



# Biosynthesis of Silver Nanoparticles from *Kepundung* Fruit Peel (*Baccauera Racemose*) and Their Application in Mercury Detection Using Digital Image Colorimetric Methods

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## Abstract

Mercury is a dangerous and toxic metal, thus necessitating an analytical method to ascertain its presence. Traditional methods for mercury analysis often involve costly instruments and specialized expertise. However, the Digital Image Colorimetry (DIC) method is an alternative for mercury detection due to its speed, simplicity, and cost-effectiveness. This research aims to synthesize silver nanoparticles (AgNPs) using *Kepundung* fruit peel (*Baccauerea racemosa*) extract as a mercury detector. *Baccauerea racemosa* has secondary metabolite compounds that can act as natural reducing agents (bioreductors) in synthesizing silver into nanoparticles. AgNPs were optimized and characterized using UV-Vis spectrophotometer, FTIR, PSA, and XRD instruments. Mercury detection was explained using RGB Detector and ImageJ. The research results revealed that the optimal conditions for synthesizing AgNPs involved a concentration of 1% extract and a pH of 7. The properties of the AgNPs included a maximum absorption at wavelengths of 400-450 nm, an average particle size of 122.7 nm, a face-centered cubic crystal structure, and characteristic functional groups at wavenumbers of 3453  $cm^{-1}$ , 1700-1600  $cm^{-1}$ , and 1445  $cm^{-1}$ . These spectral features suggested the presence of phytochemical compounds serving as bioreductants. Optimal results for mercury detection were achieved using Whatman paper no. 41 at pH 7. Mercury was detected successfully, whereas  $Pb^{2+}$ ,  $Na^+$ ,  $Mg^{2+}$ ,  $K^+$ ,  $Cu^{2+}$ ,  $Ca^{2+}$ ,  $Co^{2+}$ ,  $Zn^{2+}$ , and  $Fe^{3+}$  ions were not detected. The validation test obtained a LoD of 0.099 ppm, a LoQ of 0.330 ppm, and a coefficient value ( $R^2$ ) of 0.997, indicating good measurement linearity. Further research can be developed to increase the sensitivity of mercury detection with lower concentrations and extend its application to environmental samples.

## 1. Introduction

Mercury, classified as a heavy metal element, poses significant toxicity risks to human health. Elevated levels of mercury in the body, surpassing the tolerable threshold ( $>0.01$  ppm), can result in severe damage to the nervous system, digestive tract, and respiratory system [1, 2]. To assess the mercury levels in items like food, beverages, and beauty products, advanced analytical instruments like CV-AAS (Cold Vapor-Atomic Absorption

Spectrometry) and ICP-MS (Inductive Coupled Plasma Mass Spectrometry) are typically utilized. While offering remarkable accuracy, these methods are costly and demand specialized expertise, making mercury detection challenging for the average individual. An alternative analytical method is colorimetry, which presents several advantages. It is cost-effective, user-friendly, operates based on optical principles, and offers reliable detection of mercury presence [3].

Colorimetry is a chemical analysis method based on changes in color intensity. Typically, colorimetry involves using a UV-Vis spectrophotometer, necessitating time and financial resources for preparing mercury samples. An alternative approach is leveraging smartphones as imaging devices for color analysis. The Digital Image Colorimetry (DIC) method employs paper-based preparations, offering benefits such as reduced preparation time, cost-effectiveness, and the capacity to conduct numerous tests compared to traditional colorimetric methods [4].

The DIC method can analyze metal levels from environmental samples. The Red Green Blue (RGB) DIC model was able to measure arsenic metal levels in water and soil contaminant samples using gold nanoparticles (AuNPs) [5]. The utilization of gold particles can be relatively costly, making them unsuitable for analyses requiring affordability. On the other hand, employing AgNPs has proven effective in detecting mercury using the RGB DIC model, yielding selective results. This method demonstrates a detection limit of 10 µg/L and achieves a recovery percentage ranging from 92.5% to 96.0% [6]. However, the AgNPs produced are still synthesized using a commercial reductant (NaBH<sub>4</sub>), which is quite expensive and toxic. Therefore, further research is needed to synthesize AgNPs using natural bioreductants and explore their potential application in DIC for mercury detection.

Utilizing AgNPs in the biosynthesis process offers a sustainable approach to environmental protection compared to commercial reductants, which tend to be more toxic [7]. Bioreductants can reduce silver to nano size, increase stability, and exhibit good antibacterial activity. Bioreductant sources are obtained from plant extracts containing ascorbic acid, flavonoids, alkaloids, and other phytochemical compounds [8].

*Kepundung* fruit peel is one of the natural materials that has not been explored as a bioreductor in the synthesis of AgNPs. Menteng (*Baccaurea racemosa*), or in Lombok called *Kepundung*, is a fruit native to Indonesia with a sour and sweet taste. The ethanol extract of *Kepundung* fruit peel contains flavonoids, phenols, and terpenoids [9]. These phytochemical compounds have the potential to be used as bioreductors in the synthesis of AgNPs. Based on these problems, this study aims to investigate and optimize the potential of *Kepundung* fruit peel waste extract as a bioreductor for synthesizing AgNPs, while also evaluating the utilization of AgNPs in mercury detection through digital image colorimetry using the RGB model.

## 2. Experimental

### 2.1. Tools

Analytical detector equipment, such as smartphones: iPhone 13 (12 MP f/1.6), Oppo A12 (13 MP f/2.2), Redmi 10 (50 MP f/1.8), Mini Photo Box. RGB Color Detector software for color analysis, ImageJ, and Origin 8.5.1. Analytical instruments: UV-Vis Spectrophotometer (Orion Aquamate), Fourier-transform infrared spectroscopy (FTIR, PerkinElmer), particle size analyzer

(PSA, Microtrac PSA), X-ray diffraction (XRD, Rigaku Miniplex 600), oven (Memert UN110), centrifugator (Gemmy Table Top Centrifuge PLC-05), furnace (Thermolyne), and rotary evaporator (B-one RE-1000 VN).

### 2.2. Materials

Samples of mature *Kepundung* (*Baccaurea racemose*) were collected from Praya, Central Lombok, Indonesia. Chemicals utilized include: AgNO<sub>3</sub> (Merck), HgCl<sub>2</sub> (Merck), Pb(NO<sub>3</sub>)<sub>2</sub> (Merck), MgSO<sub>4</sub> (Merck), ZnCl<sub>2</sub> (Merck), CaCl<sub>2</sub> (Ajax Chemical), FeCl<sub>3</sub> (Ajax Chemical), KNO<sub>3</sub> (Ajax Chemical), NaNO<sub>3</sub> (Merck), CoCl<sub>2</sub> (Merck), CuSO<sub>4</sub> (Merck), PVP (Merck), NaOH (Merck), concentrated HCl (Merck), dan ethanol 70% (Merck). Whatman filter papers (No. 1, 41, and 42) (Cytiva) were used as a deposit medium for silver nanoparticles in the DIC method.

### 2.3. Procedures

#### 2.3.1. *Kepundung* Fruit Peel Extraction

One kg of ripe *Kepundung* fruit was peeled and then dried at 50°C in the oven. Dried samples were ground and filtered (50 mesh; 0.335 mm). The fine samples were macerated with 500 mL ethanol for 3×24 hours. The resulting extract was filtered and thickened using a rotary evaporator at a temperature of 50°C at 70 rpm. The thick extract was measured and then stored in a closed room. The resulting extract was tested to identify phytochemical compounds.

#### 2.3.2. Synthesis and Optimization of AgNPs

One mL of 1% PVP as a stabilizer was added to the 20 mL silver precursor AgNO<sub>3</sub> 1000 ppm. Synthesis was optimized based on the concentration of 1000 ppm AgNO<sub>3</sub> with 1%, 2%, and 3% w/v (1:1, 1:2, 1:3) extract and pH 3–11 conditions. pH conditions were adjusted by adding 0.1 M NaOH or 0.1 M HCl. The reaction was conducted for 24 hours, and the formation of AgNPs was characterized by a color change from yellow to brownish red.

#### 2.3.3. Characterization

AgNPs were analyzed using UV-Vis at a 200–800 nm wavelength range. Particle size and distribution were characterized using PSA. Functional compound groups in the extract and AgNPs were identified using FTIR with KBr pellets at different wavelengths 4000 cm<sup>-1</sup>–400 cm<sup>-1</sup>. The diffraction pattern of AgNPs was analyzed using XRD in the 2θ range from 20° to 80°. The results of the instrument analyses were compared with standard data obtained from the literature.

#### 2.3.4. Optimization of AgNPs Deposits

The optimized AgNPs were deposited on Whatman papers no. 1, 41, and 42 and HVS paper with a diameter of 1 cm. Optimization was carried out by dripping 10 ppm Hg<sup>2+</sup> solution on the detector, then photographed in a mini photo box. Various types of smartphones were used to determine the influence of the smartphone type on differences in average RGB values. The best optimization of the deposited media was further optimized at pH conditions of 5, 7, 9, and 11.

**Table 1.** Phytochemical test results of *Kepundung* fruit peel extract

Phytochemical Compound	Positive [10]	Extract
Flavonoids	Red solution	+
Tannin	Deep green or deep blue solution White precipitate (Meyer’s reagent)	+
Alkaloids	Brown precipitate (Dragendroff’s reagent) Brown or orange precipitate (Wagner Reagent)	+
Steroids	Greenish solution and blue rings	-
Terpenoids	Brownish solution and red rings	+
Saponins	Stable foam for 10 minutes	-

**2.3.5. Selectivity Test**

The selectivity of the conditions yielding the best deposit optimization results was tested with several metal ions: Cu<sup>2+</sup>, Na<sup>+</sup>, Co<sup>2+</sup>, K<sup>+</sup>, Fe<sup>3+</sup>, Ca<sup>2+</sup>, Zn<sup>2+</sup>, Mg<sup>2+</sup>, Pb<sup>2+</sup>, and Hg<sup>2+</sup> at a concentration of 10 ppm. Color changes on the detector were observed, and the mean RGB value was analyzed for each metal ions addition. The presence of mercury was indicated by the color shift from brownish yellow AgNPs to white, with the mean RGB value reaching its maximum of 255, thus demonstrating a perfect white color. Additionally, analysis of the solution preparation involved the addition of 1 mL of each metal ions solution, followed by analysis using a UV-Vis spectrophotometer.

**2.3.6. Validity Test**

Validity tests were carried out to determine the linear range of mercury concentration and the detection limit (LoD) and quantification limit (LoQ). Mercury concentrations ranging from 0.1 ppm to 2 ppm were utilized. The validation results were plotted on a graph depicting the mean RGB value against Hg<sup>2+</sup> concentration.

**3. Results and Discussion**

**3.1. *Kepundung* Fruit Peel Extract**

The extract yield was 20.23%. The phytochemical results in Table 1 indicate that the extract contains flavonoids, terpenoids, tannins, and alkaloids, consistent with findings from previous research [11]. These compounds serve as both reducing and stabilizing agents in synthesizing AgNPs. The reducing agent donates electrons to Ag ions, facilitating their oxidation within phytochemical compounds and reduction into nanoscale atomic-sized silver.

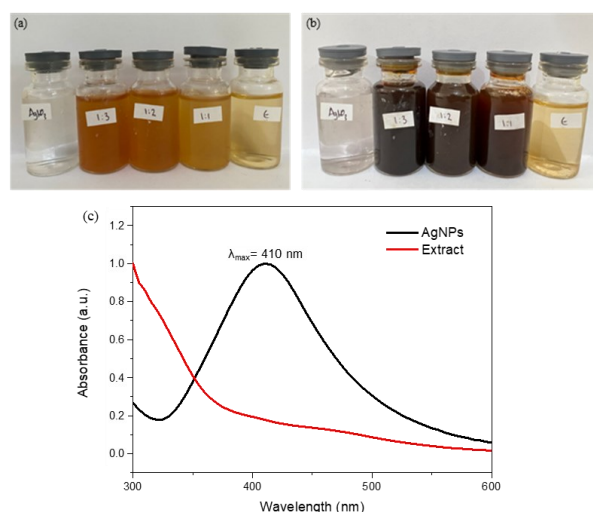
**3.2. Synthesis of Silver Nanoparticles**

AgNPs were synthesized using 1000 ppm AgNO<sub>3</sub> precursor with 1%, 2%, and 3% extract. Figures 1a and 1b show that the color of AgNO<sub>3</sub> changed from clear to reddish-brown after adding the extract and reacting for 24 hours. This color change occurs due to silver ions Ag<sup>+</sup> reduced to Ag<sup>0</sup> by phytochemical compounds originating from secondary metabolites of the extract [12]. The reaction runs spontaneously without heating treatment or additional energy because the reduction potential of phenolic compounds was smaller (0.33 V) compared to

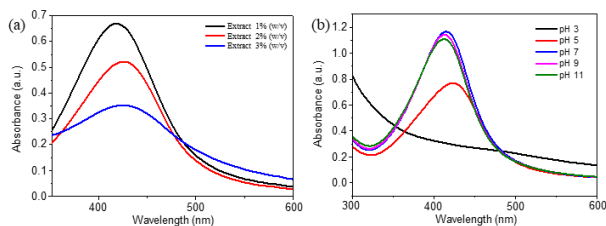
the reduction potential Ag<sup>+</sup> (0.80 V) [13]. AgNPs were formed when an absorption peak occurs in the range of 400–450 nm [14], as in Figure 1c.

Optimizing the synthesis of AgNPs aims to determine their stability under different concentrations and pH levels. Figure 2a shows that the optimal concentration for the extract was found to be 1%, surpassing the results obtained at 2% and 3% concentrations. This result aligns with previous research on *Phoenix dactylifera* L. leaf extract, which also demonstrated enhanced stability at lower concentrations [15]. In general, the higher the concentration of the extract, the higher the absorbance of silver nanoparticles due to the increased reaction speed by the bioreductor compounds from the extract.

However, in some cases, higher extract concentrations cause agglomeration and reduce absorbance. Agglomeration increases the particle size, resulting in a lack of nanoparticle formation, thereby reducing the uptake of AgNPs [16]. The pH conditions are shown in Figure 2b, where pH tends to be neutral and alkaline, which has higher nanoparticle stability because -OH ions can prevent particle aggregation, which causes the particle size to increase [17].



**Figure 1.** Synthesis of AgNPs (a) before 24 hours, (b) after 24 hours, and (c) absorbance of AgNPs after 24 hours and ethanol extract of *Kepundung* peel



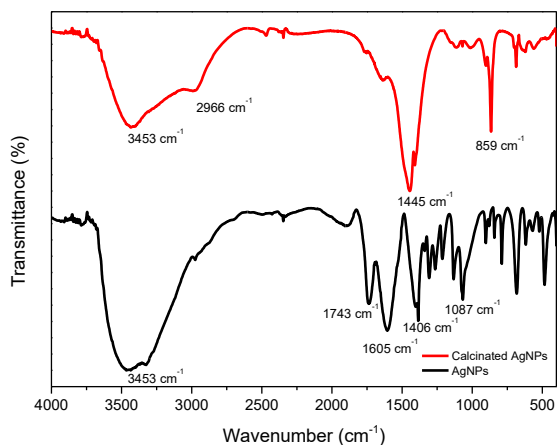
**Figure 2.** Optimization of the UV-Vis spectra of AgNPs at variations in (a) extract concentration and (b) pH at a concentration of 1%

### 3.3. Characterization of AgNPs

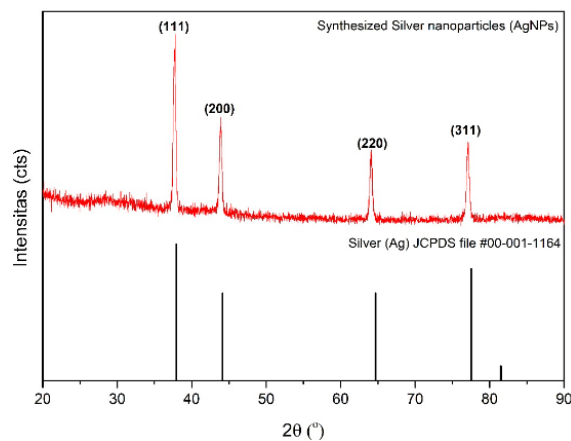
The FTIR spectra analysis of the synthesized AgNPs aims to identify specific functional groups. Prior to analysis, the AgNPs underwent two treatments: drying and calcination. These treatments aimed to discern the presence of functional groups within the secondary metabolites of the extract. In Figure 3, the absorption peak at  $3453\text{ cm}^{-1}$  indicates the vibration of alcohol or carboxylic acid molecules (O-H). Additionally, the wave number range of  $1700\text{--}1600\text{ cm}^{-1}$  signifies the presence of carbonyl groups (C=O) and aromatic carbon (C=C), which are characteristic of flavonoid, alkaloid, and tannin compounds [18].

Moreover, calcination diminishes certain absorption peaks, particularly in the range of  $1700\text{--}1600\text{ cm}^{-1}$  and in the fingerprint area below  $1500\text{ cm}^{-1}$ . This observation suggests that certain phytochemical compounds are lost during high-temperature heating. Consequently, it indicates that the extract initially contained phytochemical compounds capable of reducing  $\text{Ag}^+$  ions to  $\text{Ag}^0$  [19].

XRD diffractogram of the synthesized AgNPs is shown in Figure 4. The diffraction patterns confirm the formation of silver nanoparticle crystals with a face-centered cubic (FCC) structure, consistent with the standard JCPDS cubic crystal system no. 00-001-1164 [20]. Specifically, the diffraction peaks occur at  $2\theta$  angles of  $37.934^\circ$ ,  $44.142^\circ$ ,  $64.678^\circ$ , and  $77.549^\circ$ , corresponding to the crystal planes (111), (200), (220), and (311), respectively. These patterns signify the presence of crystalline silver [21].



**Figure 3.** FTIR spectra of AgNPs

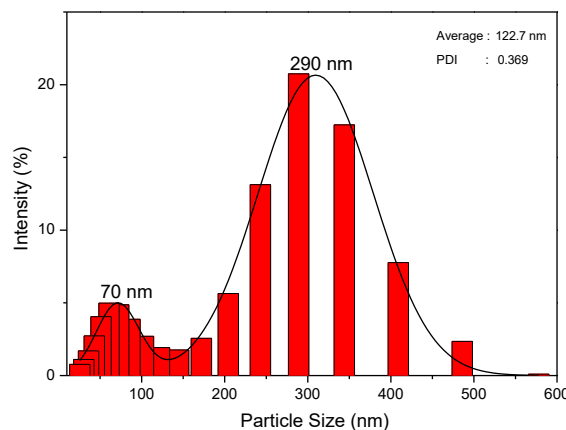


**Figure 4.** Diffraction pattern of synthesized AgNPs and comparison with the Ag theoretical database

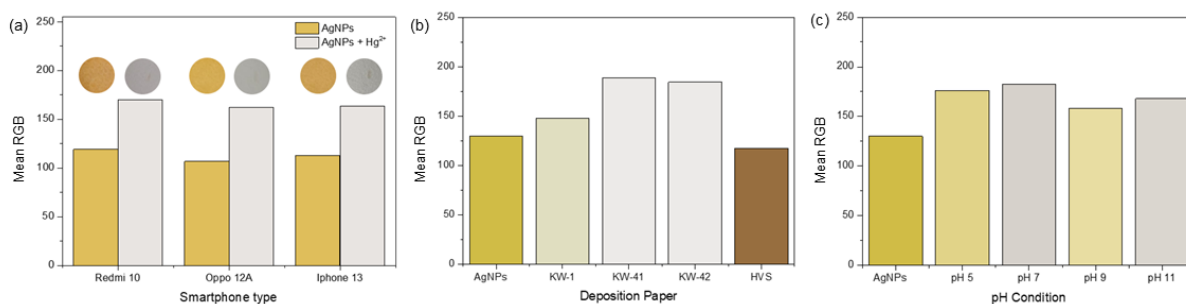
The average size and distribution of AgNP particles resulting from the optimal optimization conditions were characterized using PSA. The obtained average particle size of 122.7 nm (Figure 5) confirms the successful formation of nanoparticles. Additionally, the Particle Distribution Index (PDI) value of the synthesized AgNPs indicates that the particles are distributed uniformly or tend toward uniform size, as evidenced by a PDI value below 0.5 [22]. The appearance of two peaks in the particle distribution is attributed to the agglomeration of AgNPs, ranging from sizes  $<100\text{ nm}$  (around 60 nm) to particles  $>100\text{ nm}$  (around 290 nm). This agglomeration results from inappropriate solvents during the analysis process, which induces particle clustering [23].

### 3.4. Optimization of AgNPs Detector

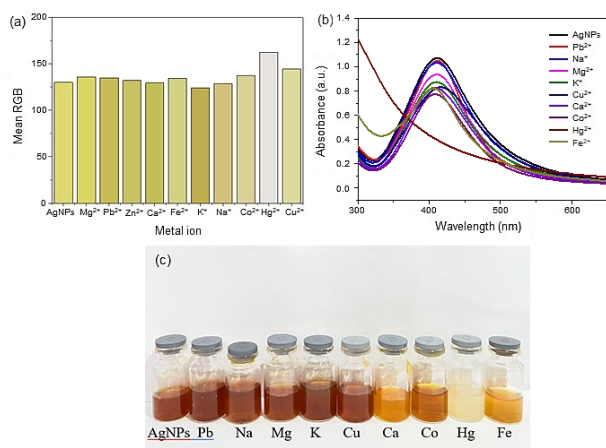
The results of mercury detection utilizing AgNPs, deposited on Whatman paper and exposed to  $\text{Hg}^{2+}$  at 10 ppm, revealed a transition in color from brownish yellow to white. The colors were analyzed using digital images captured with various smartphones. Figure 6a illustrates the variation in digital image outcomes from different smartphone models aimed at identifying potential discrepancies in color readings for the detection results. Analysis of the mean RGB values via the F-test yielded a significance value ( $>0.05$ ), indicating a lack of substantial difference in the mercury detection results.



**Figure 5.** Size distribution of AgNPs particles from optimization results

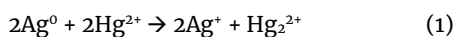


**Figure 6.** Optimization of mercury detection with AgNPs on Whatman paper (a) differences between smartphones as digital imaging devices, (b) differences in types of Whatman paper, and (c) differences in pH of AgNPs

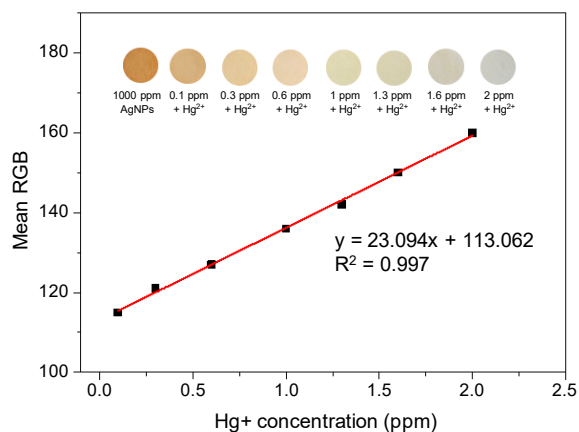


**Figure 7.** Selectivity test of AgNPs on the addition of various metals through (a) digital image, (b) absorbance of the solution preparation, and (c) change in color of the solution

The optimization of mercury detection aims to identify the most sensitive method characterized by a high mean RGB value. A higher mean RGB value means greater sensitivity of AgNPs to Hg<sup>2+</sup>. As depicted in Figure 6b, all types of Whatman paper exhibit an increase in the mean RGB value, whereas HVS paper shows the opposite trend. Variations in the mean RGB value among Whatman paper types are attributed to differences in pore size, particle retention, and surface smoothness. Therefore, optimization is essential to select the most suitable medium [24]. Whatman paper no. 41 emerged as the optimal detection medium due to its high mean RGB value, and thus, it would be utilized in subsequent mercury analyses. The maximum mean RGB value of 255 signifies a perfect white color, indicating the completion of the silver–mercury redox reaction from brownish yellow to white [25].



The subsequent optimization focuses on varying the pH of AgNPs. The results indicate that neutral conditions (pH 7) yield the highest mean RGB values (Figure 6c). This phenomenon occurs because AgNPs nanoparticles exhibit greater stability under neutral to basic conditions, thereby mitigating agglomeration, which could otherwise reduce the surface area of AgNPs. Enhanced stability of AgNPs results in a larger surface area available for interaction, rendering the AgNPs' response to mercury more sensitive [26].



**Figure 8.** Standard calibration curve for mercury using AgNPs at Hg<sup>2+</sup> concentrations ranging from 0 to 2 ppm

### 3.5. Selectivity Test

The selectivity test for mercury detection (Figure 7) reveals that the color transition from yellow (AgNPs) to white occurred visibly in the presence of Hg<sup>2+</sup> ions. No color change was observed with other ions, such as Pb<sup>2+</sup>, Na<sup>+</sup>, Mg<sup>2+</sup>, K<sup>+</sup>, Cu<sup>2+</sup>, Ca<sup>2+</sup>, Co<sup>2+</sup>, Zn<sup>2+</sup>, and Fe<sup>3+</sup>. This indicates that AgNPs exhibit selectivity in detecting mercury while remaining unresponsive to other metals. The distinct reactivity towards mercury is attributed to differences in potential reduction, whereby silver particles specifically interact with mercury through a reducing–oxidizing mechanism. The redox reaction between silver and mercury is represented in Equation (1) [27].

Certain transition metals, alkali metals, and alkaline earth metals possess a lower reduction potential than Ag<sup>+</sup>, making them incapable of oxidizing Ag<sup>0</sup> [28]. The selectivity of AgNPs is also supported in solution preparations, as in Figures 7b and 7c. Absorbance results indicate that only Hg<sup>2+</sup> was added, leading to a noticeable decrease in absorbance and visible changes in the solution color perceptible to the naked eye.

### 3.6. Validation Test

The validity test is conducted on different mercury concentrations ranging from 0.1 ppm to 2 ppm, aiming to determine the values of R, LoD, and LoQ. Figure 8 illustrates that the mean RGB value rises with increasing concentrations of mercury added to the silver nanoparticle solution. Concurrently, the color of the AgNPs fades with higher Hg<sup>2+</sup> concentrations [6].

Detection of mercury within the 0–2 ppm range yields a LoD of 0.099 ppm and a LoQ of 0.330 ppm. Additionally, the  $R^2$  of 0.997 indicates excellent measurement linearity, suggesting promising analytical potential for real sample analysis.

#### 4. Conclusion

The DIC method, employing AgNPs synthesized from *Baccaurea racemosa* fruit peel extract (*Baccaurea racemosa*), has demonstrated successful and selective detection of mercury with high sensitivity. The AgNPs synthesis was conducted using 1000 ppm  $\text{AgNO}_3$  and 1% extract under pH 7 conditions for 24 hours at room temperature. Optimized AgNPs exhibited characteristics of monodisperse particles with an average size of 122.7 nm, displaying maximum absorption at 400–450 nm, possessing a face-centered cubic crystal structure, and featuring functional groups at  $3453\text{ cm}^{-1}$ ,  $1700\text{--}1600\text{ cm}^{-1}$ , and  $<1500\text{ cm}^{-1}$ , indicating the presence of phytochemical compounds acting as bioreductors. Mercury detection utilizing the DIC principle yielded the highest mean RGB value on Whatman paper No. 41 with AgNPs at pH 7. The AgNPs detector effectively emitted radiation on mercury metal, evidenced by the color change from brownish yellow to white, while no response was observed with  $\text{Pb}^{2+}$ ,  $\text{Na}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ ,  $\text{Cu}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Fe}^{3+}$ . The AgNPs detector derived from *Baccaurea racemosa* peel extract exhibits sensitivity in detecting mercury within the range of 0–2 ppm, with a LoD of 0.099 ppm and a LoQ of 0.330 ppm. Future research may explore enhancing the sensitivity of mercury detection at lower concentrations and its application to mercury-containing samples.

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