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# Statistical Validation of The Microplate Reader for Antioxidant Activity Measurement using The DPPH Assay

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**Abstract:** Antioxidants research has been garnering increased interest among researchers, particularly in the fields of medicine and health, focusing on both natural and synthetic antioxidants. The DPPH method is the most used approach for antioxidant analysis due to its efficiency, simplicity, and accuracy. This study aims to validate the sensitivity of the microplate reader compared to UV-Vis spectrophotometer, both used for measuring absorbance in the DPPH antioxidant test. The samples used in this study include ascorbic acid, gallic acid, and quercetin standards. Statistical validation in the DPPH antioxidant test includes precision testing, T-test, and % recovery test. Based on the statistically analyzed results, the T-test values for ascorbic acid, gallic acid, and quercetin standards were 0.86, 1.52 and 0.20, respectively, all of which are less than the t-table value of 1.72. The T-test values being less than the t-table value indicates that there is no significant difference between the two methods, UV-Vis spectrophotometer and microplate reader. Meanwhile, the precision test (Horrat)r values for the two methods were 0.52, 0.33, and 0.34, respectively. These precision values (Horrat)r fall within the 0.3-1.5 range, indicating acceptable precision. Additionally, the % recovery test for gallic acid showed values in the 90-100% range, indicating that both methods possess good sensitivity. As a result, a microplate reader is equally reliable yet more practical (faster, high-throughput, smaller volumes), especially valuable for labs with limited resources.

**Keywords:** antioxidant, DPPH, T test, (Horrat)r value, % recovery test

***Abstrak:*** *Penelitian antioksidan semakin menarik minat bagi para peneliti. Khususnya di bidang kedokteran dan kesehatan, baik antioksidan yang berasal dari alam maupun hasil sintesis. Metode umum yang paling banyak digunakan untuk analisis antioksidan adalah metode DPPH karena efisien, sederhana, dan akurat. Penelitian ini bertujuan untuk membandingkan sensitivitas dua metode deteksi yaitu spektrofotometer UV-Vis dan microplate reader yang digunakan untuk pengukuran absorbansi dalam uji antioksidan metode DPPH. Sampel yang digunakan adalah standar asam askorbat, asam galat, dan kuersetin. Pengujian sensitivitas dilakukan dalam uji antioksidan DPPH meliputi uji presisi, uji T, dan uji % recovery. Berdasarkan hasil penelitian yang dianalisis secara statistik diperoleh nilai uji T untuk masing-masing standar asam askorbat, asam galat, dan kuersetin: 0,862; 1,524; dan 0,209 yang mempunyai nilai < nilai t tabel 1,728. T hitung < nilai t tabel menunjukkan bahwa kedua metode yaitu spektrofotometer UV-Vis dan microplate reader tidak terdapat perbedaan signifikan. Sedangkan hasil uji presisi (Horrat)r dari kedua metode tersebut diperoleh nilai 0,528; 0,333; dan 0,349. Nilai presisi tersebut (Horrat)r berada pada 0,3-1,5 dimana nilai tersebut masuk ke dalam keberterimaan presisi. Sedangkan untuk uji % recovery terhadap asam galat diperoleh nilai pada rentang 90-100% yang menunjukkan kedua metode memiliki sensitivitas yang baik.*

***Kata kunci:*** *antioksidan, DPPH, uji T, uji % recovery.*

## INTRODUCTION

Research on antioxidants has increasingly attracted the interest of researchers, both from natural and synthetic antioxidant sources (Abdalla & Roozen 1999; Shahidi & Ambigaipalan 2015). Antioxidant activity is widely used in food preservation and health practices (medicine) because antioxidants can eliminate reactive oxygen species, inhibit oxidation processes, and extend the shelf life of preserved foods (Carocho *et al*. 2018). The most popular method for analyzing antioxidant activity is the DPPH (1,1-Diphenyl-2-picrylhydrazyl) method (Brand-Williams *et al*. 1995; Hidayat *et al*. 2017; Silva *et al*. 2024). The DPPH method provides information on the reactivity of compounds in a simple and straightforward manner. DPPH is a stable

radical with the primary principle of showing strong absorption at a wavelength of 510 nm with a dark violet color. When a DPPH solution reacts with a compound that can donate a hydrogen atom, non-radical DPPH is formed. This can be observed through a decrease in color intensity and wavelength (Molyneux 2004; Sochor *et al*. 2010). The reduction in color intensity from purple to yellow can be measured with a spectrophotometer (Brand-Williams *et al*. 1995). One of the parameters introduced to interpret the results of antioxidant activity using the DPPH method is $IC_{50}$ (Inhibitory Concentration), which is the concentration of substrate that causes a 50% reduction in DPPH activity (Wijaya *et al*. 2023). The DPPH method is often used to measure the radical-scavenging ability of a compound because it provides accurate, reliable, and practical results. Additionally, this method is simple, fast, sensitive, and requires only a small sample size (Anton *et al*. 2021; Sánchez-Moreno 2002; Wenas *et al*. 2022).

A spectrophotometer is a commonly used instrument for measuring absorbance in the DPPH antioxidant activity test. However, the small sample size can sometimes be a limitation when testing DPPH antioxidant activity with a spectrophotometer, as the samples often come from pure compounds, extracts, or other biological sources with undetermined amounts. Antioxidant improvements in methods for determining the efficiency of antioxidant compounds are needed (Cantika & Priani 2023; Sánchez-Moreno 2002; Syamsu & Rachman 2023; Utami *et al*. 2023). With the advancement of science and technology, the use of microplate readers has emerged as an alternative to the UV-Vis spectrophotometer. Researchers like Hidayat *et al*. (2017) have used microplate readers to measure absorbance in DPPH antioxidant activity.

A microplate reader is a specialized spectrophotometer based on photodiode technology that can measure absorbance and directly read microplates / 99 micro-wells The principle of absorbance measurement with a (Obayashi *et al*. 2017; Tecan 2014). microplate reader is like that of conventional spectrophotometry, where the amount of transmitted light is proportional to the concentration of the target molecule (Botasini *et al*. 2010; Heredia *et al*. 2006). Absorbance measurement with a microplate reader involves measuring the amount of light absorbed as it passes through the wells on the microplate. The light source from the selected wavelength illuminates the sample, while a detector measures the amount of light from the opposite side of the well. The main difference between a microplate reader and a UV-Vis spectrophotometer lies in the wavelength range; the microplate reader has filters or diffraction gratings within a range of 400-700 nm. Additionally, the microplate reader's ability to read 99 wells with smaller volumes is a keys reason researchers are beginning to use microplate readers in DPPH antioxidant activity testing (Jakubczyk *et al*. 2020; Schaefer *et al*. 2016).

Based on the background above, the author conducted a statistical validation of the microplate reader by comparing it with UV-Vis spectrophotometry in the DPPH antioxidant assay. Ascorbic acid, gallic acid, and quercetin standards were used as test samples due to each compound having strong antioxidant activity with different classes measured at a wavelength of 510 nm as classified in Table 1. The $IC_{50}$ values will be determined from both tests, along with the % recovery and T-test values. To the best of the author's knowledge, this is the first study to perform a statistical validation comparing both methods.

## MATERIALS AND METHOD

### The Instruments and Materilas

The chemicals used in this study include aquabides (IKA), gallic acid (Merck), ascorbic acid (Merck), quercetin (Sigma), DPPH (Wako), and methanol (Merck). The instruments and glassware include an analytical balance, a Shimadzu UV-1800 spectrophotometer, an EZ Read 400 Biochrom microplate reader, a 99-well microplate, Eppendorf micropipettes and pipette tips with volumes of 100-1000, 20-200, and 0.1-10 µL, a pair of quartz cuvettes with a capacity of 3.5-1.7 mL, volumetric flasks (10, 25, and 100 mL), graduated cylinders, beakers, and other standard laboratory glassware.

### Methods

#### Preparation of 0.4 mM DPPH solution

A total of 4 mg of DPPH solid was weighed and placed into a 25 mL volumetric flask. Methanol was added gradually while gently shaking until the DPPH

**Table 1.** Various standards for the DPPH antioxidant method

| Compound | Class | Solubility | Key Antioxidant Feature | Common Use |
|---|---|---|---|---|
| Ascorbic Acid | Vitamin | Water | Electron donor | Food, medicine, biological studies |
| Gallic Acid | Phenolic acid | Water | Hydrogen donor via hydroxyl groups | Plant extracts, food chemistry |
| Quercetin | Flavonoid | Low (in water) | Multi-mechanism antioxidant, including radical scavenging | Pharmaceutical, nutraceutical |

was fully dissolved. More methanol was then added until the solution reached the upper calibration mark of the volumetric flask. The DPPH solution was covered with aluminum foil and stored in a room protected from sunlight.

**Preparation of 100 ppm standard solution**

Each 1 mg of ascorbic acid, gallic acid, and quercetin solid was weighed and placed into a 10 mL volumetric flask. Methanol gradually added while gently shaking until the solids were fully dissolved. More methanol was then added until the solution reached the upper calibration mark of the volumetric flask. The prepared stock solution had a concentration of 100 ppm, which was then diluted to 15 ppm.

**DPPH Method Testing with UV-Vis Spectrophotometer**

The method used was based on the studies by Maesaroh *et al*. (2018) and Sochor *et al*. (2010) with slight modifications. Methanol and the sample (ascorbic acid, gallic acid, and quercetin at concentrations of 0.5-5 ppm) were sequentially added to a test tube to a final volume of 2.4 mL. Then, 0.6 mL of 0.4 mM DPPH solution was added to the test tube containing each standard. The mixture was homogenized and incubated at room temperature for 30 minutes in the dark. The solution was then measured using a UV-Vis spectrophotometer at a wavelength of 510 nm. The absorbance of each concentration variation was recorded, and the $IC_{50}$ value was calculated. Duplicate measurements were performed for each sample over seven replicates.

**DPPH Method Testing with Microplate Reader**

The method used was based on the study by Sochor *et al*. (2010) with slight modifications. Methanol and the sample (ascorbic acid, gallic acid, and quercetin at concentrations of 0.5-5 ppm) were sequentially added to a 99-well microplate with a final volume of 240 µL. Then, 60 µL of 0.4 M DPPH solution was added to the microwell. The reaction mixture was homogenized and incubated at room temperature for 30 minutes in the dark. Measurements were taken using a microplate reader at a wavelength of 510 nm. The absorbance for each concentration variation was recorded, and the $IC_{50}$ value was calculated. Duplicate measurements were performed for each sample over seven replicates.

**Determination of % Inhibition and $IC_{50}$**

Determination of % Inhibition:

$$\frac{A_{blank} - A_{\text{standard}}}{A_{blank}} \quad ...(1)$$

Description:
$A_{blank}$ = Absorbance of the blank
$A_{tandard}$ = Absorbance of the standard

Inhibition concentration 50 ($IC_{50}$) is the minimal concentration of the extract that inhibits up to 50%. The $IC_{50}$ value is obtained from each curve by setting

Y = 50.

$$Y = ax + b \quad ...(2)$$

Description:
$Y$ = % inhibition
$x$ = Concentration
α dan $b$ = Constants

**$IC_{50}$ Value Determination for Ascorbic Acid, Gallic Acid, and Quercetin using T-Test**

The $IC_{50}$ values obtained from antioxidant activity testing of the DPPH method for ascorbic acid, gallic acid, and quercetin will be grouped based on measurements with the UV-Vis spectrophotometer and the microplate reader. These data will be statistically analyzed using a T-test to determine if there are significant differences between the measurements obtained with the UV-Vis spectrophotometer and the microplate reader.

**Sensitivity Testing using Spike Placebo Method**

Sensitivity testing will be conducted using the spike placebo method. This method involves adding a known amount of analyte to a sample (extract) with a known $IC_{50}$ value. The $IC_{50}$ value of the analyte added to the mixture (spike placebo) is then measured and compared (Harmita 2004).

Weigh 1.0046 grams of butterfly pea leaf extract (with an $IC_{50}$ value of 360 ppm). Add 0.1011 grams of analyte (gallic acid) to the extract. Dissolve the mixture in methanol and then concentrate on it using an evaporator. The mixture of extract and gallic acid is labeled as the 'spike' sample.

Weigh 0.0274 grams of the spike sample. Dissolve it in methanol in a 100 mL volumetric flask. Prepare a working solution of the spike sample at 150 ppm. Then weigh gallic acid corresponding to the amount added to the extract and prepare a working solution of gallic acid at 15 ppm. The prepared gallic acid solution is labeled as 'analyte'. Perform testing on the spike sample and the gallic acid sample. Each sample is tested in triplicate with 7 repetitions.

**RESULT AND DISSCUSION**

**DPPH method with UV-Vis spectrophotometer**

Table 2 shows the IC50 values obtained from the antioxidant activity tests of ascorbic acid, gallic acid, and quercetin using a UV-Vis spectrophotometer. The table displays the average $IC_{50}$ values: ascorbic acid 3.53±0.31 ppm, gallic acid 1.35±0.08 ppm, and quercetin 2.11±0.12 ppm. According to the %RSD values, all three standards meet the acceptance criterion for repeatability, with RSD ≤ 2/3 (RSD)r. The Horrat values for the three s are 0.67, 0.38, and 0.41, respectively. According to the Horwitz ratio (HorRat) guidelines, the acceptance precision for inter-laboratory testing (HorRat)R is between 0.5 and

2.0, while for intra-laboratory testing (HorRat)r is between 0.3 and 1.5. Based on the data in Table 2, the antioxidant testing of ascorbic acid, gallic acid, and quercetin using a UV-Vis spectrophotometer is considered precise as it falls within the acceptable range (HorRat)r.

**DPPH Method with Microplate Reader**

Table 3 shows the $IC_{50}$ values obtained from the antioxidant activity tests of ascorbic acid, gallic acid, and quercetin using a microplate reader. The table displays the average $IC_{50}$ values: ascorbic acid 3.66±0.25 ppm, gallic acid 1.04±0.11 ppm, and quercetin 2.12±0.11 ppm. According to the %RSD values, all three standards meet the acceptance criterion for repeatability, with RSD ≤ 2/3 (RSD)r. The Horrat values for the three standards are 0.52, 0.33, and 0.34, respectively.

According to the Horwitz ratio (HorRat) guidelines, the acceptance precision for inter-laboratory testing (HorRat)R ranges from 0.5 to 2.0, while for intra-laboratory testing (HorRat)r ranges from 0.3 to 1.5. Based on the data in Table 3, the antioxidant testing of ascorbic acid, gallic acid, and quercetin using a microplate reader is considered precise as it falls within the acceptable range (HorRat)r.

Based on Table 4, which shows the comparison of standard test values, it can be seen that the average $IC_{50}$ values of the three standards tested with the UV-Vis spectrophotometer (M-1) are lower than the average $IC_{50}$ values obtained using the microplate reader. Solyom *et al*. (2016) state that the difference in concentrations among various determinations (analysis results) should be 5% or less. According to the data in Table 4, the differences between the testing methods show values of less than 5%. Thus, it can be said that both methods provide nearly identical results. Figure 1 illustrated xomparison of $IC_{50}$ values for DPPH method standards.

**T-Test of DPPH Method Standards**

The data obtained from the DPPH method for antioxidant activity tests using UV-Vis spectrophotometer and microplate reader were analyzed using a T-test with MS Excel 2010. The T-test was conducted to determine whether there is a significant difference between the two tested methods. The T-test calculations were based on the average $IC_{50}$ values, absolute deviation (SD), and relative deviation (RSD) from the results of both tests. The T-test data processing is shown in Table 5. Below are the results of the T-test.

The calculated t-values for ascorbic acid, gallic acid, and quercetin are 0.86, 1.52, and 0.20, respectively. According to Julious (2019), the t-table value used for 5% significance is t-table (5%) = 1.78. The 5% significance level is used to determine whether the mean concentration differences between the two methods are statistically significant at a 95% confidence level. Based on the calculated t-values, there is no significant difference between the two

**Tabel 2**. Standard activity against DPPH with UV-Vis spectrophotometer

| No. | Standard | $IC_{50}$ Value (ppm) | | | | | | | Mean | SD | % RSD | (PRSD)r | (Horrat)r |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Repetition | | | | | | | | | | | |
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | | | | | |
| 1 | Ascorbic acid | 2.85 | 3.53 | 3.74 | 3.53 | 3.67 | 3.66 | 3.74 | 3.53 | 0.31 | 8.86 | 13.23 | 0.67 |
| 2 | Galic acid | 1.28 | 1.44 | 1.23 | 1.40 | 1.41 | 1.28 | 1.35 | 1.34 | 0.08 | 5.92 | 15.30 | 0.38 |
| 3 | Quercetin | 2.27 | 2.06 | 1.89 | 2.13 | 2.18 | 2.18 | 2.04 | 2.11 | 0.12 | 5.86 | 14.29 | 0.41 |

**Tabel 3**. Standard activity against DPPH with microplate reader

| No | Standard | $IC_{50}$ Value (ppm) | | | | | | | Mean | SD | % RSD | (PRSD)r | (Horrat)r |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Repetition | | | | | | | | | | | |
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | | | | | |
| 1. | Ascorbic acid | 3.12 | 3.89 | 3.8 | 3.68 | 3.72 | 3.63 | 3.80 | 3.66 | 0.25 | 6.95 | 13.15 | 0.52 |
| 2. | Galic acid | 1.37 | 1.53 | 1.44 | 1.31 | 1.38 | 1.36 | 1.43 | 1.40 | 0.07 | 5.07 | 15.20 | 0.33 |
| 3. | Quercetin | 2.08 | 2.21 | 2.18 | 2.13 | 2.18 | 2.16 | 1.90 | 2.12 | 0.11 | 4.98 | 14.28 | 0.34 |

**Tabel 4**. Comparison of $IC_{50}$ values, RSD, and % RSD for DPPH standard methods using UV-Vis spectrophotometer (M-1) and Microplate reader (M-2)

| No. | Standard | $IC_{50}$ (ppm) | | SD | | % RSD | |
|---|---|---|---|---|---|---|---|
| | | M-1 | M-2 | M-1 | M-2 | M-1 | M-2 |
| 1. | Ascorbic acid | 3.53 | 3.66 | 0.31 | 0.25 | 8.86 | 6.95 |
| 2. | Galic acid | 1.34 | 1.4 | 0.08 | 0.07 | 5.92 | 5.07 |
| 3. | Quercetin | 2.11 | 2.12 | 0.12 | 0.11 | 5.86 | 4.98 |

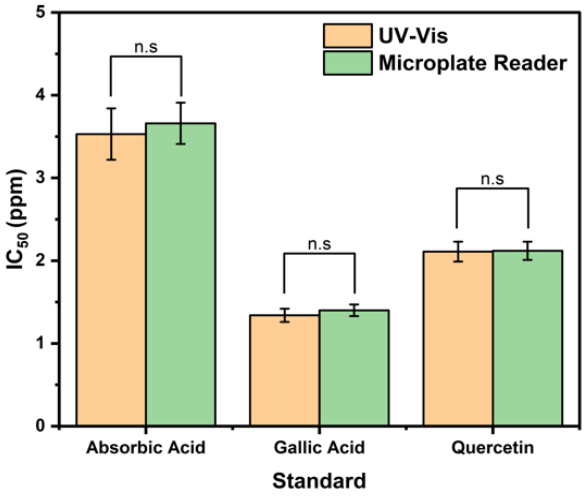


**Figure 1**. Comparison of $IC_{50}$ values for DPPH method standards using UV-Vis Spectrophotometer and Microplate reader

**Tabel 5**. Results of the T-Test for DPPH Standard Methods Using UV-Vis Spectrophotometer (M-1) and Microplate Reader (M-2)

| No. | Standard | $t_{calculated}$ | $t_{tabel}$ |
|---|---|---|---|
| 1. | Ascorbic acid | 0.86 | |
| 2. | Galic acid | 1.52 | 1.78 |
| 3. | Quercetin | 0.20 | |

**Table 6**. Results of recovery testing for antioxidant activity using DPPH method with UV-Vis spectrophotometer and Microplate reader

| Standard | $IC_{50}$ analyte (ppm) | | $IC_{50}$ spike (ppm) | | Recovery Value (%) | | Average Recovery (%) | |
|---|---|---|---|---|---|---|---|---|
| | M-1 | M-2 | M-1 | M-2 | M-1 | M-2 | M-1 | M-2 |
| Galic acid | 1.28 | 1.37 | 1.28 | 1.31 | 100.3 | 104.5 | 99.7±0.3 | 103±0.3 |
| | 1.28 | 1.37 | 1.29 | 1.34 | 99.6 | 102.2 | | |
| | 1.23 | 1.36 | 1.25 | 1.31 | 98.4 | 103.8 | | |
| | 1.35 | 1.38 | 1.34 | 1.35 | 100.7 | 102.2 | | |
| | 1.35 | 1.38 | 1.34 | 1.31 | 101.1 | 105.3 | | |
| | 1.28 | 1.36 | 1.34 | 1.34 | 95.5 | 101.4 | | |
| | 1.35 | 1.36 | 1.33 | 1.34 | 101.8 | 101.4 | | |

methods at the 5% significance level because the calculated t-values are smaller than the t-table value. According to the T-test acceptance criteria: H0 (null hypothesis) means no significant difference between the two tests if t calculated < t table, while H1 (alternative hypothesis) indicates a significant difference if t calculated > t table. Based on the T-test results from Table 5, it can be concluded that t calculated < t table, meaning that the two methods (H0) do not differ significantly. Thus, the microplate reader can be considered a suitable alternative to the UV-Vis spectrophotometer.

**Sensitivity Test (Recovery) of Gallic Acid Using the DPPH Method with Simulation (Spike Placebo) Method**

The simulation test was performed only on the standard gallic acid (Table 6), if one test can represent other test samples. Below are the recovery values from the simulation test for gallic acid using UV-Vis spectrophotometer (M-1) and microplate reader (M-2).

The comparison of recovery values illustrated in Figure 2 shows an average of 99.7±0.3% for measurements with the UV-Vis spectrophotometer and 103.0±3% for the microplate reader. According

to the % recovery acceptance guidelines outlined in the "Guidelines for The Validation of Analytical Methods for Active Constituents, Agricultural and Veterinary Chemical Products", the % recovery values for both the UV-Vis spectrophotometer and microplate reader are acceptable. Both values fall within the acceptable range for analyte addition ≥ 1% (9.14%) and % recovery between 90-110% (99.7±0.3% for UV-Vis and 103.0±3% for microplate reader). However, based on method accuracy, the UV-Vis spectrophotometer Shimadzu UV-1800 is more accurate than the microplate reader EZ Read 400 Biochrom because it has a value closer to 100%. The % recovery value for the microplate reader is above 100% but does not exceed the acceptance criteria. According to the accuracy measurement requirements, if the difference is no more than 5%, the measurement is still acceptable, meaning the microplate reader's sensitivity is also acceptable (Hidayat *et al.* 2017; Kalogerakis *et al*. 2022). The comparative table between both methods can be seen in Table 7.

**Table 7**. Comparative table: Microplate Reader vs. UV-Vis Spectrophotometer

| **Parameter** | **Scanometry** (Hidayat *et al*. 2017) | **UV-Vis Spectrophotometer** (Kalogerakis *et al*. 2022) |
|---|---|---|
| **Method** | Flatbed scanner used as a microplate reader for antioxidant (DPPH-based) assay | Absorbance measured using Infinite 200Pro microplate reader (TECAN) for persulfate analysis |
| **Gallic Acid Concentration** | 10 ppm | Not specifically mentioned |
| **% Recovery** | 91% – 96% (average 93% – 96%) | Not specifically mentioned |
| **Accuracy Criteria (%)** | 80% – 110% | 80% – 120% |
| **Reproducibility** | RSD ≤ 1% | Not specifically mentioned |
| **Response Time** | 9 minutes | Not specifically mentioned |
| **Advantages** | Cost-effective, accessible, suitable for high-throughput assays | High accuracy, ideal for standard laboratory analysis |
| **Limitations** | Lower sensitivity, requires software optimization (e.g., ImageJ for analysis) | Requires specialized equipment, higher operational cost |

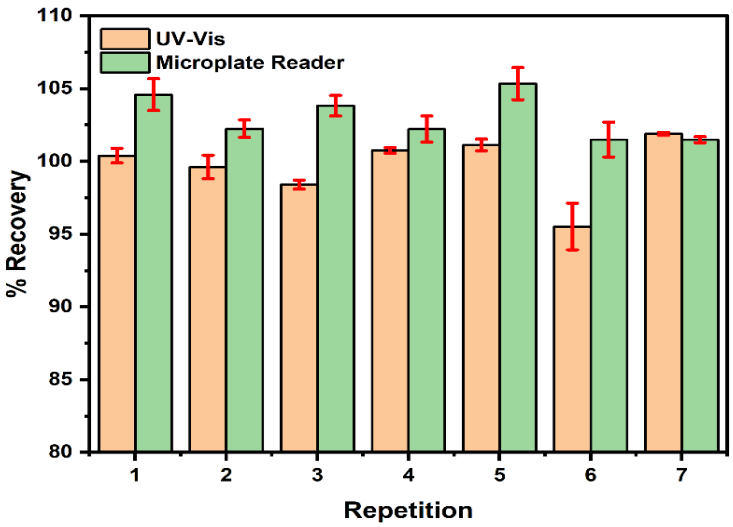


**Figure 2**. Comparison of % recovery values for antioxidant activity using DPPH method for gallic acid standard with UV-Vis Spectrophotometer and Microplate Reader

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## CONCLUSION

Based on the results of this study, it can be concluded that both the UV-Vis spectrophotometer (Shimadzu UV-1800) and the EZ Read 400 Biochrom microplate reader are reliable and suitable instruments for measuring antioxidant activity using the DPPH method. The sensitivity and accuracy of both methods were evaluated using statistical tests and analytical performance parameters. The T-test results for ascorbic acid, gallic acid, and quercetin were 0.86, 1.52, and 0.20, respectively. all of which are lower than the t-table value of 1.72. This indicates that there is no statistically significant difference between the two methods in terms of measured antioxidant activity. Furthermore, precision testing using the HorRat method showed values of 0.52 for ascorbic acid, 0.33 for gallic acid, and 0.34 for quercetin, all within the acceptable range of 0.3 to 1.5. These results confirm that both methods demonstrate good intermediate precision.

In terms of accuracy, the percent recovery for gallic acid was 99.7 ± 0.3% when measured with the UV-Vis spectrophotometer and 103.0 ± 3% with the microplate reader. Although the recovery value for the microplate reader slightly exceeds 100%, both results fall within the generally accepted recovery range of 90–110%, demonstrating good analytical accuracy for both instruments. While the spectrophotometer showed slightly higher precision and a recovery value closer to 100%, the microplate reader offered comparable results with the added advantage of higher throughput and ease of use. Therefore, both instruments can be considered sensitive, accurate, and precise for DPPH-based antioxidant activity measurement, and the choice between them may be determined by practical considerations such as available equipment, sample volume, and the need for throughput.

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