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ANTIMICROBIAL POTENTIAL OF GOLD NANOPARTICLES BIOSYNTHESIZED USING WATER EXTRACT OF DIOSPYROS CELEBICA SEEDS: SYNTHESIS AND CHARACTERIZATION

N. Ariani[1], Y.A. Devy[1], Aspiyanto[1], Andreas[1], A. Darmawan[2], Juliansyah[3], M. Azzumar[3], M.H. Habibie[3], N. Hamim[3], O. Zuas[3,✉], T. Sudiro[4], Hardono[5] and G. Soehadi[5]

[1]Research Centre for Chemistry, BRIN, K.S.T. Baharudin Jusuf Habibie, 456, Kota Tang-Sel, 15314, Indonesia

[2]Research Centre for Pharmaceutical Ingredients & Traditional Medicine, BRIN, K.S.T. Baharudin Jusuf, 452, Kota Tang-Sel, 15314, Indonesia

[3]Research Centre for Testing Technology and Standard, BRIN, K.S.T. Baharudin Jusuf, 417, Kota Tang-Sel, 15314, Indonesia

[4]Research Centre for Advanced Material, BRIN, K.S.T. Baharudin Jusuf, 420-422, Kota Tang-Sel, 15314, Indonesia

[5]Research Centre for Industrial & Manufacturing Technology, BRIN, K.S.T. Baharudin Jusuf Habibie, 420-422, Kota Tang-Sel, 15314, Indonesia

✉Corresponding author: oman003@brin.go.id

ABSTRACT

This investigation explores gold nanoparticles (Au-NPs) biosynthesis employing *Diospyros celebica* seeds water extract and evaluates their antimicrobial potential. Au-NPs were synthesized by mixing chloroauric acid with the plant extract, followed by incubation at 60°C, leading to a color change indicative of nanoparticle formation. Characterization using UV-Vis spectrophotometry, zeta potential analysis, and transmission electron microscopy confirmed successful synthesis, revealing an average particle size of 28.10 nm and a negative zeta potential of -1.20 mV, suggesting stability due to bioactive compounds in the extract. High-magnification TEM images showed Au-NPs with diverse morphologies, including spherical, rod-shaped, triangular, cubic, pentagonal, hexagonal, and diamond-like structures. Fourier-transform infrared spectroscopy (FTIR) analysis identified hydroxyl, carboxylic acid, carbonyl, amide, and carbon-hydrogen functional groups as key contributors to nanoparticle biosynthesis. Antimicrobial action was valued using the minimum inhibitory concentration (MIC) method toward *Bacillus subtilis, Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa*. Results proved no antibacterial activity, indicating that the biosynthesized Au-NPs are non-toxic to living organisms. This research highlights *D. celebica* as a sustainable resource for eco-friendly Au-NP synthesis through green chemistry approaches.

Keywords: Macassar Ebony, Gold Nanoparticle, Biogenic, Biosynthesis, Plant Extract, Antimicrobial.

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INTRODUCTION

The synthesis of metallic or metal oxide nanoparticles has been widely studied.[1-4] Gold nanoparticles (Au-NPs), in particular, have been extensively researched for biomedical and non-medical applications due to their unique physicochemical properties.[5] Functionalized Au-NPs show promise in biomedicine, especially as antibiotics, exhibiting antimicrobial, antifungal, and antiviral activities against various pathogens.[6,7,8] Au-NPs are synthesized using physical-chemical and biological methods.[9] While physical-chemical approaches allow precise control over size and shape, they often involve toxic compounds, high energy, and costly processes.[10-13] In contrast, biological synthesis, using plant extracts or microorganisms, offers an eco-friendly alternative. Plant-mediated synthesis is advantageous due to its simplicity, low cost, and ability to operate at low temperatures,[3] unlike microbial methods that require strict growth conditions.[14] Plant

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extracts contain phytochemicals like flavonoids, polyphenols, and proteins, which reduce gold ions and stabilize Au-NPs.[5,15,16] Bioactive compounds from leaves,[6,17] bark,[18,19] fruits,[20,21] flowers,[22] and seeds[23,24] have been used for Au-NP synthesis. *Diospyros celebica*, an endemic Sulawesi plant, is valued for its durable, insect-resistant wood used in furniture and crafts.[25] Phytochemical studies reveal its wood and leaves contain alkaloids, flavonoids, and terpenoids,[26,27] similar to other plants like *Annona muricata*,[6] *Populus alba*,[17] *Mimusops elengi*,[18] *Aegle marmelos*,[20] *Achillea biebersteinii*,[22] and *Carica papaya* L.,[23] known for Au-NP synthesis. While *D. celebica* extract has been used for silver nanoparticle synthesis,[28] its application for Au-NPs remains unexplored. This study biosynthesizes Au-NPs utilizing *D. celebica* seed extract as a reducer and stabilizer, characterizes the nanoparticles, and evaluates their antibacterial potential.

EXPERIMENTAL

Material and Methods

The seeds of *D. celebica* were purchased from Berkah Nursery Store in Bogor, Indonesia. A 1000 ppm chloroauric acid ($HAuCl_4$) solution in hydrochloric acid (Cat. 170216, Supelco) was obtained from Merck, KGaA, Darmstadt, Germany. Pathogenic bacteria: *Bacillus subtilis* (FNCC 0059), *Staphylococcus aureus* (FNCC 0043), *Escherichia coli* (FNCC 00195), and *Pseudomonas aeruginosa* (ARCC 9027), were sourced from Gadjah Mada University, Yogyakarta, Indonesia. Double-distilled (D.D.) water from a Merck Millipore purification system (USA) was used in this investigation. All other reagents were of analytical grade.

Preparation of Water Extract of *D. Celebica* Seeds

The extraction of *D. celebica* was done with modifications to a previous method.[28] A 7 g sample of dried *D. celebica* seeds was placed in a 100 mL stainless-steel small autoclave containing 70 mL of deionized water, then tightly closed and heated in an oven at 105 °C for 1.5 hours. Centrifugation was conducted (6000 rpm and 10 minutes) to collect the aqueous phase. The resulting yellowish-brown extract was kept at 4 °C for later use in the biosynthesis of colloidal Au-NPs.

Biosynthesis of Au-NPs

Gold nanoparticles (Au-NPs) were fabricated using a 2.3 mg/L $HAuCl_4$ solution, prepared by diluting a 1000 mg/L stock solution. Specifically, 400 µL of aqueous *D. celebica* seed extract was transferred into 200 mL of the $HAuCl_4$ solution in a glass vessel. The mixture was incubated at 60 °C in an oven for two hours. Visual inspection was conducted before and after heating, followed by UV-Vis absorbance measurement.

Characterization of Biosynthesized Au-NPs

The biosynthesized Au-NPs were characterized using various analytical instruments. A UV-Vis spectrophotometer (Genesys 150, ThermoFisher Scientific, USA) recorded absorbance in the 300–800 nm wavelength range. The zeta potential and particle size of the colloidal Au-NPs were determined using a dynamic light scattering method (SZ-100, Horiba Scientific, Japan). Functional groups in the water extract of *D. celebica* seeds and the biosynthesized Au-NPs were analyzed using an FT-IR spectrophotometer (Invenio, Bruker, USA). The structure of the Au-NPs crystal was confirmed by XRD (Rigaku SmartLab, Japan) with CuKα radiation (1.541862 Å) over a 2θ range of 10°–90°. High-resolution images were captured employing a transmission electron microscope (TEM, Tecnai G20 S-Twin, USA) by placing a drop of colloidal Au-NPs onto carbon-coated copper grids, allowing it to evaporate, and analyzing at an acceleration voltage of 200 kV. Elements were mapped via a scanning transmission electron microscope (STEM, Talos F200X, USA) at 200 kV.

Antimicrobial Evaluation of Biosynthesized Colloidal Au-NPs

The minimum inhibitory concentration (MIC) method was used to assess the efficacy of biosynthesized colloidal Au-NPs. The MIC test evaluated bacterial growth in a 96-well plastic plate containing Mueller-Hinton (MH) broth. A series of twofold dilutions of colloidal Au-NPs, ranging from 2.3 mg/L to 0.575 mg/L, was prepared with an adjusted microbial concentration of 5×10^7 CFU/mL. The incubation of the plate was carried out at 37 °C for 24 hours. Bacterial growth was assessed by visually inspecting turbidity changes in the wells before and after incubation to determine the MIC value.

RESULTS AND DISCUSSION

Characterization of Biosynthesized Au-NPs

Adding a water extract of *D. celebica* seeds to an $HAuCl_4$ solution (2.3 mg/L), followed by 120 minutes of incubation at 60 °C, induces a noticeable color change from transparent to light purple (Fig.-1a), confirming the development of Au-NPs. The Au-NPs formation was also observed by using UV-Vis spectrophotometry,[21,30] pointing a surface plasmon resonance (S.P.R.) peak at 540 nm (Fig.-1b), indicating the reduction of trivalent gold ions to metallic Au by bioactive compounds in *D. celebica* seed extract. Similar results were reported by Folorunso *et al*. (2019) for Au-NPs using *A. muricata* extract (peak at 530 nm)[6] and by Guliani *et al*. (2021) for Ag-NPs using *P. alba* extract (peak at 540 nm).[17] Figure-2a shows the zeta potential of Au-NPs biosynthesized using a *D. celebica* seed extract, with a -1.20 mV, indicating a negative surface charge due to the bioactive molecular layer. This charge creates repulsive forces that prevent aggregation.[5,17] The zeta size of the Au-NPs (Fig.-2b) is approximately 28.10 nm and a polydispersity index (P.D.I.) of 0.714, suggesting a wide size distribution.[31] This was confirmed by TEM analysis (Fig.-3a and 3b).

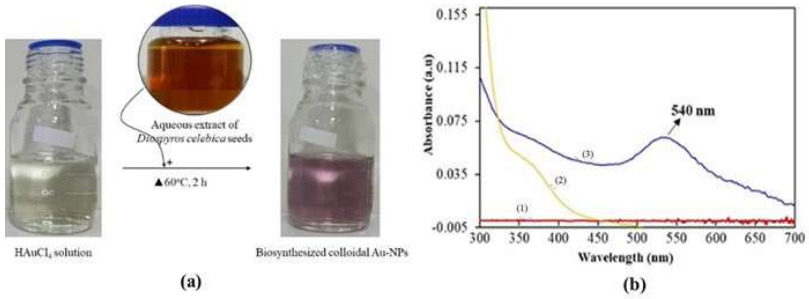

Fig.-1: (a) Schematic Diagram of Colloidal Au-NPs Biosynthesized from $HAuCl_4$ using Water Extract of *D. celebica* Seeds, and (b) U.V.–vis Spectra of (1) D.D. Water, (2) Diluted Water Extract of *D. celebica* Seeds, and (3) Biosynthesized Au-NPs with an S.P.R. Peak at 540 nm

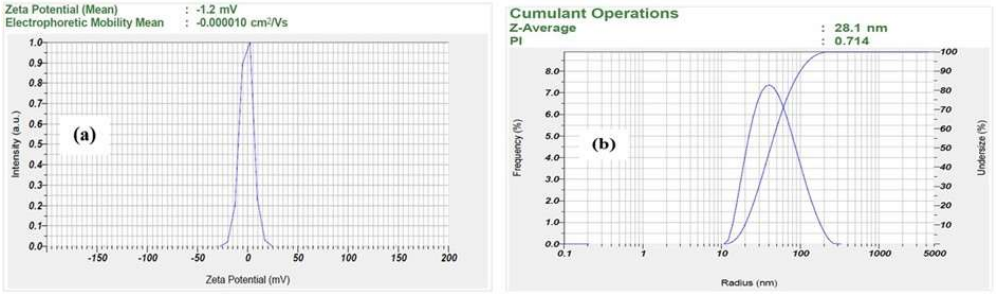

Fig.-2: (a) Zeta Potential, and (b) Zeta Size of Au-NPs Biosynthesized using Water Extract of *D. celebica* Seeds

High-magnification TEM (H.M.T.E.M.) images at 50 nm resolution (Fig.-3a) show Au-NPs with sizes between 8 and 27 nm and diverse shapes, including spherical, rod, triangular, cubic, pentagonal, hexagonal, and diamond-like structures (Fig.-3a). The particles are spatially distributed with visible gaps between them (Fig.-3a1-a7), likely due to active biomolecules preventing aggregation. Similar findings on biosynthesized Au-NPs with high distribution and low aggregation, attributed to biomolecular capping, have been reported.[20,21] Scanning T.E.M. (S.T.E.M.) analysis at 50 nm resolution (Fig.-3b1-b2) corroborates these results, with elemental mapping confirming the presence of metallic gold (Fig.-3b3), consistent with the X-ray diffraction (XRD) data (Fig.-3c).

The structure of biosynthesized Au-NPs crystal was examined using the XRD technique. Fig.-3c1 displays an XRD pattern of Au-NPs displaying specific peaks 2θ at 38.33º, 44.24º, 64.43º, 77.72º and 81.98º, corresponding to Miller indices (111), (200), (220), (311), and (222). The characteristic peaks are consistent with the J.C.P.D.S. card No. 04-0784 (Fig.-3c2), indicating that Au-NPs have a face center cubic (F.C.C.) shape.[7,32] The prominent peaks at 38.33° indicate a preferential alignment of Au-NPs along the (111) plane.[32] Furthermore, according to the calculation result employing Debye-Scherrer's method, the average

size of biosynthesized Au-NPs crystal is approximately 11.30 nm.[33] The FTIR spectra of the water extract of *D. celebica* seeds and biosynthesized Au-NPs are shown in Fig.-4a and 4b, respectively, with characteristic bands listed in Table-1. All bands in the seed extract spectrum (Fig.-4a) are present in the Au-NPs spectrum (Fig.-4b), except for the C-H aromatic ring vibration. The vibration band associated with O-H, -COOH, C=O, Amide, and C-H may indicate the possibility of active molecules, such as alkaloids, flavonoids, phenolics, and quinones from *D. celebica* seeds, that might be involved as capping agents in Au-NPs biosynthesis. Those active molecules have been found in the leaves and wood of *D. celebica*.[26,27]

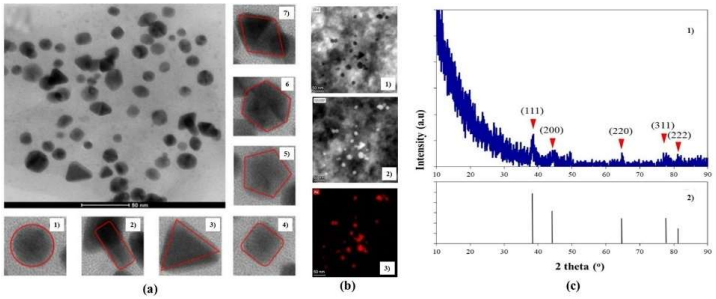

Fig.-3: (a) H.M.T.E.M. of Biosynthesized Au-NPs with Various Shapes: 1) Spherical, 2) Rod, 3) Triangular, 4) Cube, 5) Pentagonal, 6) Hexagonal, and 7) Diamond-Like Structure, (B) T.E.M. image with 1) Bright-Field Scanning Mode, 2) High-Angle Annular Dark Field Mode, and 3) Scanning Elemental Maps of Au, and (C) X-Ray Diffractogram of 1) Biosynthesized Au-Nps Using Water Extract of *D. Celebica* Seeds and 2) Metallic Au (J.C.P.D.S. File: 04-0784)

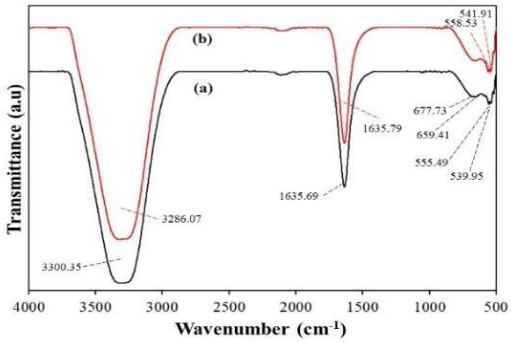

Fig.-4: FTIR Spectra of (a) Water Extract of *D. celebica* Seeds and (b) Biosynthesized Colloidal Au-NPs.

Table-1: Characteristics Absorption Bands of Water Extract of *D. celebica* Seeds and Biosynthesized Au-NPs

Bond	IR Frequency (cm^{-1})	
	Water extract of *D. celebica* seeds	Biosynthesized Au-NPs
O-H and -C.O.O.H. (stretching vibration)[34]	3300.35	3286.07
C=O and Amide group (stretching vibration)[34]	1635.69	1635.79
C-H aromatic rings (out-of-plane, bending vibration)[35]	677.73 and 659.41	-

Antimicrobial study

The M.I.C. is an in vitro assay determining the susceptibility or resistance of certain microbial strains to antimicrobial agents.[36] In this work, the microorganisms employed are *B. subtilis and S. aureus* (gram-positive) and *E. coli* and *P. aeruginosa* (gram-negative). Previous research has shown that Au-NPs impede the growth of such bacteria, implying that Au-NPs are biocidal to both.[6,30] The antibacterial impact of Au-NPs is intimately related to the release of gold ions in a microbial-water environment.[6] Au-NPs are modest in size and possess a substantial surface area, which enables gold ions to exert a more effective retardation effect. This microbial inhibition mode disrupts microbial cells' integrity by binding to carbohydrates, lipids, and proteins.[37] Gold nanoparticles can eliminate reactive oxygen species (R.O.S.) and free radicals, which damage microbial cell walls and inhibit enzyme respiration.[38,39] Table-2 shows the turbidity data of tested

microbes in 96-well after 24 h of incubation. Surprisingly, the antimicrobial tests in this study revealed that Au-NPs biosynthesized using water extract of *D. celebica* seeds had no growth inhibitory effects on all the tested microbes compared to that of both controls (tetracycline and streptomycin). Such no growth inhibitory effects were indicated by turbidity formation in the 96-wells.[29] In a word, the functionalized Au-NPs showed no antimicrobial activity under the experimental conditions, aligning with Deokar and Ingale's (2023) findings that green-synthesized Au-NPs lacked repressing effects on P. aeruginosa, *E. coli*, and *S. aureus*.[40]

Table-2: Turbidity Data after 24 h of Incubation

Sample	Microbial Tested			
	B.S.	S.A.	E.C.	P.A.
Au-NPs (2.3 mg/L)	(+)	(+)	(+)	(+)
Au-NPs (1.15 mg/L)	(+)	(+)	(+)	(+)
Au-NPs (0.575 mg/L)	(+)	(+)	(+)	(+)
Tetracycline (500 mg/L)	(-)	(-)	(-)	(-)
Streptomycin (500 mg/L)	(-)	(-)	(-)	(-)

B.S.: *B. subtilis*; S.A.: *S. aureus*; E.C.: *E. coli*; P.A.: *P. aeruginosa*; Positive (+): Turbidity indicating microbial growth; Negative (-): No turbidity indicating absence of microbial growth.

CONCLUSION

In conclusion, the water extract of *D. celebica* seeds is effective in the biosynthesis of colloid Au-NPs, which can be related to the ability of bioactive compounds in the water extract of *D. celebica* seeds to function as agents in reducing trivalent Ag^{3+} into metallic gold. The characterization results demonstrate that Au-NPs are spatially distributed, with particle sizes ranging from 8 to 27 nm and irregular shape, with low inter-particle aggregation. Antimicrobial testing revealed that the biosynthesized colloidal Au-NPs did not inhibit microbial growth. This finding implies that Au-NPs biosynthesized using a water extract of *D. celebica* seeds have no negative impact on living organisms.

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CONFLICT OF INTERESTS

No conflict of interest among the author that needs to be declared

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

N. Ariani https://orcid.org/0000-0002-1639-9058
Y.A. Devy https://orcid.org/0009-0007-1637-6896
Aspiyanto https://orcid.org/0000-0003-3325-2717
Andreas https://orcid.org/0009-0002-4344-6363
A. Darmawan https://orcid.org/0000-0001-9352-5233
J. Juliansyah https://orcid.org/0000-0002-6528-0651
M. Azzumar https://orcid.org/0000-0002-9528-5517
M.H. Habibie https://orcid.org/0000-0002-8134-4395
N. Hamim http://orcid.org/0009-0002-1301-9854
O. Zuas http://orcid.org/0000-0002-0101-5277
T. Sudiro http://orcid.org/0000-0001-7872-914X
Hardono https://orcid.org/0000-0001-8774-7040
G. Soehadi https://orcid.org/0009-0006-7263-1874

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