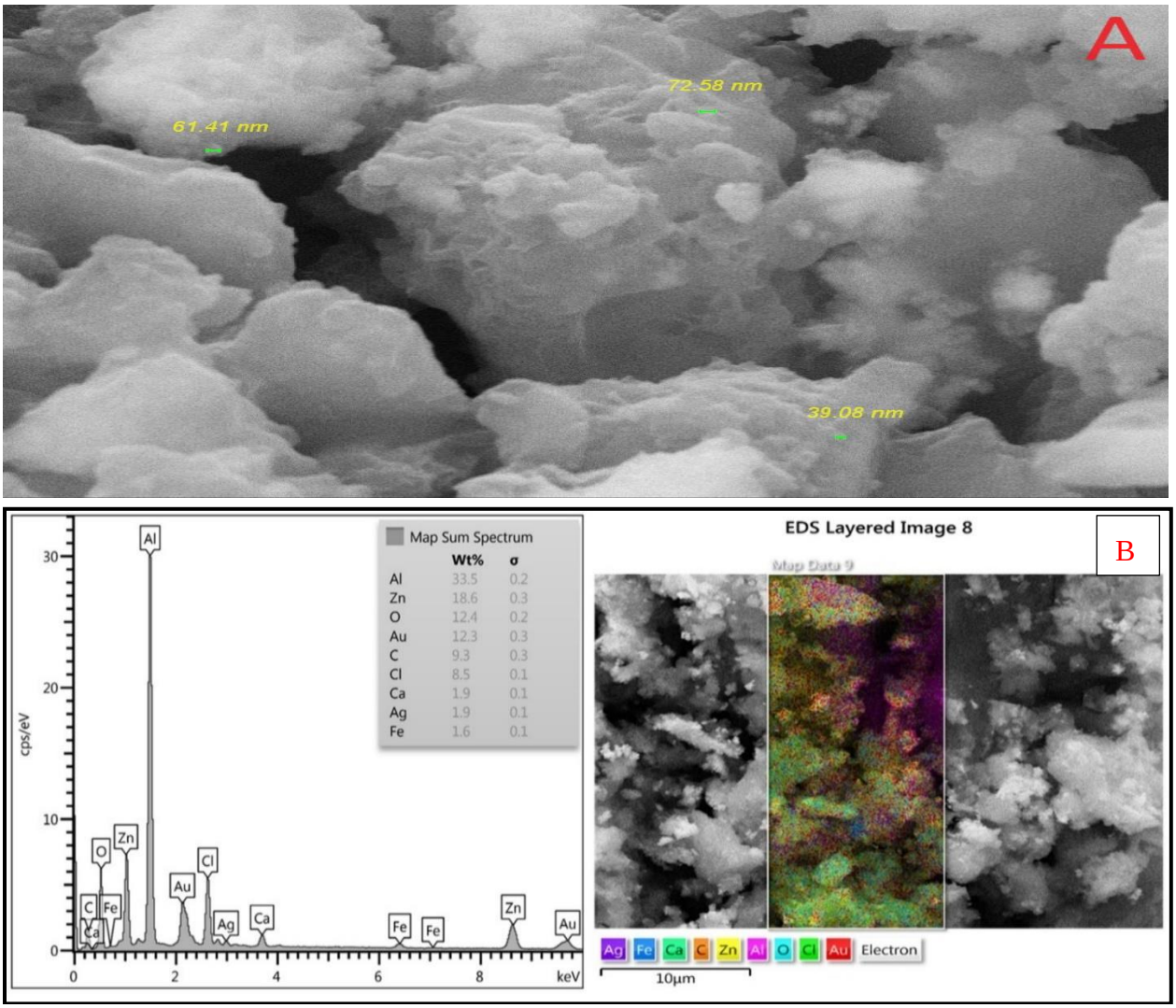

Figure (11) FE-SEM/ EDS mapping of green-synthesized AuNPs from crude extract

The data in Figure(12- A) show FE-SEM images of purified flavonoid green-synthesized AuNPs with 10 nm scan area. It determined the surface features, particle size with about 11.16~26.80nm, structural form with composition, while the SEM mapping shows the distribution of the compound and the attachment result as illustrated in Figure (12-B).

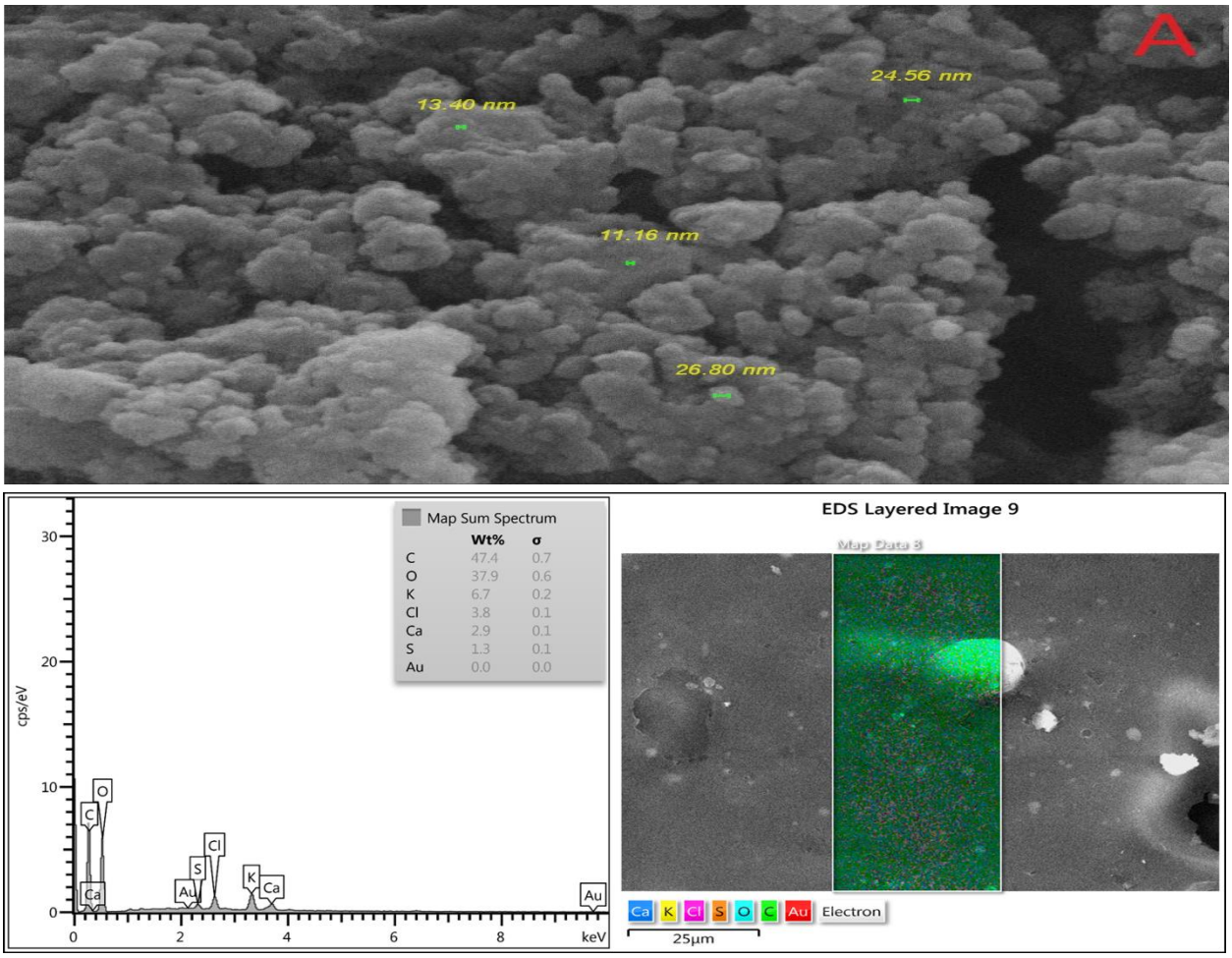

Figure (12) FE-SEM/ EDS mapping of purified flavonoid green-synthesized AuNPs

DISSCUSION

The total phenol concentration results obtained in the current study were consistent with those reported by (20), who reported that Myrtle leaf had a high value (26.3 mg/mL). Additionally, this result was consistent with that reported by (21), who found that in air-dried leaf extract, the total phenols reached 39.6 GAE/g. Also, these results were in line with (22) who reported Gallic acid with 19.0 – 132 mg/50gdw in *M. Communis* L. (Myrtaceae) Berries from Corsica across different years . Results were consistent with those obtained by (20), who reported that Myrtle leaf had high flavonoid content (18.5 mg/mL). The concentration of extracted flavonoids varied depending on the drying technique, ranging from 11.3 to 28.2 mg QE/g of extract. Similarly, the analysis of microwave-dried leaves revealed a significantly greater concentration of flavonoids ($p < 0.05$). Microwave drying of *M. communis* leaves resulted in a 1.5-fold increase in the content of flavonoids in the leaf extract, compared to air drying. The findings of (20) were consistent with the results of this investigation. (23) found that the strain G3 of *M. communis* L. had the highest flavonoid concentration, with 88.08 mg ER/g E. In comparison, G1 had a content of 63.41±2.69b, and G2 had a level of 41.01±0.14c. This result was in line with those obtained by (24), who reported that AuNPs were successfully synthesized by using Mentha spicata essential oil, as demonstrated by the color change of the reaction medium to ruby-red. Also, (25) concluded that with the increased concentration of HAuCl4 addition, the color of the lignin-AuCl4 suspension turned red, which is due to the SPR effect of the green synthesized Au-NPs. The findings were consistent with those reported by (26), who documented that AFM is used to characterize surface properties and determine topography. The Atomic Force Microscope (AFM) provides high-resolution, three-dimensional visualization of nanoparticle surfaces. The average diameter of the nanoparticles is 41.39 nm. These nanoparticles were synthesized by reducing HAuCl3 with *M. communis* L extract, and their two- and three-dimensional pictures were obtained. (27) who reported that the AuNPs were also prepared using *Pistacia atlantica* leaves extract at room temperature. AuNPs were prepared with a high reaction rate (1 min) and characterized by SEM, XRD, EDS, and AFM. AuNPs were in the 40–50 nm range. A study by (28) documented that this phenomenon is attributed to the observed shift in the absorption peak from 538 nm for 80 nm nanoparticles to 515 nm for the smallest 10 nm nanoparticles, along with a decrease in absorption as the size of the nanoparticles decreases. The extinction coefficient spectrum of the AuNPs, measured by UV-vis spectroscopy, as explained in (29), is exhibited. A wine-red colloid with a maximum absorption wavelength of 523 nm was formed when 2.5 mL of trisodium citrate was utilized, which is consistent with the findings of prior research. Nevertheless, an incremental alteration in wavelength towards the blue end of the spectrum, from 523 to 519 nm, is detected when the volume of trisodium citrate is increased to 5 mL. This wavelength shift coincides with a transition in color from a reddish hue like wine to a reddish hue resembling orange, as depicted in inset (b). The AuNFs exhibit broader absorption spectra in the wavelength range of 500 nm to 700 nm, with maximal absorption peaks falling between 540 nm and 580 nm. The results were consistent with those reported by (30), who also recorded an SEM image to determine the size and morphology of the gold nanoparticles, which resembled a TEM size of about 50 nm. The elemental mapping was done with the SEM EDAX technique, and the presence of gold, oxygen, and carbon. These results were in line with (31), who measured the size of nanoparticles by using plant leaves in *Magnolia Kobus L* to be 5~300 nm. According to (32), the basis of HR-TEM analysis, a polydispersed nanoparticle was observed under unoptimized conditions, with a size ranging from 1 to 100 nm. But, in the optimized conditions, almost uniform size and shape of the nanoparticles were achieved in both pumpkin and curled mallow leaves extract. Further, FE-SEM analysis confirmed the formation of gold nanoparticles. The presence of a strong signal identical to gold was observed in the EDS analysis of both leaves' extracts. While (33) reported that the effect of different concentrations of NaCl2 on the size of AuNPs in the presence of different reactants, such as sodium citrate and sodium borohydride, respectively. With an increase in NaCl2 concentration from 1 to 20 mM 19~47 nm, the size of particles increases whenever the concentration of NaCl2 is increased.

CONCLUSION

In conclusion, through the use of green methods in the green manufacturing of gold nanoparticles and through the use of purified active compounds such as plant flavonoids, the study demonstrated the possibility of manufacturing gold nanoparticles with unique characteristics that differ in their chemical and physical properties from the materials involved in the reaction and are safe for the environment with the possibility of using them in various medical, industrial or Environmental applications.

ACKNOWLEDGMENT

I acknowledge the technical support that provided from the chemical and physical laboratories in college of science and Plant biotechnology Lab. in Al-Nahrain university/ Baghdad/ Iraq.

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التصنيع الاخضر لجزيئات الذهب النانوية باستخدام المستخلص والفلافونويدات المنقى من نبات الياس الاخضر

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الخلاصة:

خلفية عن الموضوع: تم جمع أجزاء من نبات الياس الاخضرفي أكتوبر 2023 محليًا من عدة مناطق في بغداد، العراق، **المواد وطرق العمل:** تم تحضير المستخلص المائي بالطريقة التقليدية، ثم تم تصنيع جزيئات الذهب النانوية من المستخلص النباتي. تم إعداد أربعة تركيزات مختلفة جسيمات الذهب النانوية وتم استخدام طرق مختلفة لتوصيف جسيمات الذهب النانوية المصنعة بالطريقة الخضراء ولتقدير محتوى الفينول، تم استخدام طريقة مقايسة Folin Ciocalteu وطريقة كلوريد الألومنيوم لتقدير الفلافونويد.**النتائج:** أظهرت النتائج أنه تم تحديد الكمية المكافئة الكلية للفينولات المكافئة لحمض الغاليك باستخدام معادلة تركيز الفينولات بمقدار 33.4 ملغم في 1 غرام ووفقاً لمعادلة الخط المستقيم فإن مجموع الفلافونويدات كان 17.66 ملغم في 1 غرام من المجموع المستخرج. يوفر التغير في لون المستخلص نتيجة تفاعل الاختزال المؤشر الأول للتصنيع الأخضر لجسيمات الذهب النانوية. كما أظهرت النتائج أن قطر جزيئات المستخلص سجل 253.0 نانومتر، في حين كان 65.27 نانومتر للمركب الأخضر من المستخلص الخام و 44.99 نانومتر للجزيئات AuNPsمن الفلافونويد. تم امتصاص التحليل الطيفي للأشعة فوق البنفسجية المرئية للمستخلص المائي عند 285 نانومتر وكان 550 نانومتر لـ AuNPs الأخضر المركب من المستخلص الخام الممتص، بينما كان 555 نانومتر لـ AuNPs للفلافونويد. أيضًا، أظهرت صور FE-SEM الاختلافات بين خصائص المستخلصات الخام و AuNPsمن المستخلصات الخام أو من الفلافونويدات المنقاة.**الاستنتاج:** كانت هذه النتائج واعدة للحصول على جسيمات نانوية ذات خصائص فريدة باستخدام الطريقة الخضراء الآمنة على البيئة.

الكلمات المفتاحية: جزيئات الذهب النانوية، الياس الاخضر، التكنولوجيا الحيوية النانوية الخضراء، حمض الكالك ، الفلافونويدات الكلية.