

Original Article

The Effect of Differences in Growing Altitude on the Total Flavonoid Content Single Clove Garlic (*Allium sativum* Linn)

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Abstract: Single garlic belongs to the *Amaryllidaceae* family and contains flavonoid compounds with antioxidant activity. However, the use of herbal ingredients often shows inconsistent effects because differences in growing locations, such as soil and climate conditions, can influence the amount of flavonoids produced. The purpose of this study was to determine the difference in total flavonoid content of single garlic (*Allium sativum* Linn) based on the influence of growing locations in Boyolali Regency and Karanganyar Regency. This study used 2 kg of single garlic (*Allium sativum* Linn) samples, which were then extracted using the maceration method with 96% ethanol solvent. The total flavonoid content was determined using the UV-Vis spectrophotometry method. The results showed that the quantitative test using UV-Vis spectrophotometry obtained a maximum wavelength of 426 nm with a curve equation of $y=0.0151x - 0.0033$. The average total flavonoid content from Boyolali Regency was 0.5867 mgQE/g and from Karanganyar Regency was 0.5867 mgQE/g and 0.1495 mgQE/g. Based on statistical test data, it was found that the significance value was 0.002, where the result had a $P<0.05$ value, which means that there was a significant difference in total flavonoid content based on the growing location.

Keywords: flavonoids, maceration, single garlic, UV-Vis spectrophotometry

1. INTRODUCTION

Medicinal plants are believed by the community to have beneficial properties and have been used for generations based on experience. Every part of the plant can be used as medicine, such as the roots, stems, and leaves. Among the many medicinal plants found in nature, garlic is a very beneficial plant. Garlic is a remedy for several diseases, including respiratory tract infections, and can also increase vitality. Garlic can help treat influenza, fatigue, exhaustion, and insomnia, as it is effective in overcoming vitamin C deficiency [1].

Garlic (*Allium sativum* Linn) has many varieties, one of which is single clove garlic. Single clove garlic (single bulb) is a variety that was formed accidentally due to unsuitable planting conditions. Single clove garlic was first discovered in the Sarangan area, Magetan, East Java. The bulb of this plant contains only one small whole bulb. This is due to the failure of the main shoot to form and the suppression of the formation of shoots and buds. The leaves that normally wrap the buds are only able to wrap the whole bulb, so the skin of the whole bulb is thicker than the outer skin of the bulb with buds [2]. Prasonto's 2017 research showed that antioxidant activity tests indicated that

single garlic has higher antioxidant activity than other garlic varieties. The flavonoid content in single garlic acts as an anticholesterol agent [3].

Phytochemical studies have demonstrated that garlic contains a wide range of bioactive compounds, including organosulfur compounds (such as allicin, alliin, and ajoene), phenolic acids, flavonoids, saponins, and alkaloids. Flavonoids are an important class of secondary metabolites in garlic, contributing significantly to its antioxidant, anti-inflammatory, and cardioprotective activities. Previous studies have reported the presence of flavonoid compounds such as quercetin, kaempferol, and their derivatives in garlic extracts [4].

The levels of flavonoids and other secondary metabolites in plants are influenced by various factors, including environmental conditions, temperature, light intensity, humidity, pH, soil fertility, and post-harvest processes such as traditional processing methods and extraction techniques [5]. Planting location, particularly altitude, is an important factor that affects plant growth and development [6,7]. Differences in altitude cause variations in environmental conditions, such as temperature and humidity, which affect plant metabolism and the secondary compounds produced, including flavonoids. Previous research shows that differences in the altitude at which papaya (*Carica pubescens*) plants grow cause differences in flavonoid content. In addition, the flavonoid content of plants can vary depending on the species, organ, and stage of growth and development of the plant, which is also influenced by differences in altitude [8]. Another study on galangal (*Alpinia galanga*) plants shows that variations in growing altitude are related to differences in flavonoid levels, which are influenced by temperature and environmental humidity [9].

Single garlic (*Allium sativum* Linn) is generally cultivated in mountainous areas with cool to cold climates and relatively dry conditions, while cultivation in lowlands with high water content is less suitable as it can cause rot [10]. Differences in altitude and soil type at different planting locations have the potential to cause variations in flavonoid levels in single garlic. Therefore, research is needed to compare the total flavonoid content of single garlic from different planting locations.

Based on the above background, the researcher will conduct a comparative study of the total flavonoid content of single garlic (*Allium sativum* Linn) in different growing locations and soil types to determine the effect of growing location on the total flavonoid content of single garlic (*Allium sativum* Linn) using UV-Vis spectrophotometry.

2. MATERIALS AND METHODS

2.1. Materials

Single garlic clove (*Allium sativum* Linn) obtained from two different growing locations: Dukuh Ngemplak, Tarubatang Village, Selo District, Boyolali Regency (1500–2100 m above sea level) with regosol soil, and Pancot Hamlet, Kalisoro Village, Tawangmangu District, Karanganyar Regency (900–1000 m above sea level) with andosol soil. Other materials included 96% ethanol, distilled water, filter paper, potassium acetate, and aluminum chloride.

2.2. Method

2.2.1. Extraction of single garlic (*Allium sativum* Linn)

Samples used in this study consisted of 2 kg of single clove garlic obtained from Boyolali Regency and Karanganyar Regency. The samples were washed, sliced, and dried until a constant weight was achieved. The dried samples were then ground into powder. For extraction, 100 g of dried powder from each sampling location was subjected to maceration using 96% ethanol as the solvent

at a ratio of 1:10 (w/v) in 1000 mL for 3×24 hours at room temperature with occasional stirring. After maceration, the mixture was filtered to obtain the filtrate, which was then concentrated to obtain the crude extract. The yield of the concentrated extract obtained is then calculated using the formula:

$$\text{yield} = \frac{\text{extract weight}}{\text{initial weight}} \times 100\%$$

2.2.2. Determination of total flavonoid content

The total flavonoid content was determined using a linear regression equation obtained from the calibration curve of the UV-Vis spectrophotometer readings. The absorbance values of the samples were entered into the linear equation as variable y , and then the equation was used to calculate the x value, which indicates the total flavonoid content in the sample solution. The linear regression equation is expressed as: $Y = bx + a$

The data obtained is primary data, and the data from the comparison of total flavonoid levels is used to calculate the total flavonoid compound levels contained in the single garlic extract. The total flavonoid level is calculated using the following formula:

$$\text{Level (mgQE/g)} = \frac{\text{konsentrasi(ppm)} \times \text{volumeawalsampel}}{\text{gramsampel} \times 1000} \times \text{faktor pengencer}$$

The research conclusions and quantitative data were analyzed using descriptive percentage methods, namely by calculating the percentage of nominal data. Meanwhile, quantitative data were processed using mean \pm standard deviation (\pm SD) analysis.

Data analysis uses *the independent t-test* if the data is normal. The independent t -test is used to compare two sample means from related groups. *The Mann-Whitney test* is used if the data is not normally distributed. *The Mann-Whitney test* can be used to test whether there is a difference between two samples (groups), and the data needs to be normally distributed. p value < 0.05 = significant, p value > 0.05 = not significant.

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1. Plant determination

Plant identification was carried out to ensure the accuracy of the plant identity used so as to prevent errors in sampling. In this study, the plant used was single garlic (*Allium sativum* Linn). The identification process was carried out at the Mathematics and Natural Sciences Laboratory of Ahmad Dahlan University, Yogyakarta. Based on the identification results, the plant used in this study was correctly identified as single garlic (*Allium sativum* Linn).

3.1.2. Extraction of single garlic (*Allium sativum* Linn)

Boyolali and Karanganyar were selected as sampling locations because they represent distinct growing environments for single clove garlic in terms of altitude and soil type. Boyolali is characterized by higher elevation (1500–2100 m above sea level) with regosol soil, while Karanganyar is located at a lower elevation (900–1000 m above sea level) with andosol soil. These differences in altitude and soil characteristics are known to influence environmental conditions such as temperature, moisture, and nutrient availability, which can affect plant metabolism and flavonoid accumulation. Therefore, selecting these two locations allows for a comparative evaluation of the effect of growing location on the total flavonoid content of single clove garlic.

The extraction of single clove garlic (*Allium sativum* Linn) was carried out using the maceration method with 96% ethanol as the extraction solvent. Ethanol was selected due to its effectiveness in extracting flavonoid compounds and its suitability for polar and semi-polar phytochemicals. The dried garlic samples were ground into powder to increase the surface area and facilitate solvent penetration during extraction. Maceration was conducted for 3 × 24 hours at room temperature with occasional stirring to ensure optimal contact between the solvent and plant material. The extract was then filtered, and the filtrate was concentrated to obtain a crude extract. The extraction process yielded 3.02 g of extract (3.02% w/w) from the Boyolali sample and 3.21 g of extract (3.21% w/w) from the Karanganyar sample.

3.1.3. Determination of total flavonoid content

a. Maximum wavelength

Determination of the maximum wavelength aims to find the wavelength in the standard solution that produces maximum absorption. The maximum wavelength is 426 nm with an absorption of 0.8359.

Table 1. Maximum wavelength and absorbance

| Wavelength (nm) | Absorbance |
|-----------------|------------|
| 426 | 0.8359 |

b. Operating time

The purpose of determining the operating time is to determine how long it takes for the solution to reach a constant absorbance. The results of the study show that the absorbance began to stabilize at 30 minutes.

c. Results of concentration and absorbance of standard curve solutions

Table 2. Concentration and absorbance of standard curve solutions

| Concentration (ppm) | Absorbance |
|---------------------|------------|
| 15 | 0.2106 |
| 25 | 0.3800 |
| 35 | 0.5461 |
| 45 | 0.6519 |
| 55 | 0.8359 |

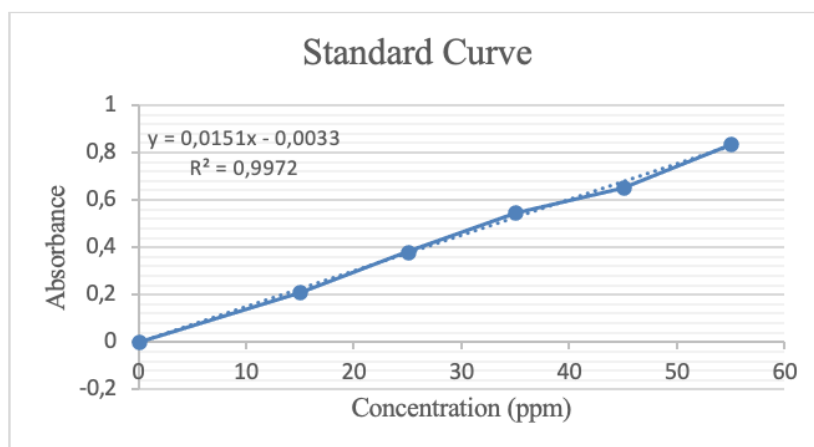


Figure 1. Standard curve graph

The use of varying concentrations aims to observe differences in absorbance values, where higher concentrations result in higher absorbance values. Based on Table 2, the standard curve was created using 5 concentration series, namely 15, 25, 35, 45, and 55 ppm, resulting in $y = 0.0151x - 0.0033$ with an R^2 value of 0.9972, indicating that the values are linear.

d. Determination of flavonoid content

Based on Table 3, the total flavonoid content of single garlic extract from Boyolali Regency was higher than that from Karanganyar Regency. The Boyolali sample exhibited an average total flavonoid content of 0.5867 ± 0.0172 mgQE/g, whereas the Karanganyar sample showed a lower average value of 0.1495 ± 0.0007 mgQE/g.

Table 3. Average total flavonoid content of single garlic extract from different regions

| Region | Total Flavonoid Content (%) | Total Flavonoid Content (mgQE/g) |
|-------------|-----------------------------|----------------------------------|
| | Average \pm SD | Average \pm SD |
| Boyolali | 0.0293 ± 0.00085 | 0.5867 ± 0.0172 |
| Karanganyar | 0.0074 ± 0.00005 | 0.1495 ± 0.0007 |

The higher flavonoid content observed in the Boyolali sample suggests that geographical and environmental factors play an important role in influencing the biosynthesis and accumulation of flavonoid compounds in garlic. Factors such as soil nutrient composition, altitude, temperature, rainfall patterns, and agricultural practices may contribute to the differences in secondary metabolite production between regions [11]. In addition, genetic variability and post-harvest handling conditions may also affect flavonoid stability and concentration [12].

Although the UV-Vis spectrophotometric method provides a reliable and practical approach for estimating total flavonoid content, the results of this study are based on duplicate measurements, which may limit statistical robustness. Therefore, further studies with a higher number of replications and complementary analytical techniques are recommended to confirm these findings.

3.1.4. Data analysis

The total flavonoid content obtained from each sample was then analyzed for normality using an *independent t-test* to determine whether there was a significant difference in the total flavonoid content of single garlic extracts originating from two different regions, and to determine whether differences in the growing location of single garlic plants could affect the total flavonoid content.

Table 5. Results of total flavonoid content *independent sample t-test* of single garlic extract

| Sample | <i>Levene's test for equality of variances</i> | <i>t-Test quality of means sig</i> | Description |
|-------------|--|------------------------------------|-------------------------|
| Karanganyar | 0.000 | 0.002 | Significantly different |
| Boyolali | 0.000 | 0.002 | Significantly different |

Based on the results of the total flavonoid test of single garlic extract, as shown in Table 5, the value of *Levene's test for quality of variance* of flavonoid levels in the Karanganyar and Boyolali samples was $0.00 < 0.05$, indicating that the two samples were not homogeneous. Then, based on the results of the *t-test for equality of means (sig 2-tailed)*, the significant value is $0.002 < 0.05$, meaning that there is a significant difference in the total flavonoid content of single garlic from the two different regions.

3.2. Discussion

Extraction was performed using the maceration method, which avoids damage to compound components due to heating. This method was chosen because flavonoid compounds are easily damaged at high temperatures. The solvent used was 96% ethanol because it can extract compounds optimally [13]. The maceration process used 100 grams of powdered single white onion simplisia. The maceration results obtained were in the form of a thick extract.

The concentrated extract of single garlic (*Allium sativum* Linn) obtained from Boyolali Regency was 3.02% w/w and the concentrated extract from Karanganyar Regency was 3.21% w/w. The extract yield is considered good if the result obtained is more than 10% [14]. The extract obtained from this study was less than 10%, which may be influenced by several factors such as the stirring and filtering processes. The stirring process aims to allow the solvent to bind the compounds contained in the powder. Stirring during maceration was carried out every 6 hours for a short duration. This stirring process can affect the results obtained; the longer the stirring time or the more repeated stirring, the higher the extract yield obtained [15]. The filtering process also affects the results obtained. The more maceration solvent that is wasted, the lower the extract yield obtained.

Sample drying was carried out by baking to reduce the water content and ensure that the flavonoid compounds were properly extracted. The baking method was chosen because the baking temperature could be controlled. Oven drying of single garlic cloves requires 72 hours or 3 days, while samples from Karanganyar require 120 hours or 5 days at a temperature of 50°C. Drying shrinkage tests showed results of 1.35% for samples from Boyolali and 5.42% for samples from Karanganyar. According to the Indonesian Herbal Pharmacopoeia, the drying shrinkage content of single garlic samples to become dry simplisia is less than 10%, so the simplisia obtained can be considered good. Residual water in crude drugs with a content of more than 10% can become a breeding ground for microbes. In addition, the presence of water can trigger enzymatic reactions that break down active substances, thereby potentially reducing quality and causing damage to simple medicines [16].

The determination of total flavonoid content in single garlic extract was carried out by measuring the wavelength using a UV-Vis spectrophotometer. The purpose of determining the maximum wavelength was to determine the measurement wavelength at which the complex between quercetin and AlCl_3 provided optimum absorbance. [17]. The maximum wavelength of quercetin was determined in the wavelength range of 400-450 nm [18]. The maximum wavelength obtained was 426 nm with an absorbance value of 0.8359.

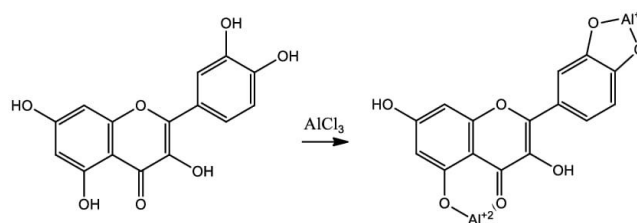


Figure 2. Formation of quercetin and AlCl_3 complex compounds

The operating time aims to determine the most stable measurement time when the sample reacts with the color reagent [19]. The results show that the absorbance begins to stabilize at 30 minutes.

The analysis results obtained a linear equation of $y=0.0151x-0.0033$ with a correlation coefficient $R^2=0.9972$, indicating that the value is linear, which is used to determine the total flavonoid content

in the sample. The average total flavonoid content of single garlic extracts from Boyolali Regency was 0.5867 mgQE/g and from Karanganyar Regency was 0.1495 mgQE/g.

Data analysis used an independent *t*-test to determine whether there was a significant difference in total flavonoid content between Boyolali Regency and Karanganyar Regency. The flavonoid content of single garlic from Boyolali Regency and Karanganyar Regency had a significant value (*p*) of 0.002, which means $p < 0.05$. This indicates a difference in the total flavonoid content of single garlic from Boyolali Regency and Karanganyar Regency.

Although specific studies examining the effect of growing altitude on the total flavonoid content of single clove garlic (*Allium sativum* Linn) are still limited, the findings of this study are consistent with previous research on garlic and other *Allium* species. Several studies have reported that environmental factors associated with altitude such as temperature, light intensity, and ultraviolet (UV) radiation play a significant role in regulating the biosynthesis of flavonoids and other phenolic compounds. Flavonoids function as protective secondary metabolites under stress conditions due to their antioxidant and UV-absorbing properties [20]. Increased exposure to UV radiation at higher altitudes has been shown to stimulate the phenylpropanoid pathway, leading to enhanced flavonoid accumulation in plants. Similar trends have been observed in garlic and related species, where variations in growing conditions resulted in significant differences in phenolic and flavonoid contents [21]. Therefore, the higher total flavonoid content observed in garlic grown at higher altitudes in this study may be attributed to adaptive physiological responses to environmental stress.

The difference in total flavonoid content in single garlic from Boyolali Regency and Karanganyar Regency in this study shows that the total flavonoid content of single garlic from Boyolali Regency is higher. The difference is influenced by the different altitude, soil, air temperature, and rainfall in the two regions. The altitude of the single garlic growing location in Ngemplak Hamlet, Tarubatang Village, Selo Subdistrict, Boyolali Regency is 1341 meters above sea level, while the altitude of the single garlic growing location in Pancot Hamlet, Kalisoro Village, Tawangmangu Subdistrict, Karanganyar Regency is 1128 meters above sea level. The optimal altitude for planting single garlic is 700-1400 meters above sea level [22].

Single garlic requires an air temperature between 15° C-25° C. The average temperature in Boyolali Regency is 23° C and in Karanganyar Regency is 25° C. Rainfall in Boyolali Regency is around 2000 mm/year and rainfall in Karanganyar Regency is around 2,722 mm/year. Single garlic requires rainfall of around 800-2000 mm/year. The type of soil used for growing single garlic in Boyolali Regency is regosol soil. Regosol soil is a type of soil that originates from the weathering of material ejected by volcanic eruptions, while Karanganyar Regency is dominated by andosol soil. Andosol soil is volcanic soil formed from volcanic ash and minerals resulting from volcanic weathering [23].

The flavonoid content or other secondary metabolites in plants are influenced by several factors, including the environment, temperature, light, humidity, pH, soil quality, post-harvest processing such as simple medicine manufacturing, extraction, and others [8]. The total flavonoid chemical content can also be influenced by the altitude at which the plant grows. Safrina's research shows significant differences in comparison of growing altitude [8].

The shortcoming of this study is that during the maceration process, stirring should have been carried out periodically, but the researchers neglected to do so, resulting in sedimentation during the maceration process, which could affect the yield. Another limitation of this study is that the drying process for single garlic from Karanganyar Regency takes longer than the drying process for single

garlic from Boyolali Regency. An excessively long drying process for single garlic can cause a decrease in flavonoid content, due to prolonged exposure to heat, which can damage flavonoid compounds.

4. CONCLUSION

Single garlic extract from Boyolali Regency has a total flavonoid content of 0.5867 mgQE/g, while single garlic extract from Karanganyar Regency has a total flavonoid content of 0.1495 mgQE/g. There is a significant difference in total flavonoid content between single garlic from Boyolali Regency and Karanganyar Regency with a value of 0.002, which means $p < 0.05$.

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Conflict of Interest: The authors declare no conflict of interest

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