BULETINUL INSTITUTULUI POLITEHNIC DIN IAŞI
Publicat de
Universitatea Tehnică „Gheorghe Asachi” din Iaşi
Volumul 71 (75), Numărul 2, 2025
Secţia
CHIMIE şi INGINERIE CHIMICĂ
DOI: 10.5281/zenodo.15617768

BIOSYNTHESIS OF GOLD NANOPARTICLES USING *AVERRHOA BILIMBI* FOR CHROMIUM DETECTION IN WASTEWATER

BY

ALFIAN FADHILAH ALMINDA[1], ANISSYA DWI KUSUMANINGRUM WIBISONO[1], AQILAH NURSAKHA[1], MITHA YUSTIKA SYARI[1], SYIFA NUR’AFIYAH[1], YUDHISTIRA DIMAS NUGROHO[1], ZALFA JAWZA KAMILA[1], MOHAMMAD JIHAD MADIABU[2] and ACHMAD NANDANG ROZIAFANTO[1,*]

[1]Department of Food Nanotechnology, Politeknik AKA Bogor, Jl. Pangeran Sogiri No.283, Bogor 16154, Indonesia
[2]Department of Analytical Chemistry, Politeknik AKA Bogor, Jl. Pangeran Sogiri No.283, Bogor 16154, Indonesia

Received: March 18, 2025
Accepted for publication: May 20, 2025

Abstract. Heavy metal chromium (Cr) is one of the substances that can cause water pollution. Although chromium is highly useful in various aspects of life, including in the industrial sector, its excessive use also generates waste that has the potential to damage the environment and pose risks to human health. The analysis of chromium (Cr) presence in water was conducted using the biosynthesis method of gold nanoparticles with *Averrhoa bilimbi* as a bioreductant. Chromium, as one of the heavy metals, has many industrial applications; however, its excessive use can pollute the environment and endanger human health. The method used in this study is colorimetry, which is known to be simple, effective, economical, and accurate. The analysis results showed that gold nanoparticles synthesized from *Averrhoa bilimbi* could detect chromium at a concentration of 10 mM under optimal conditions at pH 5, temperature 75°C, and incubation time of 5 minutes. The synthesized gold nanoparticles proved to be selective and

---

[*] Corresponding author; *e-mail*: anandangr@yahoo.com

sensitive in detecting the presence of chromium, thus offering a potential solution for monitoring chromium pollution in aquatic environments.

Keywords: Chromium, Heavy metal, Bioreductant, Gold nanoparticles, *Averrhoa bilimbi*, Sensitivity and selectivity, Biosynthesis.

1. Introduction

Water is a fundamental resource for life, and its quality is of paramount importance for human health and environmental sustainability. However, rapid industrialization has led to the contamination of water bodies, with heavy metals being among the most persistent and hazardous pollutants. Among them, chromium, a transition metal widely utilized in various industries, poses a significant environmental threat due to its toxicity, mobility, and persistence in aquatic ecosystems. The presence of chromium in the environment, particularly in its hexavalent form (Cr^{6+}) and trivalent form (Cr^{3+}), is of critical concern due to its carcinogenic, mutagenic, and teratogenic properties. Cr^{6+} is extensively used in electroplating, leather tanning, textile dyeing, metal finishing, and wood preservation, leading to its widespread release into water bodies through industrial effluents (Das *et al*., 2021; Swathanthra and Rao, 2015).

The discharge of Cr ions into water sources occurs primarily through industrial activities such as mining, metal processing, and chemical manufacturing. Due to its high solubility and oxidative nature, Cr^{6+} readily contaminates surface water and groundwater, posing severe risks to aquatic life and human health (Tumolo *et al*., 2020). Exposure to Cr^{6+} has been linked to a range of adverse health effects, including skin irritation, respiratory disorders, oxidative stress, and DNA damage, ultimately increasing the risk of cancer (Saha *et al*., 2017). Chronic exposure, even at low concentrations, can result in liver and kidney dysfunction, neurological disorders, and reproductive toxicity. Moreover, epidemiological studies indicate that prenatal exposure to Cr^{6+} can negatively impact fetal development, further emphasizing the need for stringent monitoring and remediation strategies (Sazakli, 2024).

Conventional methods for Cr ions detection, such as colorimetric assays, spectrophotometry, electrochemical sensors, and chromatography-mass spectrometry, while accurate and reliable, have several limitations (Chumkong *et al*., 2025; Akhdhar, 2023; Tu *et al*., 2018; Nam *et al*., 2012). Colorimetric assays, though cost-effective and adaptable for field use, may suffer from interference and lower sensitivity (Alonso *et al*., 2017). Spectrophotometric methods require specific reagents and controlled conditions, limiting their practicality for on-site analysis (Li *et al*., 2021; Dawra *et al*., 2024). Electrochemical sensors, despite their high sensitivity, often require sophisticated modifications with nanomaterials, increasing complexity and cost (Chen *et al*., 2015b; Man *et al*., 2024). Meanwhile, advanced techniques like ion chromatography coupled with

ICP-MS offer exceptional precision but are restricted to laboratory settings due to expensive instrumentation, complex sample preparation, and time-intensive procedures (Mohana *et al*, 2021; Michalski, 2004). These limitations highlight the need for alternative, cost-effective, and field-deployable strategies for Cr^{6+} detection.

Among the emerging techniques for chromium detection, biosynthetic nanotechnology has gained considerable attention due to its potential for rapid, sensitive, and selective analysis. Gold nanoparticles (AuNPs) have been widely explored as colorimetric sensors due to their unique optical properties, particularly their localized surface plasmon resonance (LSPR), which enables visible color changes upon interaction with target analytes (Chen *et al.*, 2015a; Hazarika *et al.*, 2023; Tu *et al.*, 2018). The biosynthesis of AuNPs using plant extracts as bioreducing agents represents a green and sustainable alternative to conventional nanoparticle synthesis methods, significantly reducing the use of hazardous chemicals while maintaining high efficiency (Bagheri *et al.*, 2023; Madhumitha and Roopan, 2013; Rani and Shanker, 2022; Varma, 2012).

Averrhoa bilimbi, commonly known as bilimbi, is a tropical fruit rich in bioactive compounds such as ascorbic acid, flavonoids, and polyphenols, making it a promising candidate for the eco-friendly synthesis of AuNPs (Hublikar *et al.*, 2023; Isaac *et al.*, 2013; Ramanarayanan *et al.*, 2018). Its high content of reducing agents facilitates the rapid reduction of gold ions, leading to the formation of stable and functional AuNPs suitable for metal ion detection (Isaac *et al.*, 2013; Liu *et al.*, 2020). The use of *Averrhoa bilimbi* for nanoparticle biosynthesis aligns with the principles of green chemistry, providing a cost-effective and environmentally benign approach for nanomaterial production.

This study aims to investigate the biosynthesis of gold nanoparticles using *Averrhoa bilimbi* extract and evaluate their application as a colorimetric sensor for Cr^{6+} detection in wastewater. The synthesized AuNPs are expected to offer a rapid, cost-effective, and eco-friendly approach for monitoring Cr^{6+} contamination in aquatic environments. By leveraging the bioreductive properties of *Averrhoa bilimbi*, this study seeks to contribute to the advancement of sustainable nanotechnology-based solutions for water quality assessment and heavy metal detection.

2. Methodology

Instruments and Materials

The instruments used in this study include laboratory glassware, a thermometer, a hotplate (Heidolph MR Hei-Tec), a magnetic stirrer, a stirring rod, a universal pH meter, Whatman No. 42 filter paper, a UV-Vis spectrophotometer (Analytik Jena Specord 200 Plus), and a microwave oven (MK 2005 L). The materials used in this research include $HAuCl_4$, *Averrhoa* *bilimbi*, $Cr(NO_3)_3$, $FeSO_4$, $MgCl_2$, $HgSO_4$, KCl, NaCl, $ZnCl_2$, NaOH 10%, and distilled water.

Preparation of 0.25 mM $HAuCl_4$ Solution

A 0.25 mM $HAuCl_4$ solution was prepared by diluting a 10 mM stock solution of $HAuCl_4$. A total of 2.5 mL of 10 mM $HAuCl_4$ solution was added to a 250 mL volumetric flask and diluted with demineralized water up to the mark. The solution was then homogenized.

Preparation of *Averrhoa bilimbi* Extract

Fresh *Averrhoa bilimbi* fruit was chopped and weighed to obtain 20 grams. The chopped fruit was placed in a beaker glass, and 100 mL of water was added. The mixture was heated at 60°C for 15 minutes. The extract was then filtered using Whatman No. 42 filter paper to obtain the filtrate, which was ready for use in the biosynthesis of gold nanoparticles.

Biosynthesis of Gold Nanoparticles

The biosynthesis of AuNPs was carried out by mixing *Averrhoa bilimbi* extract and $HAuCl_4$ solution in specific ratios. A total of 50 mL of 0.25 mM $HAuCl_4$ solution was placed in a beaker glass, and 10 mL of *Averrhoa bilimbi* extract was added. The mixture was then stirred and heated using a microwave at 703.5 watts for 2 minutes. Additionally, different ratios of extract and $HAuCl_4$ were tested, namely 1:5, 2:5, and 3:5. After a color change occurred, the temperature was reduced to room temperature. The synthesized AuNPs were stored at room temperature and visually observed before measuring their absorbance at wavelengths of 200-800 nm. The most optimal biosynthesis result was determined as a colorimetric indicator for Cr^{6+}.

Preparation of Chromium Stock Solution

A 10 mM chromium stock solution was prepared by weighing 0.25 g of $Cr(NO_3)_3 \cdot 9H_2O$ powder and dissolving it in a 25 mL volumetric flask. The solution was then diluted with demineralized water up to the mark and homogenized. This stock solution was then diluted to obtain concentrations of 8 mM, 6 mM, 4 mM, 2 mM, and 1 mM. A standard metal stock solution was prepared by pipetting and dissolving the metal in a 25 mL volumetric flask with demineralized water.

Preparation of Standard Metal Solutions

A 6 mM standard metal solution was prepared by weighing 0.3 grams of Fe^{2+} powder and dissolving it in a 50 mL volumetric flask. The solution was then diluted with demineralized water up to the mark and homogenized. The procedure was repeated by replacing Fe^{2+} powder with Ba^{2+}, Hg^{2+}, Zn^{2+}, Mg^{2+}, Ca^{2+}, Pb^{2+}, Cr^{3+}, Cr^{6+}, K^{+}, Na^{+}, Mn^{2+}, and Ni^{2+} powders.

Preparation of Calibration Curve for UV-Vis Spectrophotometry Method

A blank solution of distilled water and a mixture of 0.25 mM AuNPs with standard chromium solutions at 0, 1, 2, 4, 8, and 10 mM were prepared. The blank solution and the prepared mixtures were then measured using a UV-Vis spectrophotometer at a maximum wavelength of 578 nm. From the absorbance results, a standard calibration curve was created by plotting absorbance versus chromium concentration.

Determination of Regression Equation for UV-Vis Spectrophotometry

The calibration curve for the UV-Vis spectrophotometry method was constructed by plotting concentration against absorbance values obtained from spectrophotometric measurements. A regression equation was determined in the form of $y = mx + C$ (Riyanto, 2020) using Microsoft Excel 2010.

Determination of Limit of Detection (LOD) in UV-Vis Spectrophotometry Method

The determination of the LOD for liquid AuNPs was conducted by adding chromium standard solutions at μM concentrations and measuring absorbance using UV-Vis spectrophotometry. The measurement was repeated seven times. The LOD was calculated by determining the standard deviation (SD) of the red color intensity absorption for each repetition and the gradient value from the AuNP calibration curve. Once the SD and gradient values were obtained, the LOD was calculated.

Determination of AuNP Sensitivity to Cr(III)

The sensitivity test of AuNPs was carried out by adding 1 mL of each AuNP solution in its optimum condition into a cuvette, followed by the addition of 3 mL of Cr(III) solution at varying concentrations (1 mM, 2 mM, 4 mM, 6 mM, 8 mM, 10 mM). Absorbance was then measured using a UV-Vis spectrophotometer. AuNP sensitivity was determined by observing the change in absorbance after the addition of Cr(III) solution compared to the absorbance of the blank solution (without Cr(III) addition).

Determination of Selectivity for Heavy Metals

A total of 1 mL of colloidal AuNP solution, prepared under optimal conditions, was placed in a test tube. Then, 3 mL of standard metal solutions containing Hg^{2+}, Fe^{2+}, Zn^{2+}, Mg^{2+}, Cr^{3+}, K^{+}, Na^{+}, Mn^{2+} at a concentration of 10 mM were added to the cuvette. Absorbance was measured using a UV-Vis spectrophotometer at wavelengths ranging from 200-800 nm. The selectivity of AuNPs towards metals was determined by observing the most noticeable color changes in nanoparticles and the most significant absorbance changes after adding the standard metal solution.

Analysis of Wastewater Samples

A total of 1 mL of colloidal AuNP solution, prepared under optimal conditions, was placed in a test tube. Then, 3 mL of wastewater samples collected from the wastewater treatment plant at Politeknik AKA Bogor were added to each test tube. Sampling was conducted using the grab sampling technique at the influent. The obtained results were measured for absorbance using a UV-Vis spectrophotometer. These results can be used to determine the precision and accuracy of the AuNP sensor for Cr(III) analysis in wastewater samples.

3. Results and Discussion

Biosynthesis of Gold Nanoparticles

The biosynthesis method is a synthesis technique that uses plant extracts as bioreductants (Siddiqi and Husen, 2017). One plant extract that has the potential to be used as a bioreductant is *Averrhoa bilimbi*. This plant contains a high level of secondary metabolites in the form of ascorbic acid (vitamin C), which plays a role as a bioreductant in the synthesis of gold nanoparticles. In addition to functioning as a bioreductant, *Averrhoa bilimbi* also acts as a stabilizer or capping agent (Siddiqi and Husen, 2017). The addition of a stabilizer is essential to maintaining nanoparticle stability and preventing coagulation (Algotiml *et al*., 2022).

Figure 1. showed colour of mixture $HAuCl_4$ solution and *Averrhoa bilimbi* extract before microwave heating and after microwave heating. In this experiment, the formation of AuNPs after mixing *Averrhoa bilimbi* extract and $HAuCl_4$ solution was indicated by a color change from colorless to burgundy and purplish hues. This color change occurred after heating in a microwave for approximately ±2 minutes. The formation of AuNPs through the reaction of plant extract with $HAuCl_4$ is indicated by a color change from colorless to burgundy and purplish hues, a phenomenon attributed to the Surface Plasmon Resonance (SPR) effect. SPR occurs due to the collective oscillation of electrons at the metal-medium interface when exposed to incident light, with the resonance effect being highly sensitive to the size, shape, and surrounding refractive index of the nanoparticles (Illyashenko-Raguin, 2021; Prajna and Sinha, 2024). This effect leads to strong electric field enhancement and extinction, causing the observed color variations. The color transition signifies nanoparticle formation and their aggregation state, with changes in SPR peaks influenced by particle size and distribution (Isnaeni, 2021). Additionally, microwave-assisted synthesis offers a rapid and efficient approach for producing AuNPs, enabling uniform particle sizes and significantly reducing synthesis time to just a few minutes by enhancing the reduction process of gold ions by the plant extract (Ngo *et al*., 2016; Seol *et*

al., 2011). After the colloidal AuNPs were formed, pH adjustment was carried out. According to the literature, the optimal pH condition for AuNPs in detecting chromium is at pH 5. This is because, in overly acidic conditions (pH 2 and 3), protonation occurs in the citrate/capping agent functional group, leading to aggregation of the gold nanoparticles (Dewi *et al*., 2024).

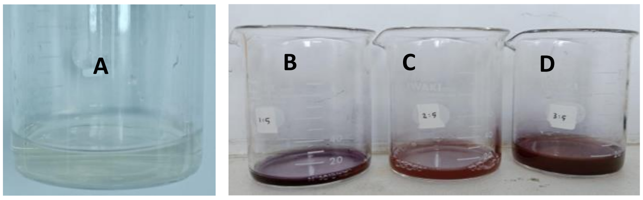

Fig. 1 – Mixture of $HAuCl_4$ solution and *Averrhoa bilimbi* extract before microwave heating and after microwave heating with ratios: (B) 1:5, (C) 2:5, (D) 3:5.

The selection of AuNPs to be used for Cr^{3+} detection was carried out. Variations in AuNPs were based on the ratio of *Averrhoa bilimbi* extract to $HAuCl_4$. Three synthesis ratios were used for AuNPs: 1:5, 2:5, and 3:5. The volume of $HAuCl_4$ used for all variations was 10 mL. The selection of the most optimal AuNPs was determined by identifying the maximum absorbance peak in the 500–600 nm wavelength range. The highest absorbance peak was found in AuNPs synthesized with an extract-to-$HAuCl_4$ ratio of 1:5, at a wavelength of 578 nm.

Particle Size Analysis (PSA) Characterization

Based on characterization using a Particle Size Analyzer (PSA), the average particle size of the gold nanoparticles (AuNPs) synthesized using *Averrhoa bilimbi* extract was 60.3 ± 2.9 nm. This indicates that the synthesized gold nanoparticles fall within the nanoscale range (1–100 nm). The relatively small particle size allows AuNPs to have a high surface area, thereby increasing interaction efficiency with chromium ions. The obtained polydispersity index (PDI) was 0.1105 ± 0.04, indicating a relatively uniform particle size distribution. A PDI value close to 0 signifies uniform particle distribution, whereas a value closer to 1 indicates a more heterogeneous distribution. In this study, the low PDI value suggests that the biosynthesis method using *Averrhoa bilimbi* extract was effective in producing AuNPs with consistent and evenly distributed sizes.

The size stability and distribution of gold nanoparticles are strongly influenced by phytochemical compounds present in *Averrhoa bilimbi* extract, such as flavonoids, tannins, and organic acids, which function as reducing and stabilizing agents in the biosynthesis process. These compounds not only assist in gold nanoparticle formation but also contribute to colloidal stability (Isaac *et al*., 2013; Stozhko *et al*., 2019). With an average size of 60.3 nm and a low PDI, the nanoparticles demonstrate optimal interaction with Cr^{3+}, thereby enhancing the sensitivity and selectivity of the analysis process.

Table 1

Particle Size Analyzer (PSA) Characterization

Sample	Particle Size (nm)	PDI
AuNPs	60.3 ± 2.9	0.1105 ± 0.04

Linearity of AuNP-Based Detection for Cr(III)

Figure 2 showed linearity of AuNPs towards Cr(III) using a UV-Vis spectrophotometer at a wavelength of 578 nm. The graph illustrates the relationship between concentration (mM) as the independent variable and absorbance as the dependent variable. The obtained intercept value was 0.2457 ± 0.00374, while the slope was 0.02454 ± 0.00062. The regression coefficient (R^2) was 0.9975, indicating that 99.75% of the variation in the data is explained by the linear model, showing a very strong correlation between concentration and absorbance. Additionally, the correlation coefficient (r) of 0.9994 reflects the strength and direction of the linear relationship, confirming a strong positive correlation.

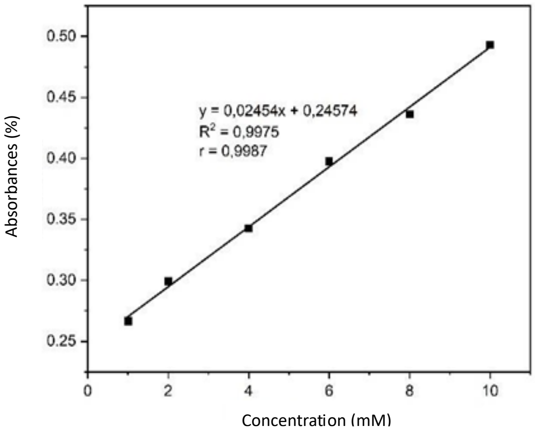

Fig. 2 – Linearity Curve of AuNPs Towards Cr(III).

Overall, these data support the hypothesis that an increase in chromium concentration is directly proportional to an increase in absorbance, with the color of the solution turning slightly purple after the addition of the chromium solution. The method's sensitivity to variations in chromium levels confirms its potential for environmental analysis and monitoring of heavy metal contamination in water and industrial waste. The high degree of linearity suggests that gold nanoparticles synthesized from *Averrhoa bilimbi* provide precise optical responses in detecting and measuring chromium concentrations in solution. The high linearity results have significant implications for the validity of this nanoparticle-based detection method. The exceptional linearity demonstrates that the gold nanoparticles synthesized from *Averrhoa bilimbi* offer precise optical responses in detecting and quantifying chromium concentrations in solution. The sensitivity of this method to variations in chromium concentration confirms its potential for environmental analysis and heavy metal contamination monitoring in industrial water and wastewater.

With correlation and determination coefficients close to 1, it can be concluded that this gold nanoparticle-based method not only offers an environmentally friendly approach to material synthesis but also possesses excellent analytical characteristics. The high accuracy and precision make it a potential method for chromium detection, both for laboratory purposes and environmental quality analysis, especially in monitoring hazardous heavy metal pollution that threatens ecosystems and human health.

Theoretical Limit of Detection (LOD) and Limit of Quantification (LOQ)

The theoretical LOD and LOQ values are used as detection limits for testing. The LOD is the minimum concentration of a substance that can be reliably distinguished from the absence of that substance (a blank value) (Snow, 2021). The LOQ is the lowest concentration at which the analyte can not only be reliably detected but also quantified with acceptable precision and accuracy (Belter *et al.*, 2014). However, the theoretical LOD and LOQ values obtained need to be confirmed to determine the actual response. The Sy/x value of 0.004806 is relatively small, indicating very low data variation and high measurement precision. LOD is the smallest concentration that can theoretically be detected by this method. The calculated LOD result is 0.59 mM, meaning this method is sensitive enough to detect chromium concentrations as low as 0.59 mM. LOQ is the smallest concentration at which measurements can still be conducted with adequate accuracy and precision. The LOQ value obtained is 5.88 mM, meaning that measurements below this value may result in less accurate or precise outcomes.

Table 2
Theoretical LOD and LOQ Values

Concentration Cr (mM)	Absorbance	Yc	Yi-Yc^2
1	0.2665	0.2702	0.000014
2	0.2992	0.2947	0.000020
4	0.3427	0.3437	0.000001
6	0.3975	0.3927	0.000021
8	0.4363	0.4417	0.000033
10	0.4931	0.4907	0.000004
Total			0.000092
Sy/x			0.004806
Theoretical LOD			0.59

LOQ Confirmation Results

The calculated average analyte concentration is 4.6146 mM. The standard deviation (SD) of 0.6559 indicates a moderate level of data variation or deviation from the mean concentration. This variation is still within an acceptable range for quantitative analysis. The %RSD value of 14.21% indicates the precision level of the method. For good quantitative methods, %RSD is generally expected to be below 5–10%, but for complex analyses involving nanoparticles, values up to 15% are still acceptable. Regarding recovery values, the lowest recovery was 58.75% (in LOQ Replication 3, 3.4515 mM), while the highest recovery was 91.90% (in LOQ Replication 6, 5.3993 mM). Most replications fall within the 58.75–91.90% range, which is close to the ideal range (80–120%) for quantitative analytical methods. A %RSD value of 14.21% suggests that the method has moderate precision. This is acceptable, but additional care is needed to reduce data variation in repeated measurements. The average % recovery value indicates fairly good results, although some replications (such as LOQ replication 3) showed lower-than-ideal recovery values. This may be due to nanoparticle instability in detecting the analyte at certain concentrations. The measured concentration results show considerable variability within the range of 3.4515–5.3993 mM. This difference may be due to the sensitivity of nanoparticles, which can be influenced by environmental factors or measurement conditions.

Table 3

LOQ Measurement Confirmation Results

Confirmation LOQ			
Repetition	Absorbance	Concentration	%Recovery
1	0.2352	4.3032	73.25
2	0.2400	5.0204	85.45
3	0.2290	3.4515	58.75
4	0.2231	4.7840	81.43
5	0.1972	5.0693	86.29
6	0.1972	5.3993	91.90
7	0.2466	4.2747	72.76
Average			4.6146
SD			0.6559
%RSD			14.21

Selectivity Results of AuNPs Toward Cr (III) Ions

The purpose of this selectivity test is to determine whether metals other than chromium significantly affect the absorbance of AuNPs, which will be used as a colorimetric indicator for detecting chromium in wastewater samples. This selectivity test is also an important parameter because the test sample does not only contain the analyte to be detected but also many other analytes. For example, wastewater samples do not contain only chromium but may also include other metals.

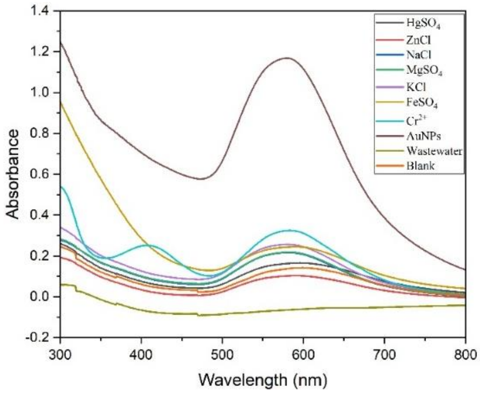

Fig. 3 – Selectivity Curve of AuNPs Toward Cr (III).

Figure 3 showed selectivity Curve of AuNPs Toward Cr (III). From Fig. 3 above, it can be observed that biosynthesized AuNPs are selective toward chromium. This is indicated by the shift in the absorbance peak of AuNPs when chromium ions are added. Additionally, the change in absorbance intensity between the solution containing chromium and the solution without chromium also indicates that AuNPs are selective toward chromium. The appearance of a new absorbance peak may also suggest the formation of a complex between AuNPs and chromium ions. Apart from the absorbance peak, the color change after the addition of chromium ions can also be a parameter to determine whether the synthesized AuNPs are selective toward chromium. AuNPs are selective for Cr (III) ions, as indicated by the colour change to grayish blue, same result is showed by Firdaus *et al.* (2023) and Zhang *et al.* (2020). This colour change occurs because trivalent metal ions can easily interfere with metal ions like Cr (III). Chromium ions can cause AuNP aggregation due to the interaction of citrate ions on the AuNPs, accompanied by intramolecular charge transfer. This is because the outer electron configuration of Cr (III) acts as a strong electron receptor to fill its half-empty d orbitals. Moreover, Cr ions have a higher effective charge, a smaller radius, and stronger coordination ability compared to other transition metal ions, making this method highly selective for chromium (Li *et al.*, 2017).

Figure 4 showed color of AuNP solution mixed with interfering metal ions. Each test tube contains AuNPs mixed with a specific metal ion solution at a concentration of 6 mM. The different solution colors indicate varying levels of interaction between AuNPs and the metal ions. In test tube (a) containing Cr ions, the solution remains a darker purple compared to the others, indicating a strong and specific interaction between AuNPs and Cr ions. Chromium ions interact with AuNPs through a redox reaction. Cr^{6+}, as a strong oxidizing agent, can cause more significant AuNP aggregation, which is visually observable through increased color intensity. In test tube (b), where $HgSO_4$ ions were added, the solution color is lighter than Cr, suggesting that Hg ions also interact with AuNPs, but not as strongly as Cr. In test tubes containing ZnCl (c) and $FeSO_4$ (g), the solution appears lighter, indicating minimal interaction. NaCl (D) produces the most stable purple color among all ions, showing that Na^+ and Cl^-, which are monovalent, have minimal effects on the structure and stability of AuNPs.

Next, the $MgSO_4$ (E) solution appears lighter than NaCl. This is because Mg^{2+} ions are divalent and tend to disrupt AuNP stability more than monovalent ions, causing partial aggregation of gold nanoparticles. Meanwhile, the KCl (F) solution appears lighter than NaCl but remains more stable than $MgSO_4$. The larger K^+ ions slightly affect the nanoparticle surface, slightly reducing AuNP stability. Based on this observation, the stability ranking of AuNPs against interfering ions is as follows: NaCl (highest stability) > KCl > $MgSO_4$ (lowest stability). This difference demonstrates that ion valency and size influence the degree of disturbance to gold nanoparticles. AuNPs are selective for Cr (III), as indicated by the color change to grayish blue. This occurs because trivalent metal ions can easily interfere with metal ions like Cr (III), confirming the AuNPs' selectivity toward Cr (III). This phenomenon can also be attributed to the interaction of capping agent groups that selectively bind Cr (III) ions (ionophore) (Barreto *et al.*, 2015; Fjordbøge *et al.*, 2019; Vivek and Burgess, 2008). Chromium ions induce AuNP aggregation due to interactions between citrate ions on AuNPs, accompanied by intramolecular charge transfer. The outer electron configuration of Cr^{3+} acts as a strong electron receptor to fill its half-empty d orbitals. Additionally, Cr ions have a higher effective charge, a smaller radius, and stronger coordination ability compared to other transition metal ions, further enhancing the selectivity of this method toward chromium (Chen *et al.*, 2014; Li *et al.*, 2017).

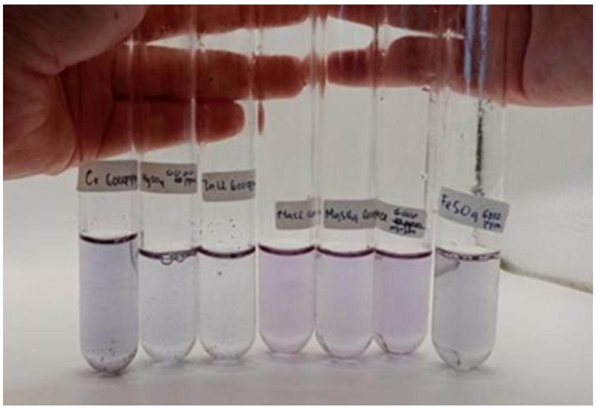

Fig. 4 – Color of AuNP Solution Mixed with Interfering Metal Ions (a) Cr, (b) $HgSO_4$, (c) ZnCl, (d) NaCl, (e) $MgSO_4$, (f) KCl, (g) $FeSO_4$ (from left to right).

Sensitivity Results of AuNPs Toward Cr (III)

A sensitivity test was conducted to evaluate the ability of gold nanoparticles (AuNPs) to detect chromium (Cr(III)) ions. Six different concentrations (1, 2, 4, 6, 8, and 10 mM) were utilized to assess the sensitivity of AuNPs in detecting Cr(III). As illustrated in Fig. 5, an increase in chromium concentration corresponds to a proportional increase in absorbance, indicating greater light absorption at higher concentrations. This observation aligns with Beer-Lambert's Law, which states that absorbance is directly proportional to the concentration of an analyte in solution. Notably, the curve does not exhibit saturation, suggesting that the sensing mechanism retains its responsiveness even at elevated concentrations. Additionally, visible color changes were observed in the Cr(III)-AuNP solutions, with increasing Cr(III) concentration resulting in progressively more intense coloration, ranging from 1 mM to 10 mM.

In the blank sample, the absorbance spectrum exhibited a primary peak at approximately 595 nm, characteristic of AuNPs undergoing Surface Plasmon Resonance (SPR) under normal conditions, in the absence of metal ion interactions. However, upon the introduction of Cr(III) ions, the absorbance spectrum underwent significant alterations. Two new peaks emerged consistently at 409 nm and 547 nm. The peak at 409 nm is likely associated with the formation of a Cr(III)-AuNP complex or redox interactions occurring within the system. Meanwhile, the peak at 547 nm suggests a shift from the original SPR peak at 595 nm toward a shorter wavelength (blue shift). This spectral shift indicates structural modifications or aggregation of AuNPs due to interactions with Cr(III) ions, further confirming the responsiveness of this nanoparticle-based sensing approach.

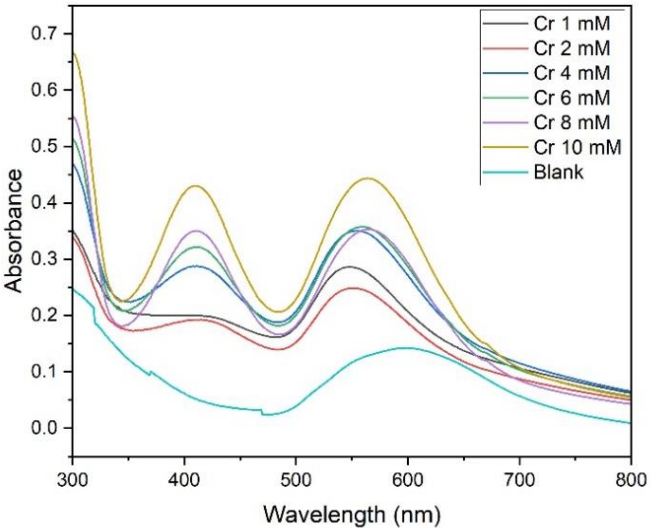

Fig. 5 – Sensitivity Curve of AuNPs Toward Cr (III).

4. Conclusion

The biosynthesis of gold nanoparticles (AuNPs) using *Averrhoa bilimbi* extract as an eco-friendly bioreductor was successfully carried out. The biosynthesis process produced nanoparticles with an average size of 60.3 ± 2.9 nm and a homogeneous particle size distribution (PDI 0.1105 ± 0.04). The synthesized nanoparticles exhibited high selectivity for Cr (III) ions, as indicated by a color change to grayish blue and the emergence of a new absorbance peak in UV-Vis spectrophotometry. The sensitivity of this method was also quite high, with the ability to detect chromium concentrations up to 10 mM and a linear relationship between chromium concentration and absorbance ($R^2 = 0.9975$). The values for linearity and selectivity demonstrate the potential of these gold nanoparticles as an accurate colorimetric indicator for chromium analysis in environmental samples.

REFERENCES

Akhdhar A., A *Novel, Simple, and Reliable Spectrophotometric Determination of Total Hexavalent Chromium by Complexation with a New Reagent of Thiazole Linked to 2H-Chromen-2-One*, International Journal of Analytical Chemistry, 2023(1), 2042221, doi: 10.1155/2023/2042221 (2023).

Algotiml R., Gab-Alla A., Seoudi R., Abulreesh H.H., Ahmad I., Elbanna K., *Anticancer and antimicrobial activity of red sea seaweeds extracts-mediated gold nanoparticles*, J Pure Appl Microbiol, **16**(1), 207-225, https://doi.org/10.22207/JPAM.16.1.11 (2022).

Alonso A.C., Jimenez G.C., Martinez L.L., Lopez G.E.R., Aguilar F.J.A., *A rapid, eco-friendly, and reliable microplate method for determination of Cr (VI)*, Turkish Journal of Chemistry, **41**(3), 420-425, https://doi.org/10.3906/kim-1609-53, (2017).

Bagheri A.R., Aramesh N., Hasnain M.S., Nayak A.K., Varma R.S., *Greener fabrication of metal nanoparticles using plant materials: A review*, Chemical Physics Impact, **7**, 100255, 10.1016/j.chphi.2023.100255 (2023).

Barreto Â., Luis L.G., Girão A.V., Trindade T., Soares A.M., Oliveira M., *Behavior of colloidal gold nanoparticles in different ionic strength media*, Journal of Nanoparticle Research, **17**, 493, 1-13, doi.org/10.1007/s11051-015-3302-0 (2015).

Belter M., Sajnó A., Barałkiewic D., *Over a century of detection and quantification capabilities in analytical chemistry–historical overview and trends*, Talanta, **129**, 606-616, https://doi.org/10.1016/j.talanta.2014.05.018 (2014).

Chen M., Cai H.-H., Yang F., Lin D., Yang P.-H., Cai J., *Highly sensitive detection of chromium (III) ions by resonance Rayleigh scattering enhanced by gold nanoparticles*, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 118, 776-781, https://doi.org/10.1016/j.saa.2013.09.058 (2014).

Chen W., Cao F., Zheng W., Tian Y., Xianyu Y., Xu P., Jiang X., *Detection of the nanomolar level of total Cr [(III) and (VI)] by functionalized gold nanoparticles* *and a smartphone with the assistance of theoretical calculation models*, Nanoscale, **7**(5), 2042-2049, doi.org/10.1039/C4NR06726F (2015a).

Chen Y., Dong Y., Wu H., Chen C., Chi Y., Chen G., *Electrochemiluminescence sensor for hexavalent chromium based on the graphene quantum dots/peroxodisulfate system*, Electrochimica Acta, **151**, 552-557, 10.1016/j.electacta.2014.11.068 (2015b).

Chumkong S., Sapyen W., Imyim A., Bhawawet N., *Green colorimetric detection of Chromium (VI) using deep eutectic solvent coated Nylon-Based device*, Microchemical Journal, **212**, 113266, doi.org/10.1016/j.microc.2025.113266 (2025).

Das B.K., Das P.K., Das B.P., Dash P., *Green technology to limit the effects of hexavalent chromium contaminated water bodies on public health and vegetation at industrial sites*, J Appl Biol Biotechnol, **9**(2), 28-35, 10.7324/JABB.2021.9203 (2021).

Dawra N., Dabas N., *Advances in spectrophotometric determination of Chromium (III) and Chromium (VI) in water: a review*, International Journal of Environmental Analytical Chemistry, **104**(13), 2994-3015, doi.org/10.1080/03067319.2022.2076224 (2024).

Dewi R.K., Firdaus M.L., Nursa'adah E., *Biosynthesis of Gold Nanoparticles Using Wuluh Star Fruit (Averrhoa bilimbi) for Chromium Analysis*, Hydrogen: Jurnal Kependidikan Kimia, **12**(1), 44-55, 10.33394/hjkk.v12i1.10188 (2024).

Firdaus M.L., Apriyoanda H., Isnan I., Wyantuti S., Eddy D.R., *Quantitative analysis of Cr (III) and Cr (VI) using gold nanoparticles with UV-vis spectrometry and smartphone colorimetric-sensing*, Iran. J. Chem. Chem. Eng., 42(3), 10.30492/ijcce.2022.545954.5087 (2023).

Fjordbøge A.S., Uthuppu B., Jakobsen M.H., Fischer S.V., Broholm M.M., *Mobility of electrostatically and sterically stabilized gold nanoparticles (AuNPs) in saturated porous media*, Environmental Science and Pollution Research, **26**(28), 29460-29472, doi: 10.1007/s11356-019-06132-8 (2019).

Hazarika U., Roy R.K., Bezboruah T., *An Accurate Sensing Technique for Estimation and Detection of Cr (III) in Water by Using Smartphone*, IEEE Sensors Letters, **7**(2), 1-4, 10.1109/LSENS.2023.3238513 (2023).

Hublikar L.V., Ganachari S.V., Patil V.B., *Phytofabrication of silver nanoparticles using Averrhoa bilimbi leaf extract for anticancer activity*, Nanoscale Advances, **5**(16), 4149-4157, https://doi.org/10.1039/D3NA00313B (2023).

Illyashenko-Raguin L., *Analysis of Biological Liquids by Metal Enhanced Fluorescence from Gold Nanoparticles,* In 2021 IEEE 26th International Seminar/Workshop on Direct and Inverse Problems of Electromagnetic and Acoustic Wave Theory (DIPED) (pp. 47-50), IEEE, doi: 10.1109/DIPED53165.2021.9552313 (2021).

Isaac R.R., Sakthivel G., Murthy C., *Green synthesis of gold and silver nanoparticles using Averrhoa bilimbi fruit extract*, Journal of Nanotechnology, 2013(1), 906592, https://doi.org/10.1155/2013/906592 (2013).

Isnaeni I., *Simulation study of size and aggregation effect on colorimetric spherical gold nanoparticles*, In AIP Conference Proceedings, 2382, 1, AIP Publishing, https://doi.org/10.1063/5.0059983 (2021).

Li T., Dai L., Huang Y., Pan S., Pang Z., Zou J., *Spectrophotometric determination of Cr (VI) in water using N, N-diethyl-p-phenylenediamine (DPD) as the indicator*, Journal of Environmental Chemical Engineering, **9**(5), 105517, https://doi.org/10.1016/j.jece.2021.105517 (2021).

Li S., Wei T., Ren G., Chai F., Wu H., Qu F., *Gold nanoparticles based colorimetric probe for Cr (III) and Cr (VI) detection*, Colloids and Surfaces A: Physicochemical and Engineering Aspects, **535**, 215-224, https://doi.org/10.1016/j.colsurfa.2017.09.028 (2017).

Liu Y., Huang L., Mahmud S., Liu H., *Gold nanoparticles biosynthesized using Ginkgo biloba leaf aqueous extract for the decolorization of azo-dyes and fluorescent detection of Cr (VI)*, Journal of Cluster Science, **31**(2), 549-560, https://doi.org/10.1007/s10876-019-01673-x (2020).

Man S., Zheng J., Zhang Y., Huang L., Wu X., *Selective sensing of hexavalent chromium in food grains using an electrodeposited bimetallic Au-Pd nanoparticles modified electrode*, Electroanalysis, **36**(10), e202400028, doi: 10.1002/elan.202400028 (2024).

Madhumitha G., Roopan S.M., *Devastated crops: multifunctional efficacy for the production of nanoparticles*, Journal of Nanomaterials, 2013(4), 951858, doi: 10.1155/2013/951858 (2013).

Michalski R., *Ion Chromatography Method for the Determination of Trace Levels of Chromium (VI) in Water*, Polish Journal of Environmental Studies, **13**(1) (2004).

Mohana Rangan S., Krajmalnik-Brown R., Delgado A.G., *An ion chromatography method for simultaneous quantification of chromate, arsenate, selenate, perchlorate, and other inorganic anions in environmental media*, Environmental Engineering Science, **38**(7), 626-634, doi: 10.1089/ees.2020.0347 (2021).

Nam S.H., Kim Y.N., *An Investigation on the Extraction and Quantitation of a Hexavalent Chromium in Acrylonitrile Butadiene Styrene Copolymer (ABS) and Printed Circuit Board (PCB) by Ion Chromatography Coupled with Inductively Coupled Plasma Atomic Emission Spectrometry*, Bulletin of the Korean Chemical Society, **33**(6), 1967-1971, doi:10.5012/BKCS.2012.33.6.1967 (2012).

Ngo V.K.T., Nguyen D.G., Huynh T.P., Lam Q.V., *A low cost technique for synthesis of gold nanoparticles using microwave heating and its application in signal amplification for detecting Escherichia Coli O157: H7 bacteria*, Advances in Natural Sciences: Nanoscience and Nanotechnology, **7**(3), 035016, doi 10.1088/2043-6262/7/3/035016 (2016).

Prajna N.D., Sinha R.K., *Shape-controlled Synthesis and Bulk Refractive Index Sensitivity Studies of Gold Nanoparticles for LSPR-based Sensing*, Plasmonics, 1-14, 10.1007/s11468-024-02387-1 (2024).

Ramanarayanan R., Bhabhina N., Dharsana M., Nivedita C., Sindhu S., *Green synthesis of zinc oxide nanoparticles using extract of Averrhoa bilimbi (L) and their photoelectrode applications*, Materials Today: Proceedings, **5**(8), 3, 16472-16477, https://doi.org/10.1016/j.matpr.2018.05.150 (2018).

Rani M., Shanker U., *Green nanomaterials: an overview*, Green functionalized nanomaterials for environmental applications, 43-80, https://doi.org/10.1016/B978-0-12-823137-1.00026-9 (2022).

Riyanto R., *Validasi & Verifikasi Metode Uji*: *Sesuai dengan ISO/IEC 17025*, Laboratorium Pengujian dan Kalibrasi (2020).

Saha J., Choudhuri S., Choudhuri D., *Effect of subchronic exposure to chromium on hematological and biochemical parameters of male albino rat*, Asian J Pharm Clin Res, **10**(5), 345-348, 10.22159/ajpcr.2017.v10i5.17468 (2017).

Sazakli E., *Human health effects of Oral exposure to chromium: A systematic review of the epidemiological evidence*, International Journal of Environmental Research and Public Health, **27**;21(4):406, doi: 10.3390/ijerph21040406 (2024).

Seol S.K., Kim D., Jung S., Hwu Y., *Microwave synthesis of gold nanoparticles: effect of applied microwave power and solution pH*, Materials Chemistry and Physics, 131(1-2), 331-335, https://doi.org/10.1016/j.matchemphys.2011.09.050 (2011).

Siddiqi K.S., Husen A., *Recent advances in plant-mediated engineered gold nanoparticles and their application in biological system*, Journal of Trace Elements in Medicine and Biology, **40**, 10-23, https://doi.org/10.1016/j.jtemb.2016.11.012 (2017).

Snow N.H., *Going Low: Understanding Limit of Detection in Gas Chromatography (GC)*, LCGC, **39**,5, 232-235 (2021).

Stozhko N.Y., Bukharinova M.A., Khamzina E.I., Tarasov A.V., Vidrevich M.B., Brainina K.Z., *The effect of the antioxidant activity of plant extracts on the properties of gold nanoparticles*, Nanomaterials, **9**(12), 1655, https://doi.org/10.3390/nano9121655 (2019).

Swathanthra P.A., Rao V., *Removal of chromium from aqueous solution using low cost adsorbents*, In Chemical and Bioprocess Engineering: Trends and Developments, pp. 321-329, eBook ISBN9780429159749, Apple Academic Press, (2015).

Tu J., Gan Y., Liang T., Wan H., Wang P., *A miniaturized electrochemical system for high sensitive determination of chromium (VI) by screen-printed carbon electrode with gold nanoparticles modification*, Sensors and Actuators B: Chemical, **272**, 582-588, https://doi.org/10.1016/j.snb.2018.06.006 (2018).

Tumolo M., Ancona V., De Paola D., Losacco D., Campanale C., Massarelli C., Uricchio V.F., *Chromium pollution in European water, sources, health risk, and remediation strategies: An overview*, International Journal of Environmental Research and Public Health, **17**(15), 5438, https://doi.org/10.3390/ijerph17155438 (2020).

Varma R.S., *Greener approach to nanomaterials and their sustainable applications.* Current Opinion in Chemical Engineering, **1**(2), 123-128, https://doi.org/10.1016/j.coche.2011.12.002 (2012).

Vivek J., Burgess I.J., *Insight into chloride induced aggregation of DMAP-monolayer protected gold nanoparticles using the thermodynamics of ideally polarized electrodes*, The Journal of Physical Chemistry C, **112**(8), 2872-2880 (2008).

Zhang Z., Ye X., Liu Q., Liu Y., Liu R., *Colorimetric detection of* Cr^{3+} *based on gold nanoparticles functionalized with 4-mercaptobenzoic acid*, Journal of Analytical Science and Technology, **11(10)**, 1-7, doi.org/10.1186/s40543-020-00209-7 (2020).

BIOSINTEZA NANOPARTICULELOR DE AUR FOLOSIND *AVERRHOA BILIMBI* PENTRU DETECTAREA CROMULUI ÎN APE UZATE

(Rezumat)

Metalul greu crom (Cr) este una dintre substanțele care pot cauza poluarea apei. Deși cromul este extrem de util în diverse aspecte ale vieții, inclusiv în sectorul industrial, utilizarea sa excesivă generează, de asemenea, deșeuri care au potențialul de a deteriora mediul și de a reprezenta riscuri pentru sănătatea umană. Analiza prezenței cromului (Cr) în apă a fost realizată utilizând metoda biosintezei nanoparticulelor de aur cu *Averrhoa bilimbi* ca bioreductor. Cromul, fiind unul dintre metalele grele, are multe aplicații industriale; cu toate acestea, utilizarea sa excesivă poate polua mediul și poate pune în pericol sănătatea umană. Metoda folosită în acest studiu este colorimetria, cunoscută pentru a fi simplă, eficientă, economică și precisă. Rezultatele analizei au arătat că nanoparticulele de aur sintetizate din *Averrhoa bilimbi* pot detecta cromul la o concentrație de 10 mM în condiții optime de pH 5, temperatură de 75°C și timp de incubare de 5 minute. Nanoparticulele de aur sintetizate s-au dovedit a fi selective și sensibile în detectarea prezenței cromului, oferind astfel o soluție potențială pentru monitorizarea poluării cu crom în mediile acvatice..