Utilization of polyfloral honey in the synthesis of gold nanoparticles and evaluation of its potency as an antibacterial against \( S.\ aureus \) and \( E.\ coli \)

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Abstract. Gold nanoparticles was synthesized undergoes a green synthesis by utilising polyfloral honey as a bioreducing agents. Resulted gold nanoparticles were characterized using UV-Vis and Fourier Transform Infra Red (FTIR) spectrometer, as well as X-Ray Diffraction (XRD) and Particle Size Analyser (PSA). Spectroscopy analysis of obtained products gave the results of an absorbance of 1.095 at wave length of 543 nm for UV-Vis, while Fourier Transform Infra Red (FTIR) shown the appearance of certain type of organic functional group, which are characteristic for sugars, the major constituents of polyfloral honey, such as O-H, C=O, N-H and C-O in the gold nanoparticle synthesis. Analysis of XRD resulted of the Miller Index 111, 200, 202 and 311 of the product that corresponding to the database of Joint Committee on Powder Diffraction Standards (JCPDS). Particle Size Analyzer result average size distribution of gold nanoparticle is 219,8 nm. The evaluation of test antibacterial activity was performed using several bacteria test such as \( S.\ aureus \) and \( E.\ coli \) that show the gold nanoparticles can inhibit the bacteria.

1. Introduction

Nanotechnology, subject that focused on production and utilization of particles with nano size (\( \leq 100 \) nm), currently became the most rapidly developing and gaining more interest due to lot of its product with wide range of application in wide variety of area, such as: material science, physical sciences, environments, biomedicine, and catalyst[1].

Among entire nanoparticles, gold nanoparticles is one of the most exciting ones. Several types of gold nanoparticles have been synthesised by either physical or chemical methods, however, both
methods showed some disadvantages, like: use excessive energy, economically high cost, resulted a by-products which potentially harm or toxic, and less environmental friendly[2-4].

In order to resolved above problems an alternative method, Green synthesis, has been carried out by utilizing secondary metabolites produced by living organism; like animals, plants, and microorganisms either from terrestrial or marine environment; as bioreducing agents [5]. One of the natural product that currently used as metal bioreductor is honey, which produced in abundant of glucose and fructose, both important monosaccharide, that could function as bioreducing agents [6].

Due to the problem of bacterial resistance, gold nanoparticles assisted by polyfloral honey and other living organism sources as bioreductor can become a promising alternative to produce potential antibacterial because of its non-toxic properties[7-10]. Phillip (2009)[6] have reported a synthesis of gold nanoparticles assisted by polyfloral honey and obtained product with average particles size 15 nm. Furthermore, Sreelakshmi et al., 2011[11] also successfully synthesised gold nanoparticles with particles size of 11.5 nm and the product significantly inhibits the growth of some bacteria, like E. faecalis, S. aureus, and E. coli.

The aims of these investigation are to utilize the polyfloral honey originated from Bone regency, South Sulawesi Province, in green and environmentally safe synthesis of gold nanoparticles; characterizing the obtaining product; and further testing its antibacterial properties against S. aureus and E. coli.

2. Materials and Methods

2.1. Material

Polyfloral honey samples for the research were taken freshly from forest area at Sadar Village, Bone regency, South Sulawesi Province, Indonesia.

2.2. Methods

The laboratory works in this research including several protocols, such as preparation of the polyfloral honey sample; preparation of gold solution; synthesis of gold nanoparticles; characterization of the obtained products; and testing of the antibacterials properties of the resulting nanoparticles against S. aureus and E. coli

2.2.1. Sample preparation

Preparation of honey solution

Honey solution were prepared by simply weighed 200 g of freshly taken polyfloral honey, dissolved, and homogenized in 700 mL double distilled water. Resulted suspension were then filtered using filter paper and transferring to into a 1000 mL beaker glass to get a clear solution of polyfloral honey.

Preparation of gold solution HAuCl₄
A stock solution of 1000 ppm of HAuCl$_4$ were prepared by weighing 1 g of pure solid gold and dissolving in 8 mL of aquaregia, the mixture of both concentrated hydrochloric acid and nitric acid with 3:1 ratio, under excessive heating until all pure gold completely dissolve. The obtained solution were then transfer into a 1 L volumetric flask and adding with double distilled water up to the 1 L volume to get the stock solution of 1000 ppm of HAuCl$_4$. Further, from these 1000 ppm stock solution of gold, 50 mL of solution were taken and transfer to a 500 mL volumetric flask and adding with distilled water up to the volume mark to obtain 0.5 mM of HAuCl$_4$ solution.

2.2.2. Optimization of the polyfloral honey concentration

Optimization of concentration, both polyfloral honey and gold metal solution, were done in order to get the optimum yield of the nanoparticles. The optimization was carried out by fill-in 10 mL of HAuCl$_4$ 0.5 mM into a 50 mL Erlenmeyer flask and added with 10 mL of polyfloral honey solution while vigorously stirred for approximately 3 hours. The colour of solution were observed until it change from colourless to violet. The absorbance of the solution were measured with UV-Visible spectrometer during 1, 2, 4, 7, and 14 days of synthesis within the range of wavelength from 200 - 700 nm. The same protocol were repeated with an addition of 20, 30, and 40 mL of HAuCl$_4$ 0.5 mM solution.

2.2.3. Synthesis of gold nanoparticles assisted by polyfloral honey

Initially 100 mL of polyfloral honey solution were added into a 1 L Erlenmeyer flask and mixed with 300 mL of HAuCl$_4$ 0.5 mM solution under stirred for approximately 3 hours until the colour of solution change from colourless to purple-violet[6]. Absorbance of the obtained gold nanoparticles solution were then measured with UV-Vis spectroscopy after 1, 2, 4, 7, and 14 days of reaction at a wave length from 200 to 700 nm. Obtained gold nanoparticles were then centrifuged 3-5 times at 10000 rpm for 25 minutes at 27 °C. The final results of gold nanoparticles were then put forward for characterization.

2.2.4. Characterization of gold nanoparticles

The characterization process of resulted gold nanoparticles were carried out using several physical and spectroscopy instrument, such as : UV-Vis (Shimadzu UV-2600) and FTIR (Shimadzu FTIR Prestige-21) spectroscopy, X-Ray Diffraction (Shimadzu XRD-7000), and Particles Size Analyser (Beckman Coulter Delta Nano C PSA).

2.2.5. Antibacterial testing of gold nanoparticles

Standard method of agar-disc diffusion[12] were apply for anti-bacterial activity testing of the gold nanoparticles. These protocol involving several steps, e.g., preparation of nutrient agar media; sub-cultured of the testing bacteria ( S. aureus and E. coli); and preparation of the positive control (Ampicillin) and empty sterile disc as a negative control.

3. Results and Discussion
There are three major results were described in this research: synthesis, characterization, and antimicrobial testing of the gold nanoparticles products. Prior to the synthesis process, an optimization of the concentration ratio between the polyfloral honey and gold solution, in form of HAuCl$_4$ 0.5 mM, was carried out. These optimization step gave a results that among the ratio of concentration between HAuCl$_4$ 0.5 mM and polyfloral honey solution from 1:1 up to 1:4, the ratio that achieved best resulted gold nanoparticles is 1:3 ratio, which was shown in the least absorbance of 1.371 at the maximum wavelength of 537 nm. These result also confirmed visually by the changing of colour of solution mixtures from colourless to darkest-violet. The complete results of the UV-Vis spectroscopy measurement of concentration ratio optimization were described in Table 1.

**Table 1. Concentration ratio of Honey and HAuCl$_4$**

| Time (days) | Concentration ratio of Honey and HAuCl$_4$ | λ$_{max}$ (nm) | Abs   | λ$_{max}$ (nm) | Abs   | λ$_{max}$ (nm) | Abs   | λ$_{max}$ (nm) | Abs   |
|------------|------------------------------------------|----------------|-------|----------------|-------|----------------|-------|----------------|-------|
| 1          | 1 to 1                                   | 534            | 0.798 | 534            | 0.975 | **543**        | **1.095** | 546            | 1.254 |
| 2          | 1 to 2                                   | 532            | 0.991 | 532            | 1.065 | **540**        | **1.314** | 544.5         | 1.47  |
| 4          | 1 to 3                                   | 537            | 1.371 | 542.5          | 1.533 |
| 7          | 1 to 4                                   | **533.5**      | **1.527** | 540.5         | 1.189 |
| 14         |                                          | **523.5**      | **1.508** | 540.5         | 1.768 |

Synthesis of gold nanoparticles were carried out by simply mixing the solution of honey and HAuCl$_4$ 0.5 mM in the optimum concentration ratio of 1:3 with vigorous stirred for approximately 3 hours until the colour of solution physically change from colourless to violet, and the measurement of UV-Visible absorbance and λ$_{max}$ results was described in Table 2. Mechanistically 2 (two) major steps involved in formation of gold nanoparticles which are, first step is the hydrolysis of the glucose by water molecules in order to open the cyclic glucose to its linear structure; second step the carbonyl aldehyde group of the glucose reduced the Au metal from HAuCl$_4$ to form an Au$^0$ solid form and gluconic acid as a final product [13].
According to Rohiman et al., 2014 [14], gold nanoparticles have specific plasmon surface absorbance peak at wavelength of 500-550 nm, so the existing of strong peak at 543 with an absorbance intensity of 1.095 clearly shown the formation of gold nanoparticles. This results also justified by the fact that the batochromic shifting in HAuCl₄ 0.5 mM solution also present with a shifted from 313.5 nm to 543 nm.

Resulted gold nanoparticles were further characterized with XRD and showed four major 20 peaks of 37.92; 44.05; 64.40; 77.51 with a Miller index of 111, 200, 202, and 311; and diameter size of 15.24; 24.74; 26.81; and 29.19 nm.

**Table 2. Diffraction Peaks of Gold Nanoparticles**

| No | 2θ   | d (Å) | Miller Index | Size (nm) |
|----|------|-------|--------------|-----------|
| 1  | 37.92| 2.37  | 111          | 15.24     |
| 2  | 44.05| 2.05  | 200          | 24.74     |
| 3  | 64.4 | 1.45  | 202          | 26.81     |
| 4  | 77.51| 1.23  | 311          | 29.19     |

FTIR spectroscopy were used to determine the major functional group that involved in the formation of gold nanoparticles. FTIR were describe in Table 3.

**Table 3. Difference of FTIR major peaks between honey solution and gold nanoparticles**

| No | Honey Wave number (cm⁻¹) | Honey Functional group | Gold nanoparticles Wave number (cm⁻¹) | Gold nanoparticles Functional group |
|----|----------------------------|------------------------|---------------------------------------|-------------------------------------|
| 1  | 3442.94                   | O-H                    | 3419.79                               | O-H                                 |
| 2  | 1643.35                   | C=O                    | 1649.14                               | C=O                                 |
The FTIR spectrum of polyfloral honey solution shows a strong absorption at wavelength of 3442.94 cm\(^{-1}\) which occurred because of the presence of O-H group; whereas 1643.35 cm\(^{-1}\) was corresponded to the characteristic peak of the carbonyl C=O; and 1055.06 cm\(^{-1}\) clearly shown C-O bonding of the alcohol functional group. In comparable, after the formation of the gold nanoparticles, there were a shifting of the wave numbers of all those major peaks above which shown a strong interaction the principal molecules of the polyfloral honey, which presumably sugars, and gold metal.

Determination of the average particles size of the products also became one of the important parameters in the synthesis of these gold nanoparticles. Underwent particles size analyser gave us a results of 219.8 nm as the average diameter size and polydispersities index of 0.249. This values is higher then the normal average particle size of nanoparticles, however the distribution intensity showed different results, as the distribution of the particles in average size in between 1 to 100 nm was the highest percentages around 37 %. The complete description of distribution intensity of resulted gold nanoparticles were described in Figure 1.

![Figure 1. Particles Size Analysis of the gold nanoparticles](image)

These resulted gold nanoparticles also tested for its efficacy as an antibacterial against S. aureus and E. coli and the results were describe in Table 4.
Table 4. Inhibition Zone of samples against S. aureus and E. coli after 48 hours

| Sample                          | Inhibition Zone (mm) |
|--------------------------------|----------------------|
|                                | S. aureus | E. coli |
| Np-Au 3.6 mg/mL                 | 7.25      | 8.4     |
| HAuCl4 0.5 mM                   | 7.4       | 8.05    |
| Honey solution 20/70 mg/mL      | 7.05      | 8.05    |
| Ampicillin (+ control)          | 24.55     | 17.1    |
| Sterile double distilled water (- control) | nil      | nil     |

Results of the antibacterial bio-assays of gold nanoparticles products were moderately inhibits both S. aureus and E. coli bacteria in comparison with the antibacterial Ampicillin as a positive control. However, compare to the both starting precursors, honey solution and HAuCl4 0.5 mM, the results are slightly higher which means that the performance of the gold nanoparticles as an antibacterial still potentially for being improve.

4. Conclusion

Synthesis and characterization of gold nanoparticles assisted by polyfloral honey had successful been conducted and gave a considerably good results. Approximately 36.5 mg of solids darkest-violet gold nanoparticles were obtained and characterized used UV-Vis and FTIR spectroscopy; X-Ray Diffraction; and Particles Size Analysis. UV-Vis spectra shown a clearly shifted of the maximum wave length of the Polyfloral extract and HAuCl4 0.5 mM which are 246.5 nm and 313 nm to 543 nm of gold nanoparticles. The FTIR spectra also confirmed these results by shown a clearly shifted of three major functional group wave numbers in polyfloral honey, O-H; C=O; and C-O, from 3442.94; 1643.35; and 1055.06 to 3419.79; 1649.14; and 1074.35 cm⁻¹ considerably. Undergoes XRD characterization the product gave four 2θ values are 37.92; 44.05; 64.40; and 77.51 which correspond to a Miller index of {111}, {200}, {202}, and {311} with average particles size of 15.24; 24.74; 26.81; and 29.19 nm.
These gold nanoparticle products were also tested for biological activity as an antibacterial against *S. aureus* and *E. coli* which gave a considerably moderate results in compare to the polyfloral honey and HAuCl\(_4\) 0.5 mM as starting precursor.

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