Facile Green Synthesis and Characterisation of Gold Nanoparticles using Fenugreek Seeds and Honey

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Abstract. Gold nanoparticles are frequently employed in a range of biological applications because of its versatility in biosynthetic pathways and complexation, good biocompatibility and ease of detection. This study discusses the application of green chemistry in the production of gold nanoparticles using fenugreek and honey. It discusses nanoparticle characterisation in order to investigate structural, morphological, and optical characteristics. The mean crystalline size of fenugreek-mediated gold nanoparticles is 12.035 nm, while honey-mediated gold nanoparticles are almost 42.2225 nm, according to XRD analysis. The absorption and fluorescence spectra correlated well with particle size variance in that the absorption and fluorescence peak positions were observed to move as particle size increased. The spherical shape of the particles was shown by SEM and TEM analysis, and the particle size was confirmed by XRD. EDAX analysis demonstrated the sample's purity. On the surface of the sample, the presence of a functional group with distinctive peaks of gold NPs is shown by FT-IR and FT-Raman analysis, indicating a high potential for hyperthermia treatment, biomarkers, and cancer diagnostics.

1. Introduction

Nanotechnology is a rapidly evolving technique that is generating significant progress in a variety of fields. Nanoparticles and nanomaterials have distinct size dependent properties they have a wide range of uses, especially in bioengineering, biomedicine, optical, and diagnostic imaging, as well as catalysis and circuitry. Metal nanoparticles have been shown to have a variety of applications [1]. Due to their unique physical and chemical characteristics resulting from the high surface to volume ratio, GNPs are a very appealing material for nanoelectronics, nanobiology and nanomedicine. It’s large area of surface and strong electron conductivity, make gold nanoparticles (AuNPs) widely used in biotechnology and biomedicine, and have been shown to be stable and less harmful as drug delivery agents. To contribute to promising biological and biomedical applications, GNPs must have optimum chemical stability and appropriate biocompatibility. It is extremely significant that the colloidal solutions prepared do not contain radioactive compounds, so green synthesis is favored. Furthermore, the particle size distribution must be as narrow as possible, with small sizes being preferable. In addition, the GNPs colloidal structures must be resistant to nanoparticle aggregation. It should also have sufficient surface functionalization to allow nanoparticles to be conjugated with biologically active molecules. Certainly, the advancement of simple, environmentally sustainable, and low-cost preparation methods is a hot topic right now, and this study on GNPs expands their possible uses in biomedicine, including drug delivery systems [2]. Because of their unusual rheological characteristics in the nano size range, plasmonic metal (noble) nanoparticles are of significant interest. Due to the inherent behavior of localized surface plasmon resonance, metal gold nanoparticles (AuNPs) have improved optical characteristics (LSPR) [3]. Metal nanoparticles can exhibit significant absorption and scattering characteristics due to the phenomena of surface plasmon resonance. Metal nanoparticles absorb light
and convert it to heat, which may be used in a number of nanophotonic applications [4]. The SPR of noble metal nanoparticles, which is absent in non-metallic nanoparticles, is particularly noticeable. The optical, magnetic, physical, chemical, electrical, and catalytic characteristics of a nanoparticle are all determined by its mean free path, which is influenced by its size and shape [5].

In the last 10 years, gold nanoparticles have been widely employed in biomedical applications such as substrates for spectroscopic techniques, sensor arrays, fluorescence, and sensor chips [6]. These innovations are based on the elastic scattering characteristics of metal nanoparticles as well as the plasmon shift of metal nanoparticles [7]. GNPs, in particular, are used for MRI, nano vehicles, and molecular adjuvants, all of which are useful for delivering ligands/drugs to specific target sites. Due to possible toxicity issues, GNPs seem to have been approved for biological uses, despite its various possibilities. GNPs toxicity is determined by their morphological features [8]. For translational applications, controlling chemical constituents on the particle surface in the nano domain is a problem. Proper encapsulating agents that offer biocompatibility and versatility for molecular docking can improve nanoparticle stability. Depending on their physico-chemical properties, the capping agent influences the absorption of nanoparticles into cells. Noble metal NPs (gold, silver, and platinum) have been encapsulated with surfactants and thiolated polymers for biomedical research purposes.

Despite the various benefits of metal nanoparticles, in-vitro and in-vivo toxicity studies have raised concerns about their safety on biological cells [9]. Gold is biocompatible and inert in its bulk form, but the way these nanoparticles are made utilizing diverse synthetic processes, as well as the type of the capping/stabilizing agents, may induce toxicity.

Due to its compatible nature in fabrication and complexation, low toxicity, and ease of monitoring, GNPs are widely used in a number of biological applications [10]. GNPs may aggregate in malignant cells and exhibit light dispersion; as a result, these NPs could be useful as a probe in cancer cell imaging. Furthermore, these nanoparticles can be employed in cancer treatment and diagnostics. In addition, AuNPs provide a useful and promising framework for medication and gene delivery [11-12].

Green chemistry is concerned with the creation of materials and technologies that can reduce or eliminate toxic contaminants while still reducing waste generation. Green nanotechnology is defined as the advancement of clean technologies that reduce the environmental and human health hazards, as well as the promotion of the replacement of new nanoproducts. Green synthesis is based on green chemistry concepts that concentrate on unusual and counterintuitive results in nanoscale materials [13].

Greek hay is the name for fenugreek. Its seeds have a pungent odor and a slightly bitter flavor. It's rich in calcium, and since it's a seed and a legume, it's high in vitamins and minerals. They're a good source of diosgenin, too [14]. Fenugreek has cardioprotective and hypoglycemic actions due to the defatted fraction or fiber. The primary hypoglycemic and antioxidant chemicals found in fenugreek seed extract included 4-Hydroxyisoleucine (modified amino-acid), trigonelline (alkaloid), galactomannans (viscouse fiber), and diosgenin (saponin). 4-Hydroxyisoleucine is a mystical molecule that has direct effects on the islets of Langerhans and increases insulin release to deal with hyperglycemia while avoiding severe hypoglycemia caused by diabetes mellitus treatment. Furthermore, fenugreek increases viscosity in the intestines and inhibits carbohydrate breakdown, delaying glucose absorption. Some of the active components in fenugreek, on the other hand, are implicated in the hypoglycemic process. Galactomannan (gelling fiber) and diosgenin are two of these substances that help to restore the antioxidant enzyme activity of SOD (superoxide dismutase), GPX (glutathione peroxidase), and catalase in hepatic tissue. Fenugreek's antioxidant activity helps diabetic rats' renal function to be restored [15].

Other active ingredients in fenugreek include alkaloids, lysine, and L-tryptophan, as well as steroidal saponins (diosgenin, yamogenin, tigogenin, and neotigogenin). Antitumor, antiviral, antibacterial, anti-inflammatory, hypotensive, and antioxidant activities have also been discovered in fenugreek [16]. Diosgenin, tigogenin, gitogenin, and saponin (fenugrin B) are among the alkaloids contained in fenugreek seed. Polyphenolic compounds such as isovitexin and rhaponticin are thought to be the most bioactive compounds in fenugreek seed. The chemical structure of fenugreek endosperm reveals that it contains significantly more protein (43.8 g/100 g) and saponin (4.6 g/100 g). In addition, seed husk has been discovered to be high in total polyphenols. Fenugreek may be able to help reduce the risk of cancer, diabetes, obesity, high cholesterol, high blood pressure, heart conditions, bacterial, fungal, and viral infections, inflammation [17].
Honey is a sweet viscous fluid made by bees, containing carbohydrates, sugars, vitamins, minerals, proteins, polyphenols and antioxidants [18]. Natural honey was applied for medicinal purposes since ancient times. Furthermore, numerous studies have shown the use of honey in cardiovascular disorders, where it affects cardiovascular risk factors such as hyperlipidemia and free radical production [19]. Honey has long been considered one of the healthiest food sources. It's made up of 80–85 percent carbohydrate (mostly glucose and fructose), 15–17 percent water, 0.1–0.4 percent protein, 0.2 percent ash, and trace amounts of amino acids, enzymes, and vitamins, as well as other antioxidants including phenolics. Natural honey's chemical composition and physical properties, on the other hand, vary depending on the plant species foraged by the bees, climatic conditions, and vegetation [20]. Fructose (32.56 to 38.2 percent) and glucose (28.54 to 31.3 percent) are the two major sugars present in honey, accounting for 85–95 percent of total sugars and are readily digested in the gastrointestinal tract. Disaccharides such as maltose, sucrose, isomaltose, turanose, nigerose, melibiose, panose, maltotriose, and melezitose are examples of other sugars. Mineral salts are found in concentrations ranging from 0.1 to 1 percent. The most abundant metal is potassium, which is supplemented by calcium, magnesium, sodium, sulphur, and phosphorus. Iron, copper, zinc, and manganese are examples of trace metals [21] are also present in honey.

The use of green chemistry in the synthesis of gold nanoparticles using fenugreek and honey is discussed in this chapter. The chapter provides a discussion of the nanoparticles characterization to study structural, morphological and optical properties.

2. Synthesis of gold nanoparticles using fenugreek seeds extract (TRIGONELLA FOENUM-GRAECUM) and Honey (APIS)

Fenugreek seed, 50 mg, was washed and dried in distilled water. The powdered material was combined with 50 milliliters of distilled water. At a temperature of 75°C, the solution was completely stirred in a stirrer for two hours. The mixture was then filtered using Whatman filter paper. Under vigorous stirring, 50 mL of Fenugreek seed extract was added to 50 mL of Hydrogen tetrachloroaurate trihydrate (HAuCl₄.3H₂O) solution. The formation of gold nanoparticles is indicated by a shift in the color of the solution from pale yellow to purple. The solution's pH was maintained at 9. After that, the mixture was centrifuged with distilled water and ethanol. The purple-colored precipitate was dried for 8 hours at 180°C, crushed into fine powder, and annealed for 4 hours at 600°C.

5 ml of honey was dissolved in 20 ml of distilled water and stirred vigorously for two hours without heating. The solution was filtered thoroughly using whatman filter paper to avoid impurities. 25ml of freshly prepared honey solution was added into 50 ml of Hydrogen tetrachloroaurate trihydrate (HAuCl₄.3H₂O) solution under vigorous stirring. The solution's hue changed from pale yellow to purple, suggesting that gold nanoparticles were forming. The pH of the solution was retained into 9. The solution was then centrifuged with distilled water and ethanol. The purple color precipitate was dried at 180°C for 8 hours, ground into fine powder and annealed for 4 hours at 600°C temperature (figure 1).

2.1. Reaction Mechanism
Material scientists use a variety of physical and chemical ways to create metallic nanoparticles. Because bacteria and fungus have demonstrated the capacity to reduce metal ions to create nanoparticles, nanotechnology necessitates collaboration between physicists, chemists, biologists, and engineers. The gold nanoparticles were made utilizing Fenugreek and honey, as revealed in this study. The phytochemical analysis proved that fenugreek and honey contain large amount of bioactives. These phytochemicals are responsible for the immediate reduction of ions and formation of gold nanoparticles by green method. It involves the formation of atoms by the nucleation process, which is followed by the formation of nanoparticle by aggregation. In the nucleation process, the hydrated electrons (e⁻_aq) behaves as strong reducing agents and can reduce Au (Au⁺) ions into zero - valent Au (Au⁰) atoms [22]. Figure 2 depicts the color change of gold nanoparticles during the synthesis procedure which attribute to the surface plasmon resonance with change in particle size.
3. Analysis of Gold nanoparticles using fenugreek seeds extract and Honey

Crystalline and phase formation of Gold nanoparticles were determined using X-ray diffractograms acquired with a Bruker AXS D8 Advance instrument employing CuKα radiation (\(\lambda=1.540598\)) in the 20-70° range. A Perkin Elmer spectrometer was used to record a Fourier Transform Infrared Spectroscopy investigation using the KBr pellet technique in the range of 4000 - 400 cm\(^{-1}\). The chemical content of the produced samples is recorded using an EDX analyzer linked to an FEI Quanta FEG 200-High Resolution Scanning Electron Microscope, and the particle size and shape of the powder is examined using a Transmission Electron Microscope (TEM) model Joel/JEM2100 (HRSEM). PerkinElmer Lambda 25 was used to create a UV-Vis optical absorption spectrum and perform PL analysis in the wavelength range of 300-1200 nm. A QE Pro Raman spectrometer with 514 nm laser light and 1.96 mW incident power was used to perform room temperature Raman spectroscopy.

4. Results and Discussions

4.1 X-Ray diffraction analysis of Gold nanoparticles using fenugreek seeds extract and Honey

The crystalline structure of the synthesized, Fenugreek capped gold nanoparticles was determined using X-ray diffraction (XRD) analysis on powdered samples. The scan was done on a scale of 10 to 80 degrees. The diffractograms indicated peaks of 2 values at 38.10, 44.30, 64.50, and 77.70, with Miller indices of (111), (200), (220), and (311) for the obtained peaks, as shown in figure 3a and 3b. According to the research, the metal gold nanoparticles exhibited face centered cubic (fcc) lattices. The findings are the same as those previously reported for JCPDS' standard powder diffraction card (gold file No. JCPDS 04-0784) [23-24]. The very high intensity peak at \(2 = 38.10\) demonstrates preferred growth in the 111 direction. From molecular-sized crystals formed from duplicated units of atoms at a predetermined distance and time intervals, the selective development in the 111 direction happened. The Debye–Scherrer formula was used to compute the mean crystalline size of fenugreek mediated gold nanoparticles, which is 12.035nm, and honey mediated gold nanoparticles, which is almost 42.225nm:

\[
D = \frac{0.89\lambda}{\beta \cos \theta}
\]

where D is the mean crystalline size, \(\lambda\) is the wavelength of the X-ray radiation, \(\beta\) is the full width at half maximum (FWHM), and \(\theta\) is the diffraction angle.

4.2 SEM and EDAX analysis of Gold nanoparticles using fenugreek seeds and honey

The SEM images of gold nanoparticles using fenugreek and honey is shown in figure 4.a and 4.b. SEM analysis show uniformly distributed gold nanoparticles that indicating spherical shape by the stabilization of capping agents. Sadeghi et al have reported spherical morphology of gold nanoparticles using Stevia rebadiauna leaf extract [25].

EDAX profile of fenugreek and honey mediated gold nanoparticles show strong gold signals for as shown in the figure 5.a and 5.b. The graph produced by the EDX study in support of the XRD data, which showed the reduction of gold ions to elemental gold.

4.3 TEM and SAED analysis of Gold nanoparticles using fenugreek seeds and honey

TEM images were used to determine the shape and size of the synthesized nanoparticles. Typical TEM images obtained for gold nanoparticles reduced with fenugreek and Honey are given in figure 6a and 7.a respectively. Figure 6.a shows a combination of spherical with rod like particles with an average particle size of 6-14 nm, while figure 7.a shows a spherical shaped morphology of the nanoparticle with an average particle size of 15-54 nm. The XRD study shows that all values are in strong agreement. It reveals that Au nanoparticles grow in the (1 1 1) plane preferentially. The nanoparticles are highly crystalline, as evidenced by clear lattice fringes in high-resolution TEM images and the standard SAED patterns of brilliant circular circles corresponding to the (1 1 1), (2 0), (2 2 0), and (3 1 1) planes (figures 6.b and 7.b).
4.4 UV-Vis analysis of Gold nanoparticles using fenugreek seed and honey

Metal nanoparticles' optical characteristics are widely recognized to be strongly impacted by their size. According to the Mie principle, tiny gold nanoparticles have just one surface plasmon resonance (SPR) absorption band, whereas anisotropic particles have two or three [26]. Figure 8.a and 8.b show the UV-Vis spectra of fenugreek and honey mediated gold nanoparticles. The bands corresponding to SPR occur at 420 nm for fenugreek mediated gold nanoparticles and 440 nm for honey mediated gold nanoparticles. As is well known, small spherical Au nanoparticles should have a single surface plasmon band. Au nanoparticles' NIR absorption might be useful in the production of photonic devices like optical sensors and NIR absorbers, and it's been shown to be beneficial in cancer hyperthermia [27].

4.5 Photoluminescence analysis of Gold nanoparticles using fenugreek seed and honey

Interband transitions in noble metals such as gold and silver have been discovered as a source of visible photoluminescence (PL) [28-33]. Fenugreek and honey-mediated nanoparticles were discovered to be photoluminescent in this study. Figures 9.a and 9.b show the PL spectra of gold nanoparticles at an excitation wavelength of 400 nm. The functionalization of gold nanoparticles with biomolecules in fenugreek and honey might explain the luminescence at 500 and 520 nm. The photoluminescence nature of gold nanoparticles produced with pseudomonas aeruginosa, whose peaks are observed to be above 500 nm, has been described by Husseiny et al [34]. The PL emission indicates use of gold NPs in bio-imaging as therapeutic applications.

4.6 FT-IR analysis of Gold nanoparticles using fenugreek seeds extract and Honey

FT-IR spectrum of fenugreek (figure 10.a) and honey (figure 10.b) capped gold nanoparticles reveal the functional group present on the surface of the nanoparticles. Fenugreek and honey act as reducing and stabilizing agents during synthesis, thus as-synthesized gold nanoparticles is surrounded by organic stabilizing molecules. Fenugreek extracts have antioxidant properties, and the chemical composition and polar nature of fenugreek seed extract include phenol component. Figure 10.a shows that the fenugreek-capped gold nanoparticle has a peak at 3450 cm⁻¹, which corresponds to phenol O-H stretching [35]. The carbonyl stretching vibrations in the amide links of the proteins cause 1679 cm⁻¹ to be recognized as the amide I. The C-N stretching or O-H bending vibrations cause the band at 1321 cm⁻¹. Protein C–OH vibrations may be seen at 1031 cm⁻¹, and C–O–C vibrations can be seen at 966 cm⁻¹ for the absorption peak. [16] [36]. Proteins can attach to gold NPs via free carboxylate groups, as is widely known. The existence of peaks at 1679 cm⁻¹ and 1031 cm⁻¹ suggests that gold NPs are linked to proteins via the carboxylate group. The flavonoids in the seed extract are powerful reducing agents, and they may be responsible for the decrease of chloroauric acid. Proteins include a carboxylate group that can act as a surfactant, binding to the surface of gold NPs and electrostatically stabilizing them. As a result, it's been revealed that fenugreek seed extract has the capacity to reduce gold NP while also maintaining stability. Fructose, glucose, sucrose, proteins, minerals, and vitamins are all major components of honey [37] [18]. FT-IR measurements were used to identify the possible biomolecules responsible for the capping and effective stability of Au nanoparticles made with honey. The FT-IR study of honey-mediated gold nanoparticles is shown in Figure 10.b. At 3409 cm⁻¹, 2332.4 cm⁻¹, 1610.15 cm⁻¹, 1075.37 cm⁻¹, and 615.70 cm⁻¹, respectively, there are intense absorptions. Around 1610 cm⁻¹, the amide I and II bands of proteins are predicted to appear as significant IR bands. The C–O stretching mode band, which came from C–O–C symmetric stretching and C–O–H bending vibrations of honey protein, was merged into a very wide envelope centered at 1075 cm⁻¹ [28]. The addition of a -OH group from sugars found in carbohydrates such as fructose, sucrose, glucose, and maltose in honey may cause a widened band at 3409 cm⁻¹ [38]. The carbohydrates bands (stretching vibrations of –C-H, –C-OH, –C-C) may be clearly recognized at lower wavenumbers, in the area 615. 70 cm⁻¹. 2332.4 cm⁻¹, which is induced by lipids in honey [39].
4.7 Raman analysis of Gold nanoparticles using fenugreek seed and honey

Optical absorption, fluorescence, Raman scattering, atomic and magnetic force, and electrical conductivity are just a few of the analytical ways for identifying gold nanoparticles, making them excellent labels for sensors. These techniques might ultimately replace PCR and fluorescence tagging as a means of identifying bacteria [34]. Raman intensity was measured from 500 cm\(^{-1}\) to 4000 cm\(^{-1}\) in our research (figure 11). The peak intensity of the spectra indicated that the peak intensity rose as the gold nanoparticle size grew (Schwartzberg et al., 2004). Peak intensities in the Raman spectral range were found at 570 cm\(^{-1}\), 788.02 cm\(^{-1}\), and 1,102.22 cm\(^{-1}\).

5 Conclusion

The bio-reducing agents' fenugreek seed extract and honey were used to successfully produce gold nanoparticles. The mean crystalline size of fenugreek-mediated gold nanoparticles is 12.035nm, while honey-mediated gold nanoparticles are almost 42.2225 nm, according to XRD examination. The absorption and fluorescence spectra were shown to have a strong relationship with particle size variation, with absorption and fluorescence peak locations shifting as particle size increased. The spherical shape of the particles was shown by SEM and TEM examination, and the particle size was confirmed by XRD. EDAX analysis demonstrated the sample's purity. The existence of the functional group present on the surface of the sample with distinctive peaks of gold NPs is shown by FT-IR and FT-Raman analysis, indicating a high potential for hyperthermia treatment, biomarkers, and cancer diagnostics.
Figure 2. Gold nanoparticle during the different stages of synthesis.

Figure 3.a. XRD graph of Gold nanoparticles with Fenugreek.

Figure 3.b. XRD graph of Gold nanoparticles with Honey.
Figure 4.a. SEM image of Gold nanoparticles with Fenugreek.

Figure 4.b. SEM image of Gold nanoparticles with Honey.

Figure 5.a. EDAX spectra of Gold nanoparticles with Fenugreek.

Figure 5.b. EDAX spectra of Gold nanoparticles with Honey.

Figure 6.a. TEM image of Gold nanoparticles with Fenugreek.

Figure 6.b. SAED pattern of Gold nanoparticles with Fenugreek.
Figure 7.a. TEM image of Gold nanoparticles with Fenugreek.

Figure 7.b. SAED pattern of Gold nanoparticles with Fenugreek.

Figure 8.a. Uv- Vis graph of Gold nanoparticle with Fenugreek.

Figure 8.b. Uv- Vis graph of Gold nanoparticle with Honey.

Figure 9.a. Photoluminescence analysis of Gold nanoparticles with Fenugreek.

Figure 9.b. Photoluminescence analysis of Gold nanoparticles with Honey.
Figure 10.a. FT-IR graph of Gold nanoparticles with Fenugreek.

Figure 9.a. FT-IR graph of Gold nanoparticles with Honey.

Figure 11. FT-Raman analysis of Gold nanoparticles with Fenugreek and Honey.

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