Development and validation of spectrophotometric methods for quantitative determination of total phenolic and total flavonoid content of lyophilizate of the leaf of sweet potato leaf

Aliyah, Muhammad Aswad, Hajrah, and Andi Dian Permana
Faculty of Pharmacy, Hasanuddin University, Makassar, 90245, Indonesia
Email: aliyah@unhas.ac.id

Abstract. Sweet potato (Ipomoea batatas L.) is one of the plants commonly used as traditional medicine, such as anti-inflammatory, anti-diabetes, anti-mutagenic and to increase platelet count. The benefits of sweet potato leaves have been reported as a result of the presence of antioxidant compounds and bioactive compounds, mainly phenolic and flavonoid compounds. In this study, we validated the spectrophotometry method of total phenolic and flavonoid compound of lyophilizate of the leaves of sweet potato juices. The results showed that the coefficient correlation (r) values were 0.97389 for gallic acid standard and 0.99869 for quercetin standard; the recovery rates ranged from 105.08% to 109.92% for phenolic, and from 87.48% to 94.36% for flavonoids respectively; precision with relative standard deviation values ranged from 0.7% to 0.4% for phenolic, and from 0.9% to 0.5% for flavonoids respectively; and the detection limit (LOD) and the quantitation limit (LOQ) for phenolic were 0.0425 µg/mL and 0.128 µg/mL respectively while for flavonoids were 0.17 µg/mL and 0.5 µg/mL respectively. The method was successfully applied in the determination of phenolic and flavonoid contents of lyophilizate of sweet potato leaf juices with the average total phenolic content of 9.83mg GAE/g lyophilizate, while the average total flavonoid level was 5.11mg QE/g lyophilizate.

1. Introduction
Sweet potato (Ipomoea batatas L.) is a high-yielding plant with short period crop time. They are generally cultivated in subtropical and tropical area. Essentially, this plant possesses an excellent tolerance in high temperature, an excellent adaptability in the soil with poor nitrogen content and needs minimum harvest management [1–3]. The leaves of the sweet potato plants, as the above-ground portion of plant, can be collected numerous intervals throughout every cultivating period. Moreover, compared to other leafy plants, they are reported to possess greater yields [4]. Nevertheless, the leaves of sweet potato have been reported to be underutilized. Only a little quantity of these parts is utilized as a fresh vegetable [5]. Several studies have shown that the leaves of the sweet potato exhibited numerous health-promoting pharmacological activities, namely hypoglycemic, antioxidant, anti-tumor and anti-inflammatory [4,6]. Furthermore, the above-mentioned activities reported in the leaves of the sweet potato are generally related to their phenolic and flavonoids contents [4,7]. To specify its antioxidant activity, Sun et al have shown that sweet potato leaf phenolic acids exhibited strong antioxidant activity, especially caffeic acid (CA) and monoaaffeoylquinic acid [5]. Liu et al. have revealed that sweet potato leaf flavonoids also possessed strong antioxidant activity [8].

Generally, despite the small utilization, the sweet potato leaves are consumed as a fresh vegetable. In this study, we used the lyophilizate of the juice of sweet potato leaves. The use of juice has shown several advantages compared to other method, especially the absence of organic solvent, resulting in non-toxic effect of the use of the sweet potato leaves. To further evaluate the main components of the sweet potato leaves, phenolic and flavonoids compounds, it is important to develop and validate the
suitable analytical methods. Amongst several analytical methods, the spectrophotometric method has been found to be the most popular and extensively applied as quantitative technique to determine the phenolic and flavonoid contents of several plants. [9]. This technique possesses several benefits to observe the concentration of phenolic and flavonoid in model solutions. For instance, particularly, with spectroscopy technique, determination of the phenolic and flavonoid content carried out in various investigational conditions, including pH, time and temperature is achievable. Importantly, spectrophotometric technique is low-cost and simple method, as well as well-known in most laboratory [10].

It is necessary to validate the analytical method before being applied in any measurements. Several parameters are important to be considered in the validation process. In the current study, we report the validation of phenolic and flavonoid compounds using gallic acid and quercetin as the standards. The parameters observed were linearity, limit of detection (LoD), limit of quantification (LoQ), accuracy and precision. Finally, the validated method was applied to determine the phenolic and flavonoid contents of lyophilizates of sweet potato leaves juices.

2. Material and Methods

2.1. Sample collection and preparation
The fresh samples of sweet potato leaves were collected at Perintis Kemerdekaan IV region, Makassar, Indonesia. The collected leaves were washed with water and were directly subjected to juicer machine, obtaining the juice of sweet potato leaves. Afterwards, the obtained juice was lyophilized using vacuum freeze dryer, obtaining dry lyophilizate of sweet potato leaves juices.

2.2. Preparation of calibration curve of gallic acid
To prepare the standard solution gallic acid, 50 mg of gallic acid was accurately weighed and dissolved in 50 mL in the volumetric flask, obtaining the stock solution with the concentration of 1000 µg/mL. Prior to the determination of maximum wavelength, 50 µL of stock solution was taken and mixed with 7.5% w/v of Folin-Ciocalteu reagent, as previously described with minor modifications [11,12]. This mixture was incubated for 8 minutes and 1 mL of 1% w/v of NaOH solution was added into the mixtures, followed by the incubation for 1 h. The absorbance was measured using a spectrophotometer (Model UV-2500, Shimadzu Co., Ltd., Tokyo, Japan) in the wavelength range between 400 and 800 nm. The calibration standard solutions were prepared in the concentration range of 3-11 µg/mL and the absorbance of all solutions were measured using a spectrophotometer in the maximum wavelength.

2.3. Preparation of calibration curve of quercetin
In order to prepare the standard solution of quercetin, stock solution with the concentration of 1000 µg/mL was prepared by dissolving 50 mg of quercetin in 50 mL of methanol. An aliquot (50 µL) of stock solution was taken and mixed with 0.2 mL of 10% w/v ammonium chloride and 0.2 mL of 1 M sodium, as previously described with minor modifications [13] The absorbance was observed using a spectrophotometer (Model UV-2500, Shimadzu Co., Ltd., Tokyo, Japan) in the wavelength range from 400 to 800 nm. The calibration standard solutions were prepared in the concentration range of 2-6 µg/mL and the absorbance of all solutions were measured using a spectrophotometer in the maximum wavelength.

2.4. Analytical Method Validation

2.4.1. Linearity. The linearity of the analytical method was evaluated by observing the correlation coefficient of the calibration curve prepared. The linearity of calibration curves of gallic acid and quercetin were observed [14].

2.4.2. Limit of detection (LoD) and limit of quantification (LoQ). To determine LoD and LoQ of the method developed, the standard deviation and the slope values of the calibration curves were determined [15]. Finally, the LoD and LoQ were calculated using the following equation:
LOD = 3.3σ/S \quad (1)
\LOQ = 10σ/S \quad (2)

Where \( \sigma \) = the standard deviation of the data response and \( S \) = the slope of the calibration curve.

2.4.3. Accuracy and precision. To determine the precision and accuracy, three quality control solutions were initially prepared. In this experiment, the concentration of low-quality control, medium-quality control, and high-quality control were 4 µg/mL, 5 µg/mL, and 6 µg/mL, respectively. The evaluation of the accuracy was carried out by comparing the concentration found with the theoretical concentration prepared. The precision was determined by observing the relative standard deviation (RSD) of the responses of all quality control solutions [16].

2.5. Application of analytical method in determination of phenolic and flavonoid contents of lyophilizate of sweet potato leave juices

Initially, the stock solution of the lyophilizates was prepared by dissolving 50 mg of lyophilizate in 50 mL of methanol, obtaining stock solution with the concentration of 1000 µg/mL. To determine the phenolic and flavonoid contents, 6 mL of stock solution was taken and mixed with the reagents described in the preparation of gallic acid and quercetin, respectively. The absorbances of samples were measured using a spectrophotometer in the maximum wavelength.

3. Results and discussion

3.1. Extraction yield and determination of maximum wavelength

In this study, 400 g of sweet potato leave was used and after subjected to juicer machine, 210 mL of juice was obtained. The juice was subjected into lyophilization machine, resulting in 22.43 g of dry lyophilizate. Therefore, the extraction yield was calculated to be 5.6%. Before the calibration curve preparation, it was crucial to determine the maximum wavelength of gallic acid and quercetin. The spectrum of gallic acid and quercetin in methanol are shown in Figure 1 and 2, respectively. The results showed that the maximum wavelength values of gallic acid and quercetin were 664 nm and 443 nm, respectively.

![Figure 1](image.png)

**Figure 1.** Determination of maximum wavelength of gallic acid.
3.2. Analytical Method Validation

3.2.1. Linearity. As previously explained, the linearity was evaluated by observing the correlation coefficient of the calibration curve. The calibration curves of gallic acid and quercetin in methanol are depicted in Figure 3 and Figure 4. As depicted, the correlation coefficient values were found to be 0.9484 and 0.9974 for gallic acid and quercetin, respectively. The values above 0.9 were considered to be linear. Therefore, the calibration curves could be used in further steps.

![Figure 2: Determination of maximum wavelength of quercetin.](image1)

![Figure 3: Calibration curve of gallic acid in methanol.](image2)
3.2.2. LOD and LOQ. The calculations of LoD and LoQ were carried out after constructing the calibration curve. The results showed that the LoD values of phenolic and flavonoid compounds were 0.0425 µg/mL and 0.17 µg/mL, respectively. For LoQ values, it was found that the values were 0.128 µg/mL for phenolic compounds and 0.5 µg/mL for flavonoid compounds.

3.2.3. Accuracy and Precision. Accuracy test was carried out by the addition of standard solution into sample solution with the concentration of 4 µg/mL, 5 µg/mL and 6 µg/mL. The accuracy evaluation results are shown in Table 1 and Table 2 for phenolic and flavonoid, respectively.

![Figure 4. Calibration curve of quercetin in methanol.](image)

**Table 1.** Accuracy test results of phenolic.

| Sample concentration µg/mL | Gallic acid concentration µg/mL | Concentration (µg/mL) | % Recovery |
|----------------------------|---------------------------------|-----------------------|------------|
|                            |                                 | Sample               | Sample + Gallic acid | |
| 600                        | 4                               | 6.2059               | 10.5019     | 107.4 |
|                            |                                 | 6.1729               | 10.4905     | 107.94 |
|                            |                                 | 6.2029               | 10.5250     | 108.05 |
| **Average**                |                                 | 6.2059               | 11.4598     | **107.79** |
| 600                        | 5                               | 6.1729               | 11.3870     | 104.28 |
|                            |                                 | 6.2029               | 11.4966     | 105.87 |
| **Average**                |                                 | 6.2059               | 12.7256     | **108.66** |
| 600                        | 6                               | 6.1729               | 12.7340     | 109.35 |
|                            |                                 | 6.2029               | 12.9082     | 111.75 |
| **Average**                |                                 | 6.2059               | **12.9284** | **109.92** |

**Table 2.** Accuracy test results of flavonoid.

| Sample concentration µg/mL | Quercetin concentration µg/mL | Concentration (µg/mL) | % Recovery |
|----------------------------|-------------------------------|-----------------------|------------|
|                            |                               | Sample               | Sample + Quercetin | |
|                            | y = 0.0902x + 0.0058          | R² = 0.9974           |             |
3.2612 6.7256 86.62
3.2370 6.7885 88.79
3.2202 6.7019 87.04

Average 87.48

3.2370 7.8109 90.99
3.2202 7.8379 92.02
3.2202 7.8789 93.17

Average 92.06

3.2370 8.9242 94.38
3.2202 8.9054 94.47
3.2202 8.8744 94.24

Average 94.36

As shown in table 1 and 2, the recovery percentage values were in the range of 105.8% and 109.92% for phenolic compounds and in the range of 87.48-94.36% for flavonoid compounds. It should be noted that the recovery percentage should be in the range of ±15% of 100% recovery, meaning that the recovery should be between 85-115%. For precision evaluation, it was found that all relative standard deviation values of samples tested were ±15%. Therefore, the method was considered to be accurate and precise.

3.3. Application of analytical method in determination of phenolic and flavonoid contents of lyophilizate of sweet potato leave juices

The validated method was finally applied to quantify the phenolic and flavonoid contents of lyophilizate of sweet potato leave juices. The results of the quantification are shown in Table 3 and Table 4.

Table 3. Determination of phenolic compounds in lyophilizate of sweet potato leave juices.

| Replication | Absorbance | Samples (g) | Phenolic content (mgGAE/g) |
|-------------|------------|-------------|----------------------------|
| I           | 0.35345    | 0.0504      | 9.85                       |
| II          | 0.35118    | 0.0504      | 9.79                       |
| III         | 0.35324    | 0.0504      | 9.84                       |
| **Average** | 9.83 ± 0.03|

Table 4. Determination of flavonoid content in lyophilizate of sweet potato leave juices.

| Replication | Absorbance | Samples (g) | Flavonoid content (mgQE/g) |
|-------------|------------|-------------|---------------------------|
| I           | 0.29996    | 0.0506      | 5.15                      |
| II          | 0.29778    | 0.0506      | 5.11                      |
| III         | 0.29627    | 0.0506      | 5.09                      |
| **Average** | 5.11 ± 0.03|

As depicted, the phenolic compound concentration was found to be 9.83 ± 0.03 mg mgGAE/g and the flavonoid content was found to be 5.11 mg ± 0.03 mgQE/g. The overall results showed that the method was successfully applied in the determination the phenolic and flavonoid contents of lyophilizate of sweet potato leave juices.

4. Conclusion

The main aimed of this study was to establish and validate a spectrophotometer method for quantification of phenolic and flavonoid contents of lyophilizates of sweet potato leaf juices. Overall,
the method was found to be linear, precise, and accurate. The validated method was successfully utilized in the evaluation of the phenolic and flavonoid contents of lyophilizate of sweet potato leave juices.

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