

# Green Synthesis of Gold Nanoparticles using Aqueous Extract of *Clitoria Ternatea* Flower

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**Abstract.** The synthesis of gold nanoparticles (Au-NPs) is being accomplished by the reduction of aqueous gold metal ions, gold(III) chloride trihydrate ( $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ ) reacted with the aqueous flower extract of *Clitoria Ternatea* (CT). CT flower extract plays an important role in synthesizing Au-NPs. It acts as a reducing ( $\text{Au}^{3+}$  to Au) and stabilizing agent that can eliminate the usage of chemicals during the production of Au-NPs. Besides that, it also reduces the production of unwanted by-products which would cause hazardous to the surroundings and environment. In this study, an absorption peak of Au-NPs is observed at the range of 540-550 nm from ultraviolet-visible spectroscopy (UV-vis) analysis. Furthermore, the diffraction peaks at  $2\theta = 38.44^\circ$ ,  $44.41^\circ$ ,  $65.03^\circ$  and  $77.58^\circ$  respectively which correspond to face-centered cubic structure with (111), (200), (220) and (311) plane confirm the successful synthesis of Au-NPs. According to transmission electron microscopy (TEM), the majority of Au-NPs are spherical in shape and having a mean particle size distribution of 18.16 nm with a standard deviation of 4.67 nm.

## 1. Introduction

The synthesis of nanoparticles can be done using either chemical or physical method conventionally where chemical synthesis always involves the usage of chemical reagents in order to act as the reducing and stabilizing agent. On the other hand, physical methods of synthesis usually involve the manipulation of surrounding properties or external forces such as temperature and pressure in order to separate the materials from larger size to nanoparticles size. The synthesis of nanoparticles can be achieved by two approaches; one is the top-down approach (bulk to nano size) while the other is bottom-up (atoms to nano size) [1]. With the different of these two approaches, scientists and researchers can produce nanoparticles of different sizes and shapes with different properties. For example, the different dimensions and their physical size of nanoparticles will provide gold nanoparticles that will yield a very different colour. These phenomena are caused by the oscillation of electric fields of a light ray propagating near colloidal nanoparticles which then interact with the free electrons causing a concerted oscillation of electron charge that is in resonance with the frequency of visible light [2].



However, most of the conventional method for synthesized nanoparticles which uses chemicals as a reducing or stabilizing agent and physical method that involve high temperature and pressure are not economically and environmentally friendly due to the toxic by-products being produced during the process that is harmful to the surroundings and environment. For example, using sodium borohydride ( $\text{NaBH}_4$ ) as a reducing agent will eventually produce several by-products such as sodium chloride ( $\text{NaCl}$ ), hydrochloric acid ( $\text{HCl}$ ), and borane ( $\text{BH}_3$ ). Thus, the synthesis method that uses natural products that act as reducing agents must be introduced in order to reduce the hazards to the environment and humans [3]. A green alternative of reducing agent can be further divided into two categories, through using microorganisms or using plants. Examples of microorganisms, such as enzyme [4], fungus [5] and algae [6] were reported successfully in the production of Au-NPs. However, synthesized Au-NPs using microorganisms having a much-complicated process especially maintaining their cultures and it is also much more expensive when compared to using plants [3,7]. Thus, plant-mediated synthesis of nanoparticles is developing due to the ease to handle and the ability of mass-production.

*Clitoria Ternatea* (CT) is commonly known as “Butterfly pea” belongs to the family of Fabaceae. The plant is a twining evergreen herb, which will grow up to 3 m high, climbing over any available prop and is distributed in tropical Asia such as Myanmar, Thailand, Indonesia, India, Philippines, and Malaysia. The stems are pubescent and spindly. The compound leaves are made of three to nine oval or elliptical leaflets and the flowers are 2–4 cm long and in various shades of blue [8]. In the olden day, the root is already being used in the treatment of various diseases, like indigestion, constipation, arthritis and eye ailments. It is also employed in cases of ascetics, enlargement of the abdominal viscera, sore throat, skin diseases, and the flower are recommended for the treatment of snake-bite [9]. It is also reported that CT flower contains high amounts of anthocyanin pigments and at the same time many pieces of evidence indicate that CT flower to be rich in antioxidant properties as compared to other flowers and medicine elements. Thus, in ancient human civilization, CT has been used to treat neurological disorders [10]. According to Ghosh 2007, CT flower also showed anti-viral, anti-inflammatory, anti-allergic, and anti-microbial properties, prevent diabetes, protect the cardiovascular system and much more health benefit most probably due to anthocyanin pigment [11]. The natural antioxidants in CT flower include flavonoids, anthocyanin, flavanol glycosides, phenolic acids and procyanidins [11]. Furthermore, the components presented in the water extract from flowers of CT were inositol (38.7%) and pentanal (14.3%) based on the gas chromatogram mass spectrometry (GC-MS) analysis [12].

In this study, Au-NPs were synthesized using CT flower aqueous extract that acts as a reducing and stabilizing agent. This green synthesis technique was chosen due to its clean and hazardous free process. In addition, this method also presents huge potential for mass-production of Au-NPs with fast reaction times, high yield and cheap.

## 2. Materials and method

All material and reagents used in this work were analytical grade and used as received without further purification. Gold(III) chloride trihydrate ( $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ ,  $\geq 99.98\%$ ) was used as the gold precursor from Sigma-Aldrich, USA. The CT flowers were purchased from one of the markets in Kuala Lumpur, Malaysia. It is harvested by the owner of the store early in the morning and let it air dry under a ceiling fan indoor to remove excess moisture from the flower for as long as 24 hours. Deionized water is utilized to prepare chemical solutions in new batches. Fresh  $\text{HNO}_3/\text{HCl}$  with the ratio of (3:1, v/v) is used to clean all glassware, followed by deionized water, and dried prior to use.

### 2.1. Preparation of aqueous CT flower extract

CT flower is collected and will be wash thoroughly with tap water first to remove dust and dirt. The flower is then washed again with deionized water before drying in an oven (Escolsotherm Forced Convection Laboratory Oven) under 40 °C for 48 hr. This is to ensure that the chemical structure in CT flower will not change due to the high temperature of drying. Besides that, all the flowers are stored at

room temperature in an airtight container for further usage. When needed, the flower aqueous is prepared from adding 1 g of CT flower in 50 mL of deionized water, 75°C for 1 hr with continuous stirring at 500 rpm to ensure maximum extraction. The extract will then undergo centrifuge at 3500 rpm for 10 min before being filtered with Whatman Grade 1 filter paper through the suction pump filtration method to further remove solid particles from CT flower extract. Fresh CT flower extract is prepared for all the experiments below.

### 2.2. Synthesis of Au-NPs

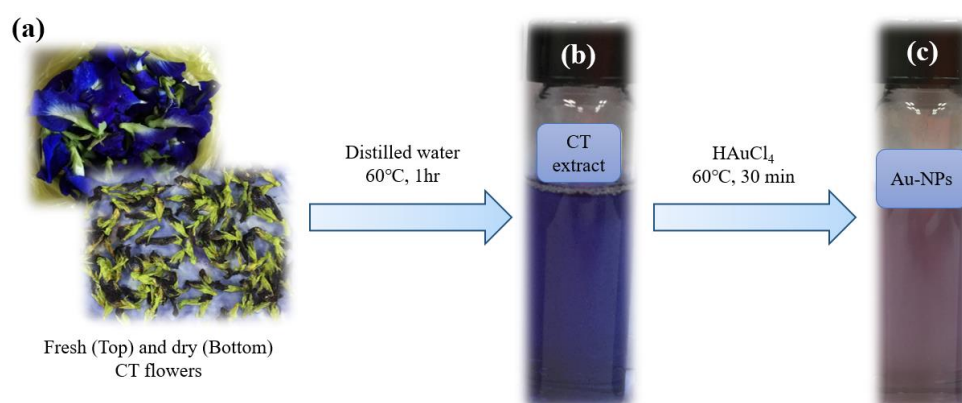
For the synthesis of Au-NPs, approximately 10 mL of CT flower extract was reacted with 10 mM of  $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$  gold metal salt with the ratio of (10:1) at room temperature, continuous stirring at 500 rpm. The colour change of the reaction was observed, and the time taken for the changes was noted. The colour of CT flower extract changes colours immediately from dark blue to purple colour which indicates the formation of Au-NPs.

### 2.3. Characterization of Au-NPs

UV-visible spectroscopy (UV-vis) at regular intervals from the range of 400 to 800 nm (Shimadzu, UV-2600) was used to confirmed the production of Au-NPs. The Au-NPs sample was then transferred to an oven and dried at 70 °C for 2 days in order to remove all the moisture. The dried sample was then collected and analyzed for the structure and composition using powder X-ray diffraction (XRD). The data was recorded using PANalytic EMPYREAN at 45 kV, 40 mA, and over a range of  $2\theta = 20\text{-}80^\circ$  with a scanning rate of  $2\theta/\text{min}$ . Transmission electron microscopy (TEM), JEOL JEM-2100F was used to study the morphology, size and distribution of the Au-NPs. NPs size distributions were measured and calculated using program ImageJ Launcher and a histogram was plotted. The samples were prepared by dispersing them in deionized water under sonication for 15 min and then were dropped onto a carbon-coated copper grid and dried at room temperature.

## 3. Results and discussion

CT flower extract (1.0g, 10 mL) acts as both the reducing and stabilizing agent when reacted with  $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$  (10 mM, 1 mL). The most obvious reduction occurred for  $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$  was regarding the colour changes as shown in Figure 1 (dark blue to purplish). CT flower is chosen due to the high amounts of anthocyanin pigment which also being used in natural supplements [13]. It also is shown having high reducing, anti-microbial, anti-inflammatory, anti-carcinogenic and antioxidant properties [14–16]. However, CT leaves and roots did not provide enough reducing power to reduce  $\text{Au}^{3+}$  to  $\text{Au}^0$  (Au-NPs).

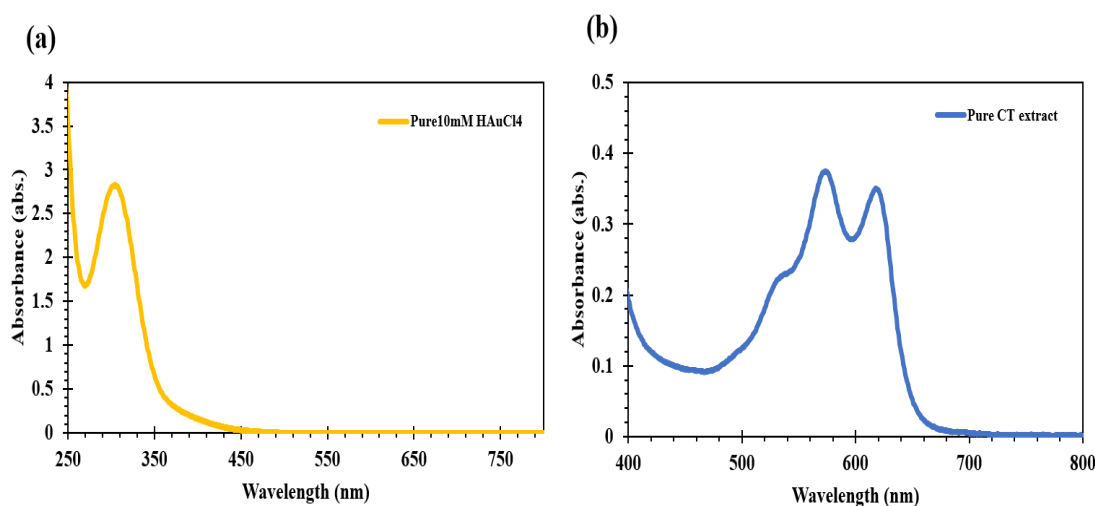


**Figure 1.** Colour changes after the addition of  $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$  where, (a) CT flower, (b) CT flower extract, and (c) Au-NPs solution after reaction

The reaction was rapid when there was enough flower extract to reduce  $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$  from  $\text{Au}^{3+}$  to Au. However, a parameter for reaction time was done in order to ensure the reaction was completed at a specific moment. CT flower extracts possessed a concentration-response relationship in 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity using ascorbic acid as a positive control.  $\text{IC}_{50}$  of ascorbic acid ( $5.34 \mu\text{g/mL}$ ), the  $\text{IC}_{50}$  of CT flower extract ( $84.15 \mu\text{g/mL}$ ). Moreover, the Ferric Reducing Antioxidant Power (FRAP) assay calibrated with standard ascorbic acid, the reducing power of the CT flower extract was ( $0.33 \text{ mmol/mg}$ ) ascorbic equivalent [17].

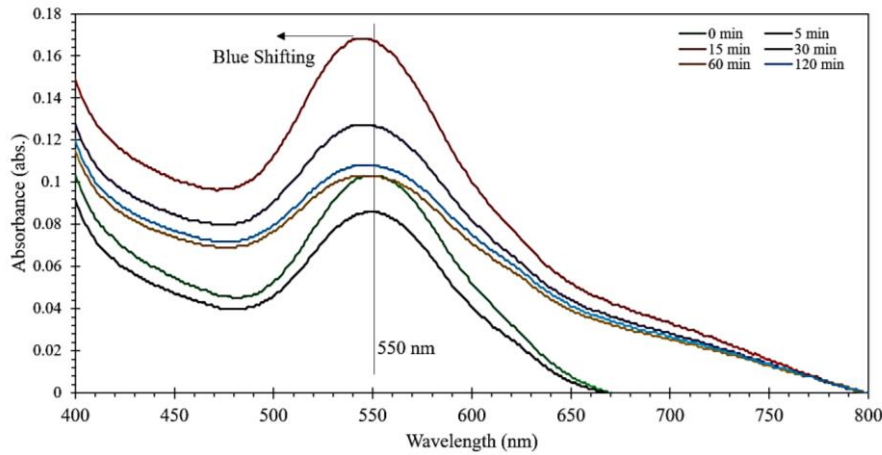
### 3.1. UV-Visible spectroscopy analysis

It is important to know the peaks that can be obtained from pure gold metal salt  $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$  alone and CT flower aqueous extract through UV-vis characterization. By doing so, the process of chemical reactions can be monitored closely as only atoms present in the reactants can be ended up in the product. As no new atoms will be created and no atoms are destroyed. Thus, in other words, the reactant peak should be decreased with the formation of new peaks. Initially, pure  $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$  with the concentration of 10 mM was measured and a sharp peak is obtained at around 310 nm as shown in Figure 2 (a). Besides that, pure CT flower extract was measured and two peaks at around 575 and 620 nm were obtained as shown in Figure 2 (b). Delphinidin which is an anthocyanidin with the colour of purplish blue was responsible for both of the peaks found in CT flower extract [18].



**Figure 2.** UV-vis absorption spectrum regarding (a) pure  $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$  metal salt with a concentration of 10 mM and (b) pure CT extract (1.0g, 1hr) at  $60^\circ\text{C}$ .

Generally, Au-NPs has an absorption peak range from 540-550 nm. The UV-vis spectroscopy analysis of CT flower extract reacted with  $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$  was shown in Figure 3, with the wavelength range between 400-800 nm. The CT flower extract did not show any absorption peak for all 6 samples at different reaction times (0, 5, 15, 30, 60, 120 min). However, Figure 3 shows the optimum time for the reaction was at 15 min with the highest absorbance at around 0.17 and at the same time the absorption peak also become sharper as compared to other samples. Besides that, the 15 min sample has a better property as the UV-vis absorption peak shifted to the left (blue shift). This proposes a decrease in the size of Au-NPs. The optical properties of Au-NPs are highly dependent on the nanoparticle's diameter. The smaller Au-NPs scatter less light because it has smaller optical cross-sections and due to its albedo [19]. Thus, 15 min sample would be selected to run the XRD and TEM characterization to confirm the crystallinity and purity, and to study the size and shape of AU-NPs respectively.



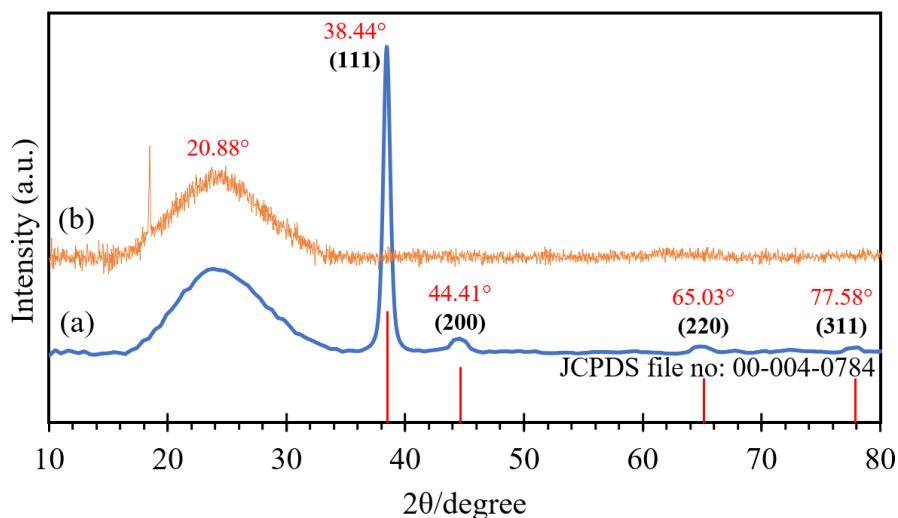
**Figure 3.** UV-vis absorption spectrum of CT flower extract with  $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$  at different time intervals.

### 3.2. X-ray diffraction analysis

The production of pure crystalline Au-NPs was confirmed again through XRD as shown in Figure 4. After comparing the spectrum with the database, it is found that the entire spectrum was compatible with the JCPDS file no. 00-004-0783 which can be found at X'Pert HighScore. These spectrums give an intense peak at  $2\theta = 38.44^\circ$ ,  $44.41^\circ$ ,  $65.03^\circ$  and  $77.58^\circ$  respectively which correspond to face-centered cubic structure with (111), (200), (220) and (311) plane [3]. Besides that, Figure 4 (b) show the XRD spectrum for pure CT flower extract. It helped to explain the peak at  $2\theta = 20.88^\circ$  which was triggered by the organic compound from CT flower extract. The particle size of Au-NPs can be estimated by using the Debye-Scherrer formula as shown in equation 1:

$$d = \frac{k\lambda}{(\beta \cdot \cos\theta)} \quad (1)$$

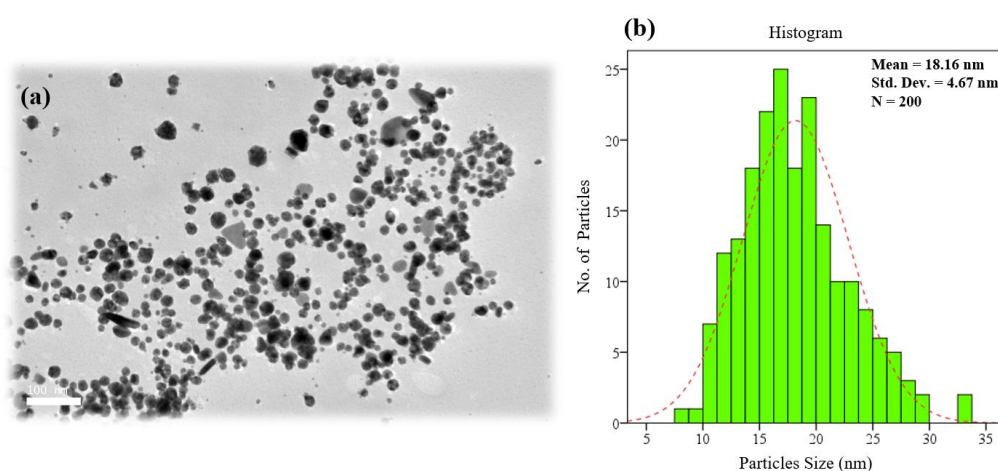
where  $d$  is the average crystallite size,  $k$  is the Scherrer constant (0.9),  $\lambda$  is the X-ray wavelength (0.154 nm),  $\beta$  is the line broadening in radians, and  $\theta$  is the Bragg angle [20]. The calculated average Au-NPs size is 17.67 nm at the angle of  $38.44^\circ$ .



**Figure 4.** XRD patterns of (a) Au-NPs using CT flower extract, and (b) pure CT flower extract.

### 3.3. Transmission electron microscopy analysis

The size and the morphology of the Au-NPs synthesized were investigated using the TEM analysis which is represented by Figure 5 (a). The Au-NPs synthesized from CT flower aqueous extract (20 mg/mL, 10mL) reacted with  $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$  (10 mM, 1 mL) was well dispersed. It was because CT flower extract did not only have the reducing power ( $\text{Au}^{3+}$  to  $\text{Au}^0$ ) but also having the stabilizing properties to minimize agglomeration of Au-NPs [21]. Moreover, the TEM image of synthesized Au-NPs also showed that most nanoparticles were in a spherical shape. A histogram of particle size distribution was drawn according to the size of 200 Au-NPs as shown in Figure 5 (b). According to the histogram, the mean particle size was 18.16 nm in diameter with a standard deviation of 4.67 nm. The crystallite size of the synthesized Au-NPs was found to be 17.67 nm in diameter from XRD analysis, which was in an agreement with the result obtained from the TEM that shows a size distribution between 13 – 23 nm in diameter.



**Figure 5.** (a) TEM image of Au-NPs forms using CT flower extract at 100 nm, and (b) histogram of the distribution of the particle size of Au-NPs.

## 4. Conclusion

In conclusion, Au-NPs were synthesized successfully by a green and simple approach using CT flower extract without utilizing any chemical that acts as a reducing or stabilizing agent. Based on the UV-vis analysis studied, the best reaction time for synthesized Au-NPs using CT flower extract was 15 min with the absorbance peak of around 0.17 at 540 nm. Furthermore, according to the XRD analysis, a high purity crystalline of Au-NPs was prepared. The TEM imaging ascertained that Au-NPs were observed as majority spherical in shape with a mean particle size distribution of 18.16 nm in diameter. The synthesized Au-NPs using CT flower extract having the potential to be applied in other applications especially biomedical applications due to its biocompatibility and nontoxicity.

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