International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 11, Issue 6, 2019

Original Article

BIO-NANOPARTICLES: GREEN SYNTHESIS OF GOLD NANOPARTICLES AND ASSESSMENT OF BIOLOGICAL EVALUATION

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Received: 08 Jul 2019, Revised and Accepted: 23 Sep 2019

ABSTRACT

Objective: The design like bio-nano particles are beneficial over chemical and physical composition due to the eco-friendly and lower-cost synthesis of nanoparticles. The current study was purposed for the biosynthesis of gold nanoparticles (GNPs) and their antioxidant evaluation.

Methods: Aqua extract of *Muntingia calabura*, Linn was applied for the synthesis of GNPs and confirmed by UV visible and ATR-Fourier Transform Infra-Red (ATR-FTIR) spectroscopy. Transmission Electron Microscope and Particle Size Analyser were used for the shape zeta potential and determination of size. Antioxidant activity was examined by DPPH (1,1 diphenyl-2-picryl-hydrazyl) radical scavenging method.

Results: The result showed that UV–vis absorption spectra of H Au Cl_4 at 290 nm while absorption spectra of biosynthesis gold nanoparticles at 540 nm. The forming of nanoparticles were spherical, having an average particle size of 88 nm, and the result of zeta potential was 9.5 mV. Analysis of ATR-FTIR revealed the possible involvement of phytochemical constituents in gold nanoparticles of aqua extract. Green synthesized nanoparticles showed enhanced antioxidant properties.

Conclusion: Green synthesized GNPs showed enhanced biological activities. Present results also support the benefit of using the biosynthesis method for the production of gold nanoparticles that have the potential of antioxidant and biology activities.

Keywords: Green synthesis, Gold Nanoparticles, Antioxidant, *Muntingia calabura*, Biological activities

DOI: http://dx.doi.org/10.22159/ijap.2019v11i6.34826

INTRODUCTION

The development of science and technology has led several studies in nanotechnology which potential breakthroughs in good fields such as materials and manufacturing, health care, and medicine. Among the nanoparticles, the bio-reduced gold and silver nanoparticles have gained the utmost importance in biomedical applications. The size and surface morphology play an essential role in controlling the chemical, physical, and electronic properties of nanomaterials [1]. Both Au and Ag nanoparticles are the main leading robust nanomaterial provides an excellent platform in biology biomedical research and applications [2]. The characteristic of gold nanoparticles is different from the mass, as a gold nanoparticle is a wine red solution while the mass gold is a yellow solid. The gold nanoparticles can be manufactured into a shapes variety. The shape and size of gold nanoparticles very influence their characters and properties. Due to their broad spread applications in diagnosis, targeted drug delivery system, and therapeutics due to their minimal small size, physical and chemical properties, high surface area, stability, non-cytotoxicity optics, gold nanoparticles have revolutionized the field of targeted medicine [3, 4]. Preparation of gold nanoparticles is not only comfortable but is also highly flexible since they can be synthesized in different sizes and shapes [2]. French scientist research in which they formulated to synthesize fluorescent gold nanoparticles inside human hair. They showed that the gold colour remained even after repeated washings [5].

Much of the study is devoted to the study of the synthesis, stabilization, and functionalization of the GNPs. The most common reduction agents reported are sodium citrate, transferrin, and Natrium Borohydride while general species used for functionalization include various oligonucleotides, peptides, antibodies, and lipids. The interest in GNPs is primarily due to the relative ease of their synthesis, with reasonable control of their shapes, size, their optical characteristics, and their high biocompatibility [6].

After the progress of the concept of green nanoparticle prepared, there has been a growing need for environmentally benign metal nanoparticle synthesis process that does not use toxic chemicals in the synthesis protocols to avoid adverse effects in medical applications [7]. In the last decade, biosynthesis of metal nanoparticles is a growing need to reveal clean, nontoxic chemicals, environmentally mild solvents, and renewable materials and hence the focus turned towards green chemistry and bioprocesses [8]. The influence for green chemistry and bioprocesses approaches from nature through fungi, bacteria, yeast, and plant extracts in the synthesis of biocompatible gold and semiconductor nanoparticles [9]. The use of non-toxic chemicals, environmentally friendly solvents and renewable materials are some critical issues that deserve important consideration in green synthetic strategies [7].

To reduce further chemical interventions and to improve the sustainability of the process, alternative biosynthetic green methods that utilize plant-based phytochemicals for reduction of metal ions provide an inherently green approach to nanotechnology, also referred to as green nanotechnology. Recently, several studies have demonstrated the dual role of whole plant extracts and pure compounds isolated from plants as active, reducing agents and as stabilizers, to provide a robust coating on the biocompatible GNPs. The reactive phytochemical species include polyphenols such as flavonoids and non-flavonoids, the abundant antioxidants in human food [10].

Muntingia calabura L is a fast-growing thin tree, native to the American continent and is commonly was conserved in warm areas of the Asian region [11]. The extract of *M. calabura* plant displayed excellent antioxidant activity and the antioxidant activity of *M. calabura* plant is may due to the presence of ascorbic acid, flavonoids, and polyphenols [12]. The current study designed for green synthesis of gold nanoparticles (GNPs) with extracts of *M. calabura*, L and their biological evaluation.

MATERIALS AND METHODS

Materials

The *Muntingia calabura* was collected from the Spice and Medicinal Plant Research Institute, Bogor, Indonesia. Au Foil (PT Antam, Indonesia), Gummy Arabic, 1,1-Diphenyl-2-Picril-Hydrazil (DPPH), Methanol, Bovine Serum Albumin (Sigma-Aldrich), Phosphate Buffer Saline (IPHA), PD-10 column (GE Health Care, England), Aqua Bidestilata (IPHA, Indonesia),Aqua Regia, Sodium Dibasic Phosphate (Merck).

Preparation of the plant extract

The collected leaves of *M. calabura* were washed thoroughly with distilled water. Dried *M. calabura* leaves were sliced into small pieces and 5.00 g of sample was added to 100 ml of double-distilled water and heated at 70 °C for 15 min. The mixture was stored to room temperature and filtered through Whatman No. 1 filter paper. The aqueous extract was cooled in a refrigerator for further studies [13].

Preparation of the $HAuCl_4$, and gummy arabic solutions

A Solution of 0.002 M $HAuCl_4$ was prepared by dissolving 20.1612 mg of Au foil in hot aqua regia that mixture from $HCl_{(aq)}$ and $HNO_{3(aq)}$ (3:1). This solution was heated, and 10 ml of an aqua pro injection was added. The heating and add aqua pro injection were operated three times. The final solution was added to a 0.01 M HCl solution to achieve the 0.002 M $HAuCl_4$ solution [14].

The Gummy Arabic solution 8.2 ml and 0.3 ml aliquot of the aqua pro injection were heated to 55 °C and continuously stirred. To this hot Gummy Arabic solution, 1.5 ml of the 0.002 M $HAuCl_4$ solution was added with continuous stirring [14].

Biosynthesis of gold nanoparticles

Synthesis of GNPs was carried out at room temperature by adding 1.0 ml *Muntigia calabura* leaves aqueous extract to 9.0 ml of 0,002 M $HAuCl_4$[15]. The result was incubated at room temperature in dark condition for 24 hr and observed for any change in colour. During the solution containing Muntingia, the color changed orange then yellow and purple–red after about 10 min. The reaction mixture was mixed for an additional 30 min [10].

Characterization

Synthesis of GNPs was done at room temperature by adding of aqueous leaves extract of *Muntigia calabura* and added to 9.0 ml of 0,002 M $HAuCl_4$[15]. The reaction was nursed at room temperature in dark condition for 24 h and GNPs formation was detected by changed colour from orange then yellow to purple-red after about 10 min. To find out the particles size distribution, the synthesized GNPs was dispersed and carried out with an SZ100 model HORIBA nanoparticle analyser (HORIBA Ltd., Kyoto, Japan). Zeta potential describes the electrical potential in the double layer of ions surrounding a particle at the boundary of the particle surface and the adsorbed ions in the diffuse layer. Zeta potentials were determined with an SZ100 model HORIBA nanoparticle analyser (HORIBA Ltd., Kyoto, Japan). The Zeta Potential describes the electrical potential in the double layer of ions surrounding a particle at the boundary of the particle surface and the adsorbed ions in the diffuse layer. Zeta potentials were determined with a SZ100 model HORIBA nanoparticle analyser (HORIBA Ltd., Kyoto, Japan).

Spectroscopic measurements

The biological reduction of H $AuCl_4$ was determined using UV-Visible spectrophotometer (TECAN, Switzerland). The formation of ruby red colour confirms the synthesis of GNPs. The confirmation of colour change was characterised by the absorption peak over the range of a from 300 nm to 700 nm.

The radical scavenging activity

The radical scavenging activity of the synthesized GNPs was determined using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay. Different concentrations of 1 ml the synthesized GNPs was mixed with 5 ml of 0,1 mmol methanolic DPPH and incubated in the dark at room temperature for 20 min. After incubation, the absorbance of the samples was measured by using UV–vis Spectrophotometer at 517 nm and methanolic DPPH reagent without sample was used as a control [16]. The ability to scavenge DPPH radical was calculated by the following equation:

$$\text{Inhibition (\%)} = \frac{\text{Absorbance Control} - \text{Absorbance test}}{\text{Absorbance Control}} \times 100 \text{ [16]}$$

Statistical analysis

Each experiment was carried out in triple replicates and the results were expressed as the mean±standard deviation (n=3). The mean and standard deviation were measured using Microsoft Excel.

RESULTS AND DISCUSSION

Biosynthesis of gold nanoparticles

The matured collected leaves are washed with distilled water, sliced into small pieces and boiled in distilled water to obtained extract. The extract can be purified by filtration with Whatman No. 1 filter paper [17]. Gold Nanoparticles (GNPs) were prepared with The aqueous extract of the M. calabura, and the synthesized GNPs were indicated from the colour change from yellow to ruby red after 1 hour after stirring. The formation of the ruby red colour showed that the completion of a synthesis process the biosynthesis of GNPs. The plant extract contains different biomolecules like enzymes, amino acids, proteins, sugars, and other traces of metals [18]. These metabolites are strongly comprised of the bio-reduction process. The usual reaction was Au^{+}ions reduction into metallic Au ° nanoparticles in the presence of metabolites and redox enzymes. The reaction is given below.

$$HAu_{+}Cl_4 + 4H_20 + \text{extracts of plant} \rightarrow Au_{o}NPs + \text{by-products [18]}$$

Colour changes during GNP synthesis are caused by excitation of surface plasmon resonance, which is expressed as characteristic features of synthesized nanoparticles in the study of diagnostic and therapeutic applications of gold nanoparticles depending on their synthesis and characterization. The absorption peak was recorded at 540 nm in the spectrum of UV-visible. The peak of the nanoparticles is broadened in the absorption spectrum. For the biological and biomedical application, it is found that the broadening of the peak in the absorption spectra indicates the poly-dispersion of the nanoparticles from a study involving the peak of absorption and properties of scattering of gold nanoparticles [2]. UV–vis absorption spectra maximum of H $Au\ Cl_4$ at 290 nm, while absorption spectra maximum of gold nanoparticles with extract of Muntingia at 540 nm (fig. 1). It displayed that these changes are consistent with an initial rapid reduction of $Au^{(III)}$ to $Au^{(°)}$ and showed that the biosynthesis gold nanoparticles had been formed. Furthermore, the presence of an absorption band at 550 nm is suggestive of the presence of gold nanoparticles.

Characterization of biosynthesized gold nanoparticles

The characterization of gold nanoparticles is an essential step in the biosynthesis of gold nanoparticles. The stability, shape, size, surface area and their dispersion of nanoparticles are the critical properties used to characterise the synthesis of gold nanoparticles. The characterization of nanoparticles obtained was performed using Attenuated total reflection-Fourier Transform Infrared (ATR-FTIR) Spectroscopy, UV–visible spectroscopy, Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS) [19].

The Attenuated total reflection-Fourier Transform Infrared (ATR-FTIR) spectrum explains the interaction of gold nanoparticles with aqueous leaf biomolecules of *Muntingia calabura*. A robust and broad peak recorded at the wavenumbers 3259.49 cm^{-1} shows the presence of O-H functional group with H bonded at 3259.49 cm^{-1} indicating the presence of phenol and NH functional groups in *Muntingia calabura*. An absorption band appeared at 1635.29 cm^{-1} correspondings to C=C stretching vibrations confirming the presence of alkenes and aromatics (fig. 2). It was stated that the presence of phenols and alcohols is indicated by the O–H stretching vibrations between 3200 cm^{-1} and 3400 cm-1 and the presence of alkenes and aromatics is shown by the C=C stretching vibrations between 1575 cm^{-1} and 1675 cm^{-1}. The depth and width of the band are indicative of the intensity and abundance of the particular functional group in that band [20].

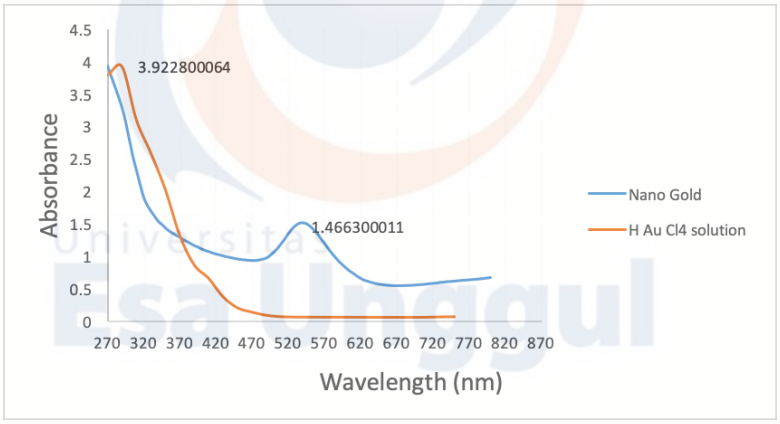

Fig. 1: UV spectra of H Au Cl_4 aqueous solution (a) biosynthesis gold nanoparticles (b)

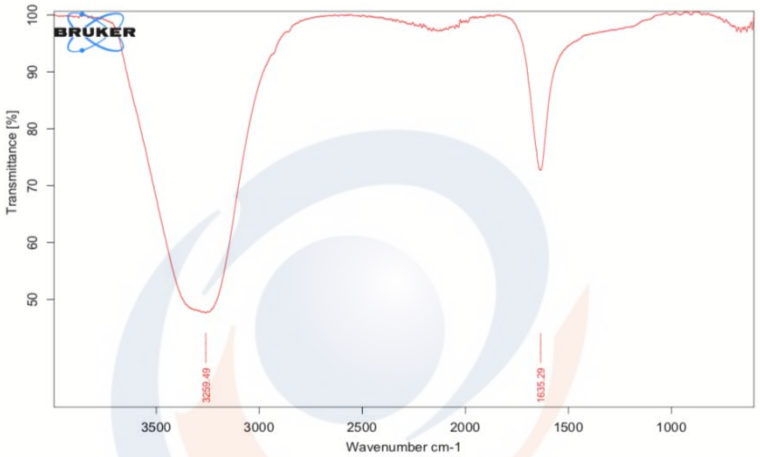

Fig. 2: FT-IR image of nano gold-*Muntingia calabura*

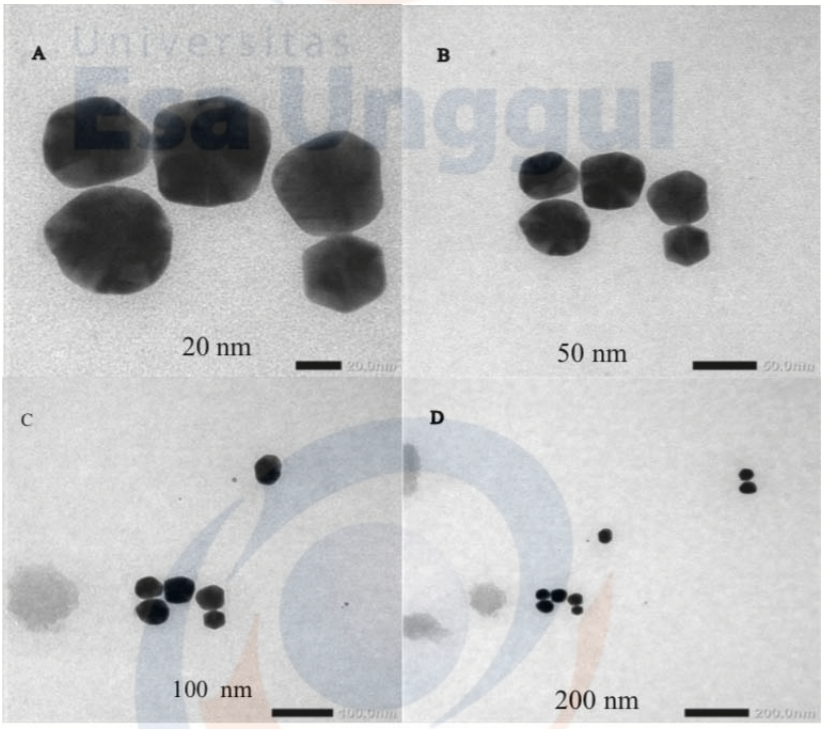

Fig. 3: Representative TEM images of the synthesized GNPs (A) on 20 nm scale (B) on 50 nm scale (C) on 100 nm scale (D) on 200 nm scale

TEM analysis of the synthesized gold nanoparticles (GNPs)

After the completion of a synthesis process, the synthesized GNPs have been sampled for TEM analysis. In this process, the samples were prepared by placing a drop of obtained suspension on the copper grids. The grids were further dried and used for TEM analysis. A typical Transmission Electron Microscope (TEM) image of the nanoparticles formed is presented in fig. 3. In general, the particles are spherical and mostly separated between gold nanoparticles. That indicated that the use of gummy Arabic in nanoparticle preparation could prevent aggregation of gold nanoparticles. These results illustrated the synthesis of Au $^{(0)}$ nanoparticles through reduction of $Au^{(3+)}$

Determination of particle size and zeta potential

The value of particle size distribution of gold nanoparticles was determined using a Particle Size Analyser (PSA) with Dynamic Light Scattering (DLS) method. Particle size characterization is of particular significance to nanomedicine. The size equality of nanoparticles to biological moieties is considered to impart many of their unique medical character [21]. DLS is the most used instrument for the size characterization of spherical particles, a commercial tool being available and widespread [22].

In DLS, the nanoparticle solution is illuminated by a monochromatic. All the same, the primary objective of a DLS measurement is still for size (distribution). The size distribution histogram of dynamic light scattering (DLS) showed that the z-average size of the synthesized GNPs is 88,9 nm with a polydispersity index is 0.109 (fig. 4). It indicated that the distribution consisted of a single size mode without aggregates. The range for the PDI is from 0 to 1. Values of PDI nearby to zero indicate a homogeneous dispersion, and those higher than 0.5 indicate high heterogeneity [23].

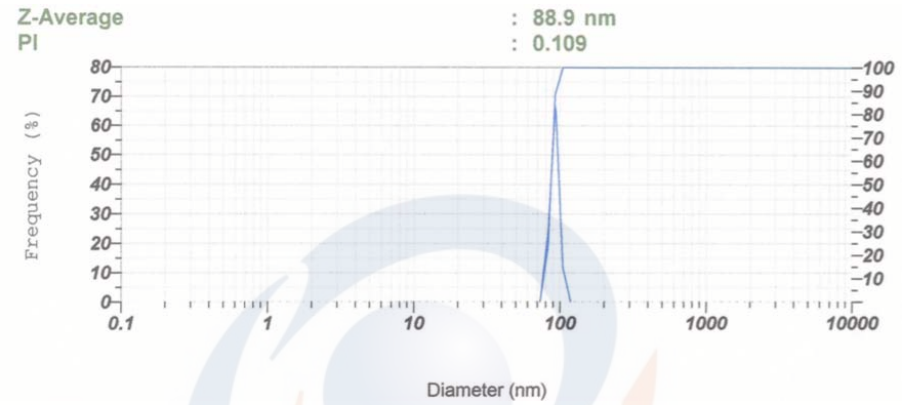

Fig. 4: The size distribution histogram of dynamic light scattering (DLS)

Also, zeta potential is an essential characteristic of nanoparticles, which is to predict the stability of the colloidal solution. The interaction among particles is critical to the stability of the colloidal solution. Zeta potential is a value that indicates repulsion force between particles. The colloidal solution stabilized by the presence of electrostatic repulsion rejected. The higher the repulsive force among particles rejected will cause the particles to be close to each other and will form aggregates. Nanoparticles with a zeta potential of+/-30 mV below stable nanoparticles [24].

The zeta potential and size of the nanoparticles can be controlled by various process parameters, including the amounts of emulsifier, drug, and polymer, the intensity and duration of homogenization, and the particle hardening profile. A reduction in the electrical charge is known to increase the flocculation and coalescence rates. Tolerable high zeta potential (>-30mV) ensures a stable emulsion by causing repulsion of adjacent droplets [25]. From the results of samples Zeta Potentials of the synthesized GNPs was-9.5 mV and below 30 mV (fig. 5), maybe it caused for Arabic gum as a stabilizer. Arabic gum constituents are principally magnesium, calcium and potassium salts of the polysaccharide of Arabic acid, which on acid hydrolysis yields L-arabinose, L-rhamnose, D-galactose, D-glucuronic acid, and D-galactose. The solution of Arabic gum in water dissociates the salts and reveals the negative charge of Arabic gum, which allows the interaction with the positive charge of gold [23].

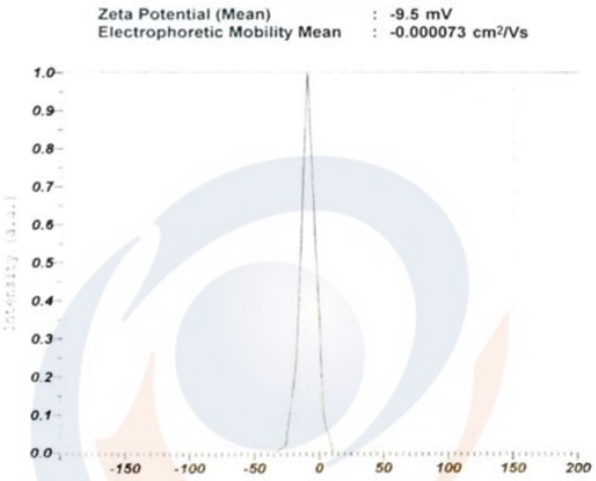

Fig. 5: Zeta potential of the synthesized GNPs

The results of samples Zeta Potentials of the synthesized GNPs was-9.5 mV also means that the solution of nanoparticles is considered an approximately neutral solution with a negative charge. Nanoparticles with a zeta potential between−10 and +10 mV are considered an nearly neutral, while nanoparticles with zeta potentials of greater than+30 mV or less than−30 mV are considered strongly cationic and strongly anionic, respectively [21].

The radical scavenging activity

DPPH is a method which has been generally used as a measure for evaluating free radical-scavenging activities for antioxidant analysis. The DPPH test method is based on the reduction of DPPH, a free radical that is stable by antioxidants. The free radical DPPH with an odd electron gives a maximum absorption at 517 nm (purple colour). When Antioxidants react by DPPH, which is a stable free becomes paired off in the presence of a hydrogen donor (e. g., a free radical scavenging antioxidant) and is decreased to the DPPH and as a result, the absorbance's reduced from the DPPH [26]. To examine the antioxidant capacity through free radical scavenging by the test samples, the change in optical density of DPPH radicals is observed [27]. DPPH scavenging activity for the synthesis of GNPs was 24.1043 ppm (table 1), while that of the quercetin as a control, was 2.5554 ppm (table 2). From table 1 indicated that synthesis of GNPs is a potent scavenger of superoxide radicals and the potential of antioxidants activities.

Table 1: Absorbance, % inhibition and IC 50 of synthesis of GNPs (The values are means±SD of three replicates)

Concentration (ppm)	Absorbance ± SD (nm)	% Inhibition ± SD (nm)	IC 50 (ppm)
0	0.967 ± 0.014	0.000±0.000	
10	0.688±0.029	28.861±1.933	24.1043
20	0.573±0.002	40.743±1.296	
30	0.366±0.032	62.191±2.743	
40	0.204±0.019	78.962±1.654	

Table 2: Absorbance, % inhibition and IC 50 of quercetin (The values are means±SD of three replicates)

Concentration (ppm)	Absorbance ± SD (nm)	% Inhibition ± SD (nm)	IC 50 (ppm)
0	0.937 ± 0.018	0.000±0.000	
1	0.752±0.006	18.559±2.058	2.5554
2	0.555±0.007	39.893±1.691	
3	0,363±0.008	60.606±1.654	
4	0,215±0.006	76.67±2.036	

CONCLUSION

Green synthesized GNPs showed enhanced biological activities and the advantages of using a green method for the production of nanoparticles having the potential of antioxidants activities.

ACKNOWLEDGMENT

The authors thanked to the Excellence Scholarships Indonesian Lecturers (BUDI-DN) from Indonesia Endowment Fund For Education (LPDP) for financial support to conduct these studies.

AUTHORS CONTRIBUTIONS

All the authors have contributed equally

CONFLICT OF INTERESTS

Declared none

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