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Green Synthesis and Characterization of Gold Nanoparticles Using Crushed Clove Buds (*Syzygium aromaticum*) Oil Extracted by Hydrodistillation

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ABSTRACT: For the synthesis of metal nanoparticles, essential oils have the potential to serve as stabilizers and reducing agents at the same time. The aim of this study was to prepare and characterize gold nanoparticles (AuNPs) by green synthesis from clove oil. The clove oil (Syzygium aromaticum) was used in the current research program to create affordable and environmentally friendly gold nanoparticles. Ultraviolet -visible, X-ray diffraction (XRD) , zeta potential, particle size, and scanning electron microscope (SEM) methods were used to characterize the nanoparticles. It was discovered that the UV-visible absorption peak was 562 nm, demonstrating the polydispersity of the nanoparticles. The XRD data indicated that the gold nanoparticles' surfaces were crystalline in nature. The zeta potential obtained was 90.26 mV, which indicates that the nanoparticles were stable and not aggregated. SEM indicates the regular spherical shape of the nanoparticles. Particle size effects were thought to be the cause of the peaks in the XRD patterns.

KEYWORDS: clove; green synthesis; Gold nanoparticles; *Syzygium aromaticum*; zeta potential.

1. INTRODUCTION

Global interest has grown in various elements of nanotechnology research, and a variety of new uses and advances for various types of nanoparticles have been found in the areas of energy, electronic engineering, space, and medication [1]. Numerous industries, including farming, ecological chemistry, veterinary medications, marine knowledge, drug revealing [including cardiac remedy, dental repair, analytical methods, dermatological therapy, and cancer remedy], and various others, use nanotechnology [2]. Numerous studies have demonstrated the ideal rheological properties, antibacterial properties, and potential for skin condition healing of nanoparticles and nanocarriers [3].

According to size, distribution, and shape, nanoparticles, which are typically clusters of atoms between one and one hundred nm size and show better characters related to the bigger particles of the materials from which they are produced, [4] Most commonly, the traditional physical and chemical techniques used to synthesize gold nanoparticles (AuNPs) involve the use of dangerous composites that involve health concerns to individuals and pollute the surroundings. Nanomaterials created using chemical synthesis have received less attention than those created through biosynthesis [5]. Gold nanoparticles (GNPs) are particularly inert in biological enviromemts , and have many physical characters that are suitable for many biomedical applications [6].

Green chemistry has originated like a feasible and modest substitute to extra multifaceted chemical artificial procedures for gaining AuNPs [7]. Plant extract was utilized to create NPs as an eco-friendly method, which has been shown to be more promising in terms of bioactivity and biocompatibility than those created using inorganic methods [8].

2. RESULTS

2.1. Extraction yield

Extraction of clove bud oil by hydro-distillation revealed a bright yellow oil with a typical clove oil smell, and the percentage of the powder yielded 50%. (This has been accomplished by the equation down

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below: Percentage yield of the extract = weight of extract (g) / weight of clove bud powder (g) × 100). The major component was caryophyllene and the lower one was trancalamene.

2.2. Clove chemical constituents

Major components of clove oil extract were determined by GC-Mass spectrometer and listed in Table 1. GC-Mass spectrum is also presented (Figure 1).

Table 1. GC-Mass major components of clove oil extraction.

Compounds	Retention time	Area percentage %
Caryophyllene	13.90	28.90
Humulene	14.50	21.06
Eugenol	12.76	13.10
Cyclohexane	16.46	7.85
Alpha copaene	13.16	6.61
1.6.10-dodecatrien	15.99	3.73
Adamantane	17.27	3.28
Alloaromadendrene oxide	17.53	2.99
Naphthalene	15.41	2.96
Caryophyllene oxide	17.73	1.53
Trancalamene	15.48	1.53

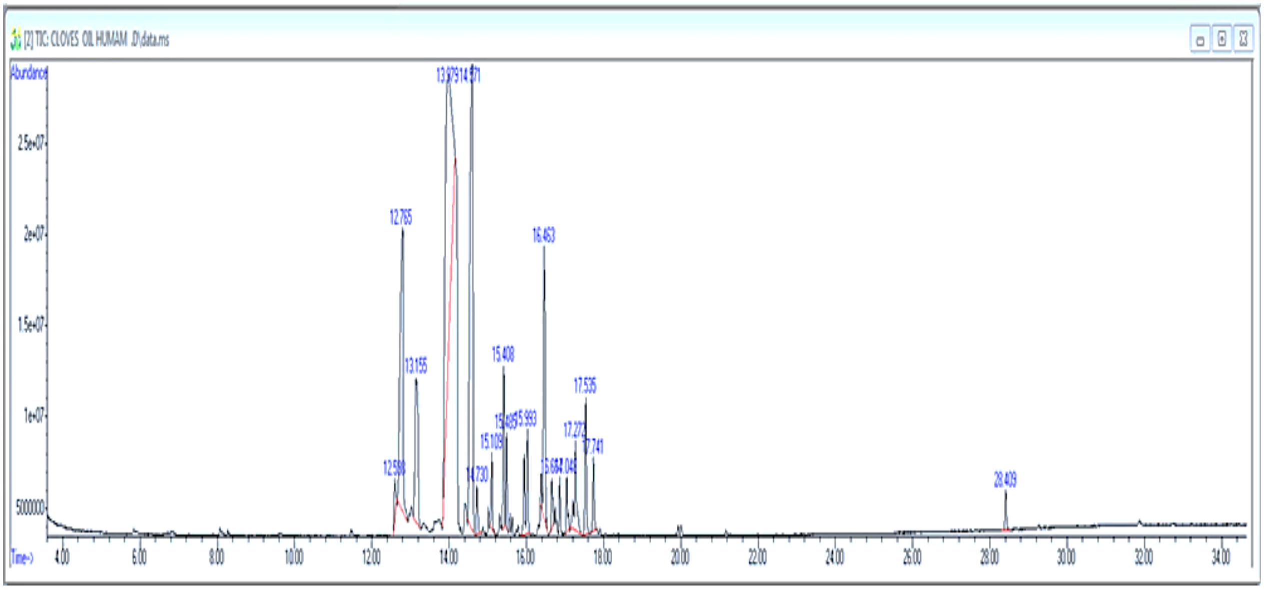


Figure 1. Phytochemicals analysis of *S. aromaticum* oil by GC-Mass spectroscopy

2.3. Preparation and characterization of gold nanoparticles

2.3.1. Preparation of gold nanoparticles

Clove oil was utilized for reduction process of chloroaurate ions. The disappearance of the faint yellow solution of gold and the revealing of a brownish color in a few minutes confirms the creation of nanoparticles of gold, which corresponds to (11) (Figure 2).

2.4. Characterization of *Sa*AuNPs

2.4.1. UV-Visible type of spectroscopy

The spectra revealed by UV-Visible for AuNPS prepared from clove oil was 562 nm (Figure 3).

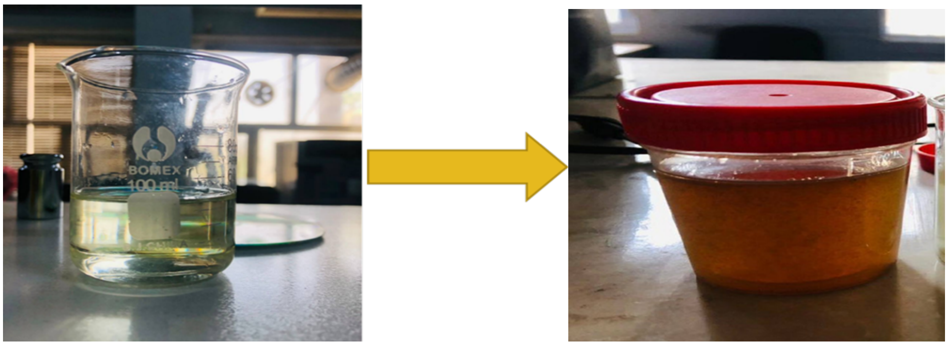


Figure 2. Formation of gold nanoparticles

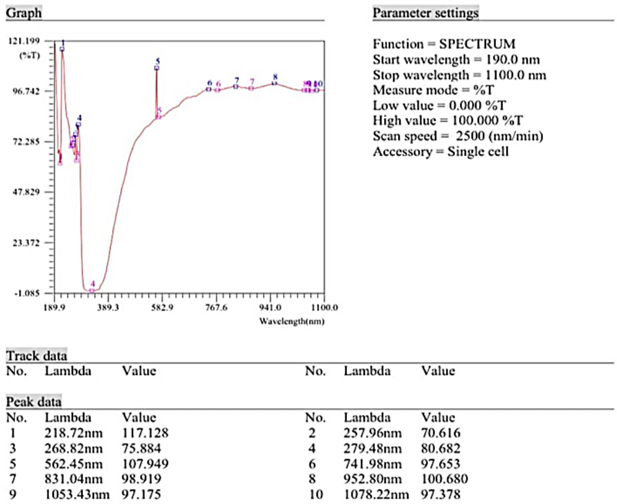


Figure 3. UV-Visible spectra for *Sa*AuNPs

2.4.2. Zeta potential

The zeta potential can be seen in figure 4.

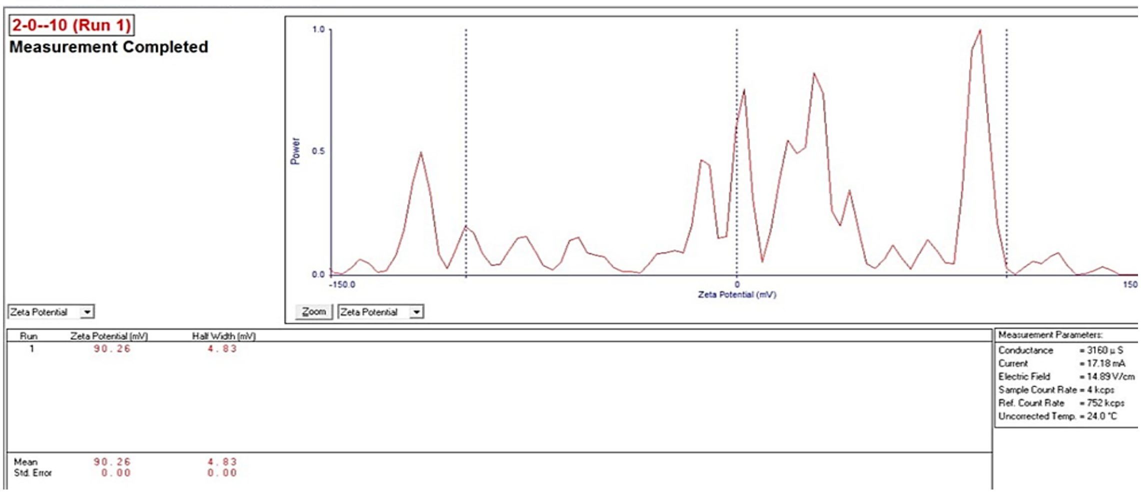
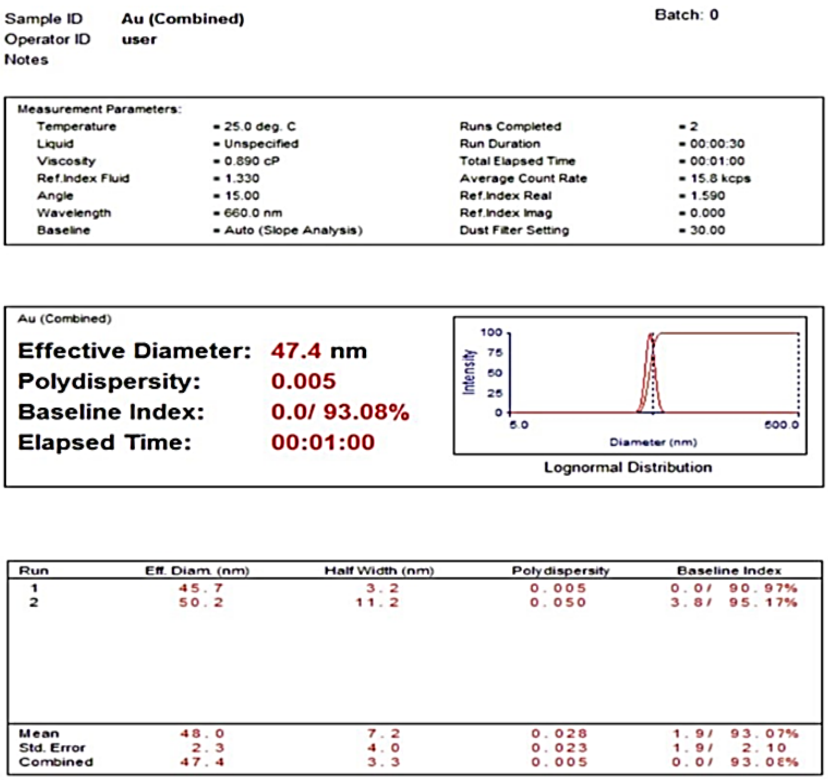


Figure 4. zeta potential for *Sa*AuNPs

2.4.3. Particle size

The *SaAuNP's* effective particle size was brought to an average diameter of 48 nm (Figure 5).



Sample ID Au (Combined) Batch: 0
Operator ID user
Notes

Measurement Parameters:			
Temperature	= 25.0 deg. C	Runs Completed	= 2
Liquid	= Unspecified	Run Duration	= 00:00:30
Viscosity	= 0.890 cP	Total Elapsed Time	= 00:01:00
Ref.Index Fluid	= 1.330	Average Count Rate	= 15.8 kcps
Angle	= 15.00	Ref.Index Real	= 1.590
Wavelength	= 660.0 nm	Ref.Index Imag	= 0.000
Baseline	= Auto (Slope Analysis)	Dust Filter Setting	= 30.00

Au (Combined)

Effective Diameter:	47.4 nm
Polydispersity:	0.005
Baseline Index:	0.0/ 93.08%
Elapsed Time:	00:01:00

Run	Eff. Diam. (nm)	Half Width (nm)	Polydispersity	Baseline Index
1	45.7	3.2	0.005	0.0/ 90.97%
2	50.2	11.2	0.050	3.8/ 95.17%
Mean	48.0	7.2	0.028	1.9/ 93.07%
Std. Error	2.3	4.0	0.023	1.9/ 2.10
Combined	47.4	3.3	0.005	0.0/ 93.08%

Figure 5. Particle size of *Sa*AuNPs

2.4.4. XRD Analysis of SaAuNPs

The XRD showed crystalline nature of the nanoparticles appeared by four peaks corresponding to standard reflections of Bragg (Figure 6).

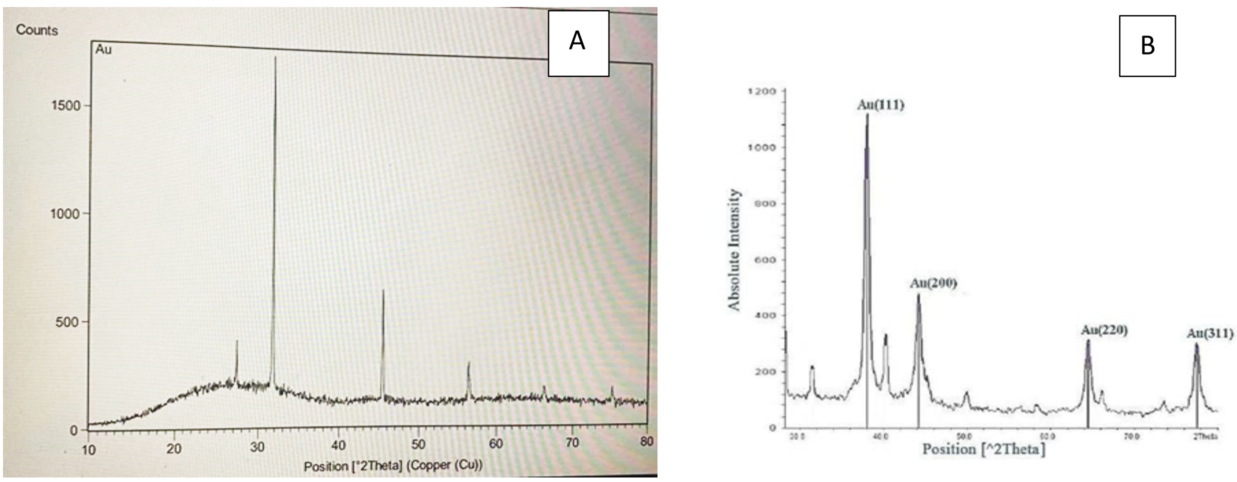


Figure 6. XRD pattern of SaAuNPs A) XRD analysis of Gold NPs B) standard Bragg reflections (16)

Figure 6 shows the XRD pattern of synthesized gold nanoparticles and shows the crystalline structure that proves the biosynthesis of gold nanoparticles from *S. aromaticum*.

2.4.5. SEM photograph of SaAuNPs

The SEM technique was utilized to easily visulize the size and shape of SaAuNPs. The image of the synthesized gold nanoparticles is shown in Figure 7. The image from SEM exhibited a spherical shape.

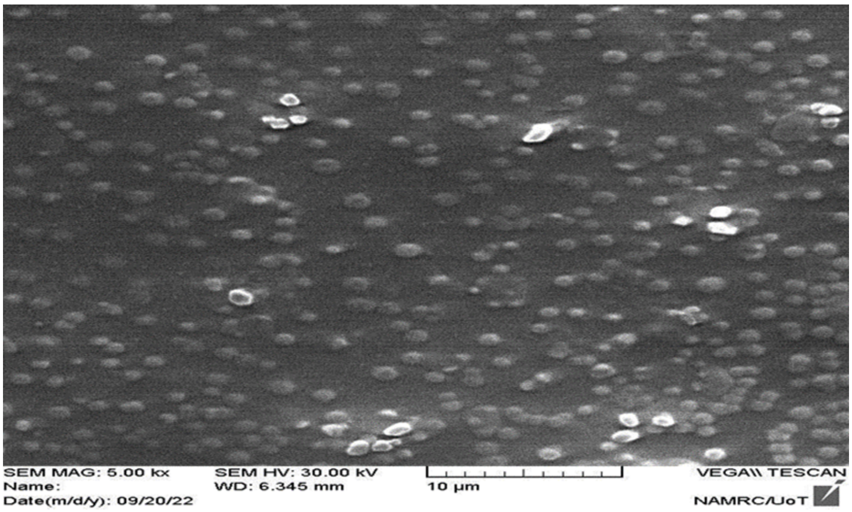


Figure 7. SEM image of *Sa*AuNPs

3. DISCUSSION

The outcome of the extraction yields nearly complied with the outcomes of a study [9], which discovered that when clove bud powder was extracted, the yield was 48.84%. Despite these findings were unlike those learned by a number of studies with yields variable from 0.18 to 7.6% [10], Harvest season, extraction method, and place of origin can all affect yield differences. The yield rises through reducing the particle size of the crushed herb [11]. Therefore, the drop in particle size was associated by a rise in the extraction yield of essential oil [12]. Mainly, twelve peaks linked to separate constituents were achieved by gas chromatography/mass spectrometry in the oil of the *S. aromaticum*, where composites were shown in Table 1.

The chief components of the essential oil observed are caryophylline (28.9%), humulene (21.06%), and eugenol (13.1%), which correspond to several studies regarding the chief components of the oil [13, 14]. In the present study, the outcomes were in disagreement with the results of many studies that worked on the characterization of buds of the oil of *S. aromaticum* by hydrodistillation and found that eugenol compound was the main constituent in clove oil. The present data revealed the effect of the grinding [crushing] technique on the essential oil composition, depending, especially, on the time of hydrodistillation [15,16].

In the current study, the components of eugenol in the whole clove oil were higher than in the crushed clove oil. Eugenol levels in whole clove oil extraction decreased after two hours in the distillation method, while the component of caryophyllene in crushed clove bud oil was higher than in whole clove oil. Therefore, crushing clove bud increased caryophyllene content but reduced the eugenol level in the oil compositions considerably [17]. This outcome agrees with a study that showed, by comparing compositions made from whole and ground cloves: While eugenol is the predominant component in entire clove buds, caryophyllene is the most prevalent compound in powdered clove [18]. Previous studies have shown that the environment, genotype, geographic origin, time of harvesting, location of drying, temperature, duration of drying, and extraction technique can all have an influence on the chemical constitution of *S. aromaticum* oil [19, 20].

An absorption band was observed by UV-visible at 562 nm that is related to the brownish color obtained, which indicates the creation of nanoparticles of gold. The emission maxima at 562 nm results from the state of inter-molecular charge/transfer. It is believed that the head-to-tail orientation of the particles (J_ aggregation) was answerable for the reveal of the emission band via long wavelengths [21]. The gold resonance of the plasmon of surface peak was seen in the UV_vis band to occur at 562 nm, and this absorbance slowly increases as response time increases. Surface plasmon resonance's redshift, broadening, and splitting were likely the result of the SPR's diminishing, which is brought on by changes in the surrounding medium's refractive index and resulting changes in the sizes of metallic nanoparticles in colloidal solution [22].

The mean of the zeta potential obtained was 90.26 mV, which specifies that the nanoparticles of gold were stable and unaggregated. The nanoparticles having a zeta potential value above +30 mV are commonly considered stable. The zeta potential of a colloidal solution is a sign of its constancy. The bigger the potential, the greater the repulsion between the particles [23]. Recent studies have demonstrated that the efficiency of synthesized nanoparticles is significantly influenced by particle size. Determining a nanoparticle's small size is crucial. Consequently, the green production of nanoparticles of gold has gathered a lot of interest [24].

The normal particle size of gold nanoparticles is generally below 100 nm. In an aqueous solution, sphere-shaped AuNPs reveal a spectrum of colors (e.g., orange, brown, purple, and red) as the central size raises from 1 headed for 100 nm [25]. The significant four peaks of the XRD were found in the 2 θ ranges at 34 °, 45 °, 66 ° and 76° of the gold nanoparticles' structure with face-centric cubicity, a representative of Bragg's reflection, which corresponds to the planes of (111), (200), (220), and (311). Similar results were obtained from the studies of [26, 27]. Thus, the XRD patterns illustrate that the AuNPs created by S. aromaticum were crystalline.

These results of SEM revealed the close association among the plasmonic features and morphology of nanoparticles of gold, which makes these spherical AuNPs ultimate for utilization in medical requests such as supply of drugs, cancer therapeutics, diagnosis of cancer, in addition to many other conditions [28]. Therefore, nanotechnology enhances the capacity to handle, quantify, and unify substance at the nanoscale level, by which these nanoparticles have many properties, especially in the medical [29] and biological [30] fields.

4. CONCLUSION

Clove oil is a convenient, economical, and environmentally friendly way to create gold nanoparticles. Several methods, including SEM, zeta potential, XRD, and UV-V is type of spectroscopy, can be used to describe the nanoparticles. These methods can offer valuable information regarding the morphology, size, crystal structure, stability, and optical features of the nanoparticles. The green-type synthesis of nanoparticles is gaining popularity as it provides an environmentally acceptable alternative to conventional procedures, which frequently include toxic chemicals and hazardous materials.

5. MATERIALS AND METHODS

5.1. Plant collection

The fresh buds of Iraqi clove (Syzygium aromaticum) were collected from a local market in Baghdad, Iraq, and classified by the Ministry of Health, the National Center for Herbal Medicine, and the Al-Razi Center for Medical Herbs. Buds were then cleaned using sodium-hypochlorite 5% solution (NaOCl), washed three to four times with distilled water, and then kept to get dry.

5.2. Preparation and extraction of clove oil (*Syzigyum aromaticum*)

Clove oil was extracted from dry buds using hydro-distillation (water distillation) by the Clevenger apparatus. 200 g of dried buds were taken, crushed, and added to 500 ml of boiled (100 °C) distilled water in a round bottom type of flask in the Clevenger apparatus until distillation of oil was ceased after 4-5 hours. The volume of the obtained oil was calculated, and the essential oil in the distillate was dried over anhydrous- sodium sulfate (Na_2SO_4) , and then kept in the refrigerator at a temperature of 4 °C.

5.3. Identification of active ingredients of the clove oil

Mass spectrometry-type of gas chromatography analysis determines the variable constituents in the oil and numerate the amunt of each constituent present as a percentage [31]. To identify the active components demonstrating antimycotic action, GC/MS was used to detrmine the phytochemicals of the S. aromaticum extracts. By injecting 1 L of the sample (0.1% in absolute methanol) and operating in scan mode and splitless on the GC/MS Thermo-Trace GC Ultra (or TSQ Quantum GC-MS), the GC/MS analysis was carried out. Using an Agelint HP-5ms Ultra Ineit capillary column (30 m, 0.25 m film thickness), the phytochemical investigation was conducted. The rates of the four ramps were as follows: ramp 1 was 60 °C hold for 3 min; ramp 2 was 60 °C to 180 °C hold for 7 min; ramp 3 was 180 °C to 280 °C hold for 8 min; and ramp 4 was 280 °C hold for 3 min. The following describes the operation's conditions: The carrier gas was helium with 99.99% purity, and the injector and detector were 250 °C hot. Comparing the findings of the GC-analysis with the specific reference-retention time and the specific spectral mass data from the database of NIST allowed the chemical components of the clove bud extract to be identified [32].

5.4. Preparation of gold nanoparticles by S. aromaticum oil:

Chloroauric acid ($AuCl_4$), purchased from (Sigma-Aldrich) , was used with no purification process . Then, 50 ml of clove oil was added to 50 mL of a 0.004 M of a solution of AuCl4 (aqueous) during continuous stirring at (200 rpm). After the completion of reduction, the solution was proceed in centrifugation and nanoparticles were then collected and re-dispersed in water. The centrifugation step was repeated by several times, so that the presence of other impurities might get washed-out [33].

5.5. Characterization of *Sa*AuNPs

5.5.1. UV-Visible spectroscopy

The stability and formation of SaAuNPs were monitored with ultraviolet-visible type of spectroscopy (UV-VIS) (performed in a Schimadzu Asia Pacific Spectrophotometer). The samples were analyzed over the wavelength spectra of 250 nm to 800 nm. The absorbance of this solution was measured at 1-hour intervals for 1–24 hours, and the revealed color change was noticed as it turned to deep-brown at the finish of the 24 hours [34].

5.5.2. Zeta potential

The dependence of the stability of nanoparticles in a given suspension is mailny on the zeta potential, which is dependent on surface charge. It is also a key element in the first adsorption step of nanoparticles onto the membrane of the cell [35].

5.5.3. Particle size

A nanoparticle of gold is usually a suspension of sub-micron particles that were mainly dispersed in water. If the size range is under the level of 100 nm, then the sample can also be named nanoparticles or called nanogold [36].

5.5.4. X-ray diffraction (XRD) analysis

The crystalline form and properties of SaAuNPs was detected by utilizing X-ray diffraction (XRD) route of analysis. Centrifugation of the developed AuNPs' solution was performed for 30 minutes at 10,000 rpm. To create powder AuNPs for X-ray powder diffraction measurements, the solid Au NP residues were twice washed with deionized distilled water. The copper radiation (Cu K, 1.5406) by the fourty kV and thirty mA was utilized to record the patterns of the powder- X-ray diffraction on the Shimadzu XRD-6000 [37].

5.5.5. Scanning Electron Microscope (SEM)

The development of thin films of carbon coating on copper grids, is necessary for sample preparation for the analysis by SEM. These types of films were made by dripping a tiny quantity of sample that was prepared onto the specific grip, wiping away the leftover solution using blotting paper, then drying for at least five minutes under a mercury lamp [38].

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