



MICROWAVE-ASSISTED GOLD NANOPARTICLES OF AJWAIN AQUEOUS EXTRACT AND THEIR OPTIMIZATION;

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ABSTRACT: In this present research study, formulation of gold-nanoparticles was done by using aqueous extract of seed of ajwain. The synthesized gold-nanoparticles complex was found to be having the average particles size of 37 nm. The confirmation of gold-nanoparticle was done firstly by visual observation as colour changes from brown to light purple and Surface Plasmon Resonance (SPR) peaks at 531.29 nm was observed using UV Spectrophotometer. FTIR spectra of gold-nanoparticles resembled to their reported values. The results of DSC confirmed that there is a conjugation between green extract on the surface of nanoparticles as they gave a sharp and broad exotherm and endotherm peaks for *Trachyspermum ammi* respectively but no peak was found for gold nano formulations and Transmission electron microscopy results showed that, most of the particles were roughly spherical in shape with particle size in the range 20 nm. It can be concluded from the results and observations that ajwain green extracts loaded gold-nanoparticles were successfully prepared and optimized.

Keywords: Gold nanoparticles, ajwain, phytochemicals, TEM

INTRODUCTION:

The expansion of green extract loaded processes for the development of nanoparticles is acquiring into an significant outgrowth of nanotechnology [1,2]. Now a days nanoformulation, particularly gold, zinc, silver have attracted the attention of researcher because of their broad application in the evolution of new applied science in the areas nanoscience [2-4]. Specially Gold nanoparticles could also have lots of new practical application in biological science for example, they are expended for the development of DNA labeling and biosensors [5, 6]. In the field of drug delivery system also green gold nanoparticles are used for example, they can act as tiny, powerful heaters and precise to kill cancer after cellular uptake, [7-8], and they are open to inducing apoptosis in lymphocytic leukemia [9]. Many research paper have been released in the literature on the biosynthesis of zinc nanoparticles, gold nanoparticles, silver nanoparticles by using several natural herbal extracts, especially *Eucalyptus camaldulensis*, *Pelargonium roseum*, *Alfa alfa* (*Medicago sativa*), *Neem* (*Azadirachta indica*) [10-14]. Moreover a method for synthesis of gold nanoparticles by using aqueous extract of a green tea has been issued. Although the development of green synthesis of gold nanoparticles, using several natural herbs have been

reported. Ajwain or *Trachyspermum ammi* L. (Apiaceae) is an important spice, medicinal, and aromatic plant. Based on literature it was confirmed that the ajwain seeds contain essential oil thymol as a major phytoconstituent along with γ -terpinene, *p*-cymene, β -pinene, α -pinene, and α -terpinene. Due to bearing of various phytochemical constituents in ajwain, it has been used as pharmacological and biological benefits.[15].

Microwave assisted method for the synthesis of gold nanoparticles by using herbal green extract is an ecofriendly impendent as it is simple, cost effective, safe, and prevent the expend of chemical solvents Microwave energy supplies uniform rapid heating of the chemical particles, which frequently gives monodispersivity and homogeneity of the nanoparticles [16] It has various advantages over established thermal method such as less time consuming, lower energy consumption and more beneficial product and [17]. In this research we observed that the aqueous extract of ajwain was a potential herb for the speedy green synthesis of gold nanoparticles. These ajwain aqueous extracts was used for the green synthesis of gold nanoparticles by following the citrate reduction of aqueous gold hydrochloride solution. The optimization of the prepared ajwain extract loaded gold nanoparticles was done by using UV-spectroscopy, FT-IR, DSC and TEM analysis and reported in this paper.

2. Materials and Methods:

2.1. Materials:

2.1.1. Collection of chemical and plant sampling:

Gold chloride(HAuCl_4), methanol, hydrochloric acid, sulphuric acid were procured from sigma (St.Louis) and The fruits of *Trachyspermum. Ammi* (Ajwain) was collected from the Darrang (Assam). The botanical authentication of plant specimen of Ajwain (*Trachyspermum ammi*) were done by a taxonomist at Department of Botany, Gauhati University. It was authenticated to be

Name of the plant species	Family	Collection no	Accession no
<i>Trachyspermum ammi</i> (L)	Apiaceae	SSUHSA	18660

2.1.2. Preparation of water (as a menstrum) extract of *Trachyspermum ammi*:

400gm of the desiccated ajwain crude powder was extracted by using water as a menstrum with the help of cold maceration process. After the finishing of the process, aqueous extract was then condensed under reduced pressure then the ajwain crude was collected and the percentage yield of water extract 6%.

2.1.3. Phytochemicals investigation :

As per the standard of Indian Pharmacopoeia (IP) different phytochemical test like test for alkaloid, glycoside, tannin, saponin, reducing sugar, protein etc were carried out.[18]

2.1.4. Preparation and characterization of green -gold Ajwain extract loaded nanoparticle Preparation of stock and gold chloride solution:

The ajwain aqueous extract stock solution was prepared by dissolving 1gm of extract in 100 ml water and another stock solution of gold chloride was prepared by adding 50mg of gold chloride to 100ml of distilled water.

Formation of gold nanoparticles:

Gold nanoparticles loaded with *Trachyspermum ammi* were prepared by using Microwave-assisted method. In this method, the reduction or synthesis of gold nanoparticles is obtained by microwave irradiation. For this method, a different ratio of the green extract to the gold chloride solution was subjected to microwave irradiation to synthesize the nanoparticles. The gold chloride solution and the tea extract mixture were microwave irradiated for 20 min in a microwave reactor at 110°C and

530W power. The gold nanoparticles was first optimized by the observing the color change of the solution [19].

2.1.5. Characterization of green-gold *Trachyspermum ammi* extract loaded nanoparticles:

2.1.5.1. UV–Visible spectroscopy: UV–Visible spectroscopy was performed for observing exact variation in absorbance at 200 nm to 750 nm at regular intervals in the samples of synthesized green-gold nanoparticles.[20]

2.1.5.2. Particle Size and Particle Sizes Distribution (PDI):

After the synthesis of the extract loaded gold- nanoparticles, determining the particle size determination and determination of the polydispersity index (PDI) are the most important to evaluation .By using dynamic light scattering (DLS) methods . In a nutshell, 1 mg of each lyophilized nano sample was distributed in 2 ml of Milli-Q water for the analysis, and the results were obtained using the ZetasizerNanoZS 90. (Malvern Instruments, Worcestershire, UK). The study was performed at 25 °C with a 90 ° scattering angle[21].

2.1.5.3. Zeta Potential (ζ):

The electric potential of a particle in suspension is known as zeta potential (ζ), and it is a crucial physical characteristic to assess the stability of thenano-formulation. Using ZetasizerNanoZS 90, the zeta potential of the nano-formulation was determined (Malvern Instruments, Worcestershire, UK). For the examination, 1 mg of the lyophilized gold nanoparticle sample from each group was dispersed in 2 ml of Milli-Q water. It was then examined with the aforementioned instrument using a clear disposable zeta cell. [22]

2.1.5.4. Fourier-Transform Infrared (FT-IR) Spectroscopy Studies:

FT-IR studies was performed for ajwain extract gold nanoparticles by using a Digilab Spectrum Excalibur FT-IR spectrometer equipped with an attenuated total reflection (ATR) accessory. The dry sample was pressed onto an ATR crystal and the infrared spectrum was recorded from 4000 to 400 cm at room temperature and 4 cm resolution.

2.1.5.5. Surface Morphology by Transmission Electron Microscope (TEM) Measurements:

The surface morphology of the optimized nanoparticles specifically for the samples TA-GNP was investigated using a transmission electron microscope (TEM) (JEOLJSM-6360, Tokyo, Japan). The study of surface morphology usually involves studying the shape of nanoparticles.

2.1.5.6. Energy dispersive X-ray spectroscopy (EDS):

Energy dispersive X-ray spectroscopy (EDS) is a process that analytically determines the elements (elemental composition) present in any material.

3. RESULTS AND DISCUSSION:

3.1. Phytochemical investigation ,

Sl no	Chemical test	Results
	Tests for Alkaloids	
	Mayer's Test	Present
	Wagner's Test	
	Hager's Test	
	Dragendroff's Test	
	Tests for Glycosides	
	Borntrager's Test	Present
	Legal's Test	
	Keller Killiani test	
	Legal's test	
	Test for Phenols	
	Ferric chloride test	

Test for Flavanoids		Present
Test for Steroids	Salkowski test	Present
Test for Tannins	Lead acetate test	Present
	Shinoda test	
Test for Terpenoids	Libermann - Burchard's	
Test for carbohydrate	Molisch's test	Present
Test for reducing sugar	Fehlings test	Present
Test for protein	Copper sulphate test	Absent

Table no. 4: phytochemical tests for *ajwain*

3.2. By using microwave assisted method:

The synthesis of green -gold *Trachyspermum ammi extract* loaded nanoparticle was primarily identified by the colour change of the solution from pale yellow to violet upon microwave heating (Figure 1). A series of nanoparticles (figure 2) at different molar ratio of gold chloride solution and extract mentioned in Table 1 was prepared for better optimization.

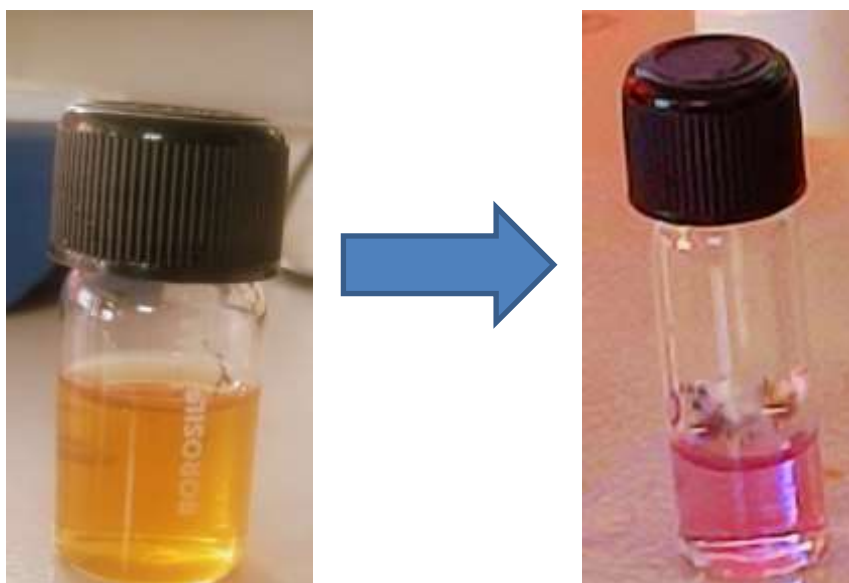


Figure 1: Changes of colour of extract

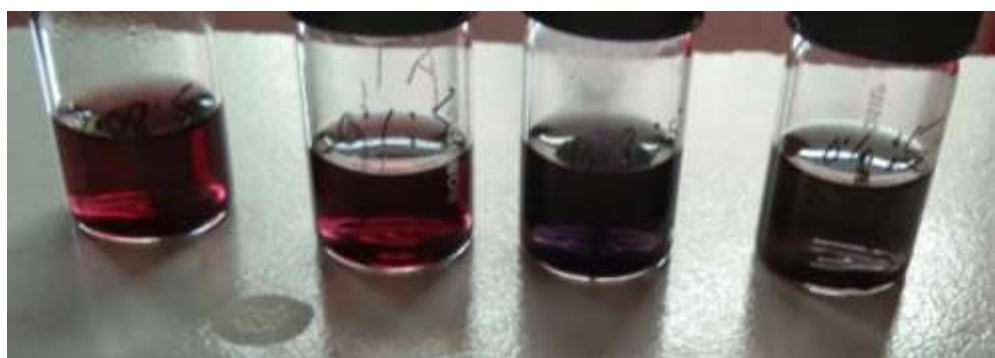


Figure 2: Series of TA-GNPs (Microwaveassisted method)

Sl no	Formulation Code	Aqueous extract: HAuCl ₄	Uv data	Zeta size	PDI	Zeta potential
1	TA-GNPs 1	2.5:1.5	541	67.15	0.304	-18.4
2	TA-GNPs 2	2:1.5	539.52	51.25	0.492	-21.12
3	TA-GNPs 3	1.5:1.5	534.26	41.01	0.486	-22.08
4	TA-GNPs 4	1:1.5	531.29	36.71	0.502	-25.1

Table 1: optimization of nanoparticle at different ratio (Microwave assistedmethod)

❖ **TA-GNPs= Trachyspermum ammi (ajwain) loaded gold nanoparticle****3.3. CHARACTERIZATION OF AJWAIN (TRACHYSPERMUM AMMI) AQUOUS EXTRACTS LOADED GOLD-NANOPARTICLE:****3.3.1. UV–Visible spectroscopy:**

UV-Visible spectroscopy analysis described a well-defined surface plasmon resonance (SPR) peak centered for TA-GNPs formulations such as at λ_{max} of 541 nm for **TA-GNP 1**, 539.52 nm for **TA-GNP2**, 534.26 nm for **TA-GNP3** & **531.29 nm** for **TA-GNP4**. These formulations contain, respectively a drug/gold chloride molar ratio of 2.5:1.5, 2:1.5, 1.5:1.5 and 1:1.5. These SPR absorbance peaks are characteristic of spherical GNPs. Decreasing the drug/gold chloride molar ratio to 2.5 :1.5 (TA-GNPs1) and 1:1.5 (TA-GNPs 4) resulted in decreasing the intensity of the SPR peaks. (Table 1

3.3.2. Particle size and polydispersity index (PDI):

Nanoparticle size and polydispersity index(PDI) are known to affect their colloidal stability and in vivo bio distribution and ability to deliver their cargo to specific sites in the body. For the successful delivery of drugs, it was shown that a smaller nanoparticle size facilitates passive accumulation increase the enhanced permeability and retention affect. The green extract /HAuCl₄ molar ratio on nanoparticle size and polydispersity index is demonstrated in the table 1. The particle sizes of the Trachyspermum ammi loaded nanoparticles (**TA-GNP**) were found in the range of 67.15 to 36.71 nm & the polydispersity index (**PDI**) were in the range of 0.304 to 0.502. Zeta potential were found in the range of -18.4 to -25.1. Described in the Table 1. Increasing in the ratio for TA-GNPs from 1:5 to 2.5:1.5 resulted in a change in GNPs size from 67.15 to 30.33 nm. Therefore, further increase in the ratio of drug / HAuCl₄ will significantly increase in the GNPs size

3.3.3. Determination of FTIR Spectrum Profile :

FT-IR studies were used to further confirm successful formation of Gold-nanoparticles and loading of Trachyspermum ammi extract in nanoparticles. The FT-IR spectrum of Trachyspermum ammi (Figure 3) shows characteristic absorption bands at 3429.43 cm⁻¹ due to N-H, 2729.20 cm⁻¹ for O-H groups stretching, 1514.12 cm⁻¹ due to N-O stretching and a number of bands in the range of 945.12–1458 cm⁻¹ due to stretching of carbon-carbon bonds in benzene ring. The spectrum of TA-GNPs 4 shows absorption bands at 3482.9 and 3620.3 cm⁻¹, and several bands in the region of 100–2628.2 cm⁻¹, (Figure 4) which are ascribed to the stretching vibrations of EGCG. The presence of these bands further confirms the successful loading of green extract onto the surface of GNPs.

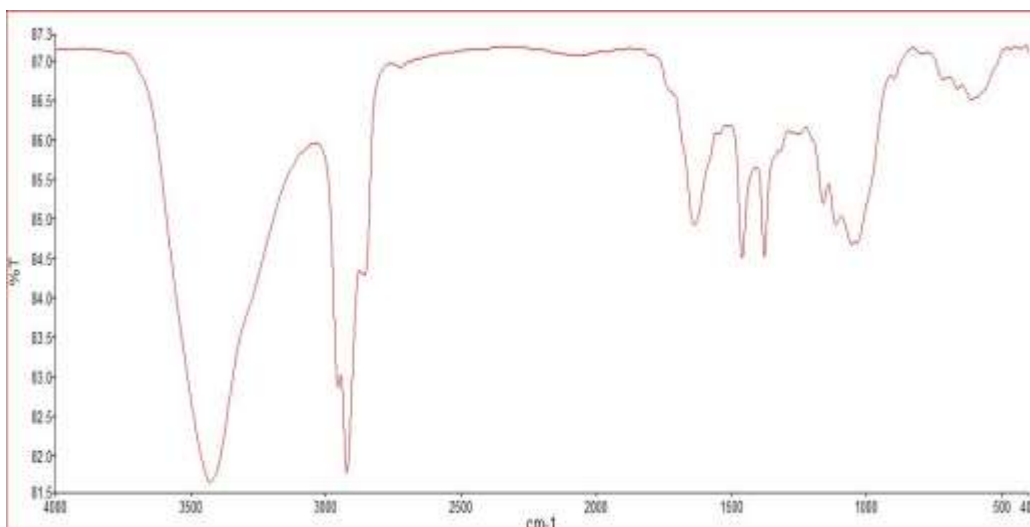


Figure 3 :FTIR Spectra of ajwain extract

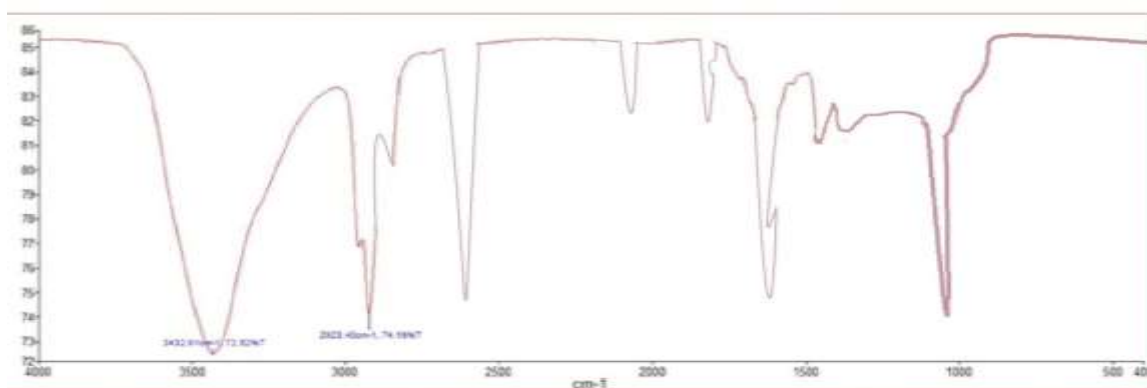


Figure 4: FTIR Spectra of TA-GNPs4

3.3.4. Transmission Electron Microscope (TEM) Measurements: TEM images of gold-nanoparticles derived from the extract of ajwain fruits are shown in (Figure 5). Gold-nanoparticles are roughly spherical in shape. Some of the nanoparticles were found to be oval and/or elliptical. This type of variation in shape and size of nanoparticles synthesized by green chemistry are common.. TEM analysis showed that most of the particles had a size in the range of 20 nm.

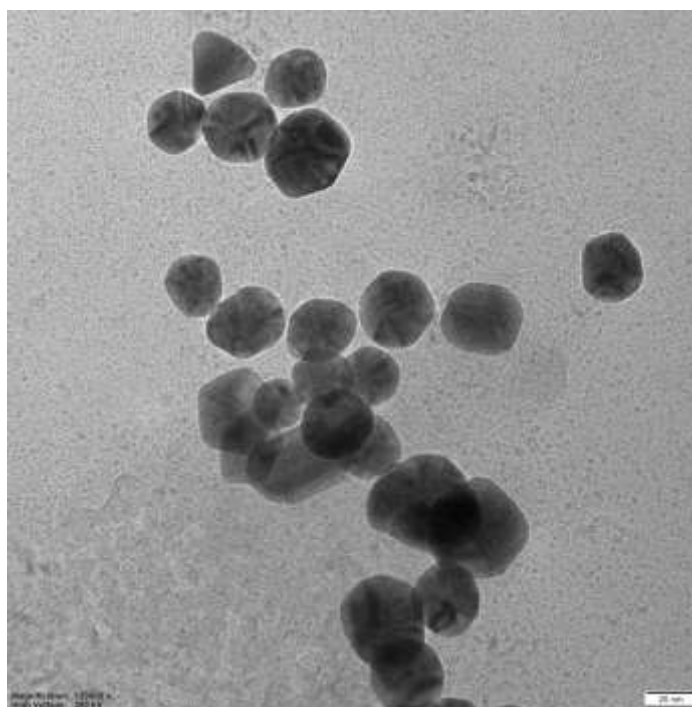


Figure5 : TEM of the sample TA-GNP 4

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

The results of EDS analysis of gold-nanoparticles derived from the ajwain extract loaded gold nanoparticle shown in **TA-GNP 2(reduction)** (figure 6) and it shows the presence of different type of element such as sodium, calcium, potassium, oxygen & chlorine.

Application Note

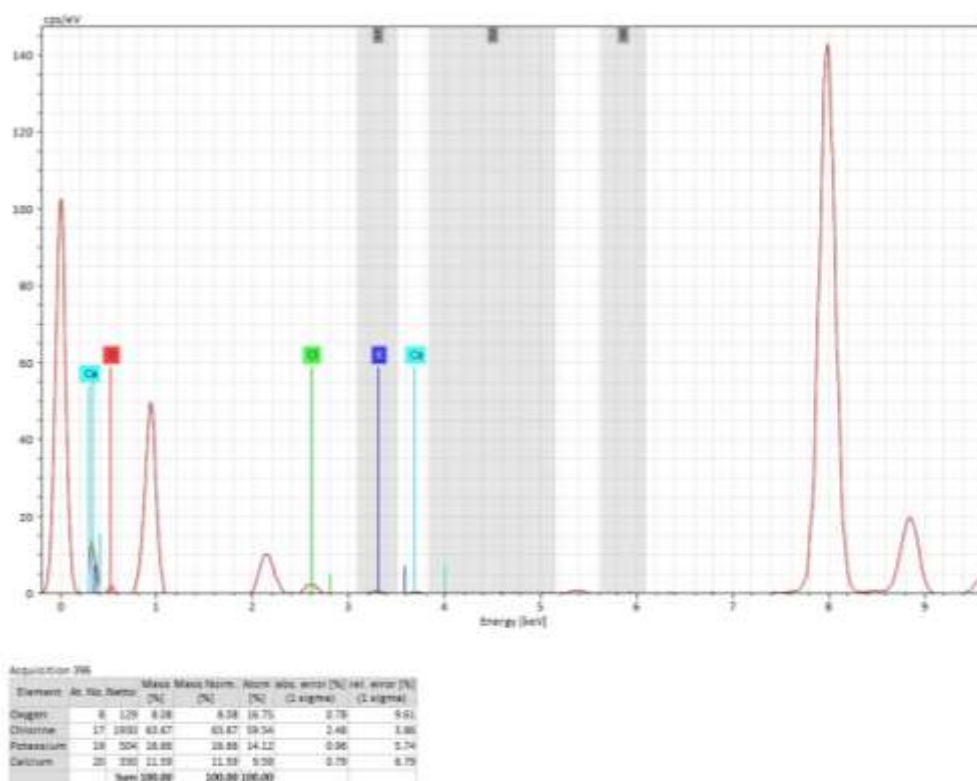


Figure 6 : EDS results of TA-GNP 4

CONCLUSION

Hence, from this research study, it was concluded that ajwain fruits aqueous extracts was efficient of developing green synthesis of gold nanoparticles with visual observed changing of colour during citrate reduction and with good particle size size of nanoparticles. Their optimization were performed by using UV–Visible spectroscopy, particle size polydispersity index (PDI), FT-IR and TEM analysis. Surface Plasmon Resonance (SPR) peaks at 531.29 nm was observed using UV Spectrophotometer. FTIR spectra of gold-nanoparticles resembled to their reported values. Transmission electron microscopy results showed that, most of the particles were roughly spherical in shape with particle size in the range 20 nm Hence, it was concluded that the ajwain aqueous extracts can be an good source for development of green gold nanoparticles to a better point as prepared by other microwave assisted methods.

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