α-Mangostin Content of Mangosteen Leaves (*Garcinia mangostana L.*) Based on Different Growing Conditions

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ABSTRACT

Environmental factors, such as soil height, plant age, soil conditions, maintenance, and plant seeds influence the content of secondary metabolites in an organism. The growing conditions can also affect the content of active compounds in plants. Xanthone is an active compound in *Garcinia mangostana* Linn. One of its derivatives is α-mangostin, which has antioxidant, anticancer, antituberculosis and antihistamine effects. This study aimed to determine the effects of growing conditions on the levels of α-mangosteen in mangosteen leaf extract using Thin Layer Chromatography-Densitometry. Standard solution of α-mangosteen and ethyl acetate extract of mangosteen leaves (A and B samples) were analyzed with Camag TLC Scanner 3 using silica gel 60 F254 stationary phase and chloroform-ethyl acetate mobile phase (9:1). The results showed that the Rf values for standard solution of α-mangostin, sample A, and sample B were 0.65, 0.62, and 0.62, respectively. Meanwhile, the levels obtained from samples A and B were 2.10% ± 0.0755 and 2.07% ± 0.0321, respectively. Different growing conditions did not affect the level of α-mangostin ethyl acetate extract of mangosteen leaves.

Keywords: α-mangosteen; Planting conditions; TLC-Densitometry

ABSTRAK

Metabolit sekunder pada suatu organisme dipengaruhi oleh aspek area seperti ketinggian tanah, umur tumbuhan, keadaan tanah, pemeliharaan serta bibit tumbuhan. Keadaan tanam tersebut juga dapat berpangaruh terhadap kandungan senyawa aktif dalam tanaman. Xanton ialah senyawa Aktif pada manggis (Garcinia mangostana Linn). Salah satu turunannya merupakan α-mangostin mempunyai aktivitas antioksidan, antikanker, antituberkulosis dan efek antihistamin. Penelitian ini bertujuan untuk mengetahui pengaruh kondisi tanam terhadap kadar α-mangostin ekstrak daun manggis menggunakan Kromatografi Lapis Tipis-Densitometri. Standar α-mangostin, ekstrak etil asetat daun manggis A dan B di analisis dengan Camag TLC Scanner 3 menggunakan fase diam silika gel 60 F_{254} dan fase gerak kloroform-etil asetat (9:1). Hasil analisis menunjukkan nilai Rf untuk standar α-mangostin, sampel A dan B masing-masing adalah 0,65; 0,62 dan 0,62. Sedangkan kadar yang diperoleh dari sampel A dan B masing-masing adalah 2,10% ± 0,0755 dan 2,07% ± 0,0321. Kadar α-mangostin ekstrak etil asetat daun manggis tidak dipengaruhi oleh kondisi tanam yang berbeda.

Kata kunci: *α-mangostin; Kondisi tanam; KLT-Densitometri*

INTRODUCTION

Secondary metabolites in an organism are influenced by several environmental aspects, including differences in morphology, light, nutrients, composition of planting media, plant tissue used, and biosynthesis processes (Nurfitriani et al., 2017). Plant maturity levels and age can also influence secondary metabolites in plants (Pantria et al., 2020; Supriatna et al., 2019; Ali et al., 2022). The factors affecting the quality of the active compounds contained in plants are external and internal. External factors include growing conditions, climate, altitude, environmental contamination, pests and diseases, temperature and humidity, and intensity of ultraviolet light. Meanwhile, internal factors include genetic quality and age (Wahyuni et al., 2021). Several examples of research have proven that external factors affect the content of secondary metabolites in the test material. Citronella stalks (*Cymbopogon nardus* L. Rendle) planted in the lowland areas of Denpasar and Bedugul highlands have different essential oil contents. The n-Hexadecanoic acid and the Driman-8,11-diol

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compounds were not found in citronella stems that grow in Bedugul highlands. However, the presence of the Selina-6-en-4-ol compound can still be identified at the two locations where these plants grow (Dacosta et al., 2017). The addition of Gandasil D-type fertilizer significantly affected the number of leaves, plant height, and fresh weight of red spinach plants (Manurung et al., 2020). The use of humic acid can not only improve the quality of the planting medium so that plant growth can increase, but it can also increase the growth and polyphenol content of binahong leaves. Polyphenols are one of the compounds that can provide antioxidant activity (Riyandi et al., 2020). The antibacterial activity of essential oil from lemongrass in inhibiting *Streptococcus mutans* is best found in the highlands (935 m above sea level) compared to the lowlands (Panaungi et al., 2019). The differences in age and environmental conditions in the epidermis of the saplings and the bark of the *Rhizophora mucronata* mangrove trees cause the levels of flavonoid and phenolic compounds contained in the two parts of the plant to be different (Supriatna et al., 2019).

Mangosteen (*Garcinia mangostana* Linn) is cultivated in Southeast Asian countries, including Indonesia (Wathoni et al., 2021; Pangow et al., 2018). In Tasikmalaya, many mangosteen plants are planted because of their high economic value. Mangosteen leaves contain saponins, triterpenoids, tannins, and flavonoids (Pangow et al., 2018; Nurfiana et al., 2018). Meanwhile, there are saponins, flavonoids, and steroids in extracts and fractions of mangosteen leaves (Turahman & Sari, 2018; Nurfiana et al., 2017). The active compounds abundant in the mangosteen plant are xanthone derivatives (Maligan et al., 2019; Maftucha et al., 2022). Xanthones have biological activity as anti-inflammatory, antioxidant, antibacterial, and antimicrobial (Turahman & Sari, 2018). Xanthones are included in the class of flavonoid compounds (Kurniawan, 2020b). The main compounds of xanthones are α-mangostin and α-mangostin (Rena et al., 2022; Maftucha et al., 2022; Maligan et al., 2019). α-mangostin is a yellow amorphous crystal with a melting point of 180-182°C, giving it absorption in UV light at αmax of 215, 243, and 317 nm. The α-mangostin compound has anticancer, antituberculosis, antihistamine, and antioxidant activity (Sri Wahyuni et al., 2018; Maligan et al., 2019).

Research on the effects of external and internal plant factors on compound content has been carried out. However, the impact of α-mangostin compound on mangosteen plants has never been studied. Thus, this research aimed to determine α-mangostin levels based on differences in growing conditions using TLC-densitometry.

MATERIALS AND METHOD

Mangosteen Leaves Sampling

The sampling consisted of two dark green mangosteen leaves (*Garcinia mangostana* Linn) from different districts. Sample A is mangosteen leaves from Karangjaya, and Sample B is Puspahiang. The two areas are included in the Tasikmalaya Regency, West Java Province. Karangjaya and Puspahiang are the cultivation centers and producers of mangosteen fruits for export commodities. There are differences in the growing conditions of the two regions. The mangosteen plants growing in Karangjaya are more than 50 years old. They are not from certified mother trees, growing on clay soil at 725 meters above sea level, and the maintenance and fertilization are not performed productively. Meanwhile, on average, the mangosteen plants growing in Puspahiang are 17 years old. They come from certified mother trees, growing on sandy loam soil at 600 meters above sea level, and the maintenance and fertilization are performed productively.

Preparation, processing, and extraction of the

samples

Mangosteen leaves were collected, wet sorted to remove dirt, and washed under running water. The samples were dried, sorted, and ground to obtain powder (Siahaan et al., 2019). 500 g of simplicia powder was extracted using the maceration method with n-hexane solvent. The residue was then extracted with ethyl acetate solvent using a macerator (Kurniawan, 2020a). The extract was evaporated using a rotary evaporator until the extract was obtained. This extraction method was selected based on several studies on the isolation of α-mangostin, which was conducted using the maceration method (Kurniawan, 2020a; Wijayanti et al., 2017).

Simplicia quality parameter testing

Testing on simplicial quality parameters includes macroscopic examination, microscopic examination, screening of flavonoid compound groups, determination of total ash content, drying shrinkage, water-soluble essence content, ethanol soluble essence content, and water content calculation. The testing on simplicia quality parameters is essential to determine the identity and quality of the samples used.

Preparation of α-mangostin stock solution

The main solution of α-mangostin mains solution was obtained from Markherb Bandung Indonesia, made in a concentration of 1000 µg/mL by weighing 10 mg of pure α-mangostin standard, put into a 10 mL volumetric flask then added methanol to the boundary mark and then made a dilution until the concentration of α-mangostin was obtained standard.

Analysis of α-mangostin levels using thin-layer chromatography-densitometry

Analysis of the levels of ethyl acetate extract samples of mangosteen leaves using thin layer chromatography-densitometry was carried out by spotting α-mangostin standard, sample A of ethyl acetate extract, and sample B of ethyl acetate extract using a 100 µL capillary pipette on an 8 x 4 com silica gel 60 F254 TLC plate with an eluent travel distance of 6 cm and the distance between spots of 1 cm. The TLC plate was then inserted into a chamber containing a saturated mobile phase of chloroform and ethyl acetate (9:1). The chamber was closed, and the mobile phase was allowed to reach its expansion limit. After drying, the TLC plate was viewed under a UV lamp, and then the Rf value obtained was scanned with a Camag TLC Scanner 3 at a maximum wavelength of 254 nm (Andayani & Ismed, 2017).

Data Analysis

The data analysis used was statistical analysis with a t-test. This analysis was used to test whether there was a significant difference in the levels of α-mangostin in the ethyl acetate extract of mangosteen leaves A and the ethyl acetate extract of mangosteen leaves B. The type of t-test used was a separate t-test (Independent Sample t-test) performed with SPSS 16.0 for Windows.

RESULTS AND DISCUSSION

Macroscopic testing was conducted on mangosteen leaves Simplicia powder, samples A and B. This test was carried out by observing the organoleptic of the simplicial, such as shape, color, taste, and smell. The macroscopic test results for samples A and B had the same organoleptic results, which were dark green in color, a slightly black taste, and a distinctive smell. In microscopic testing, which can be seen in Figure 1 and Figure 2, both samples show the same fragment shape. The results of this test indicate that the two samples come from the same species, namely *Garcinia mangostana* L.

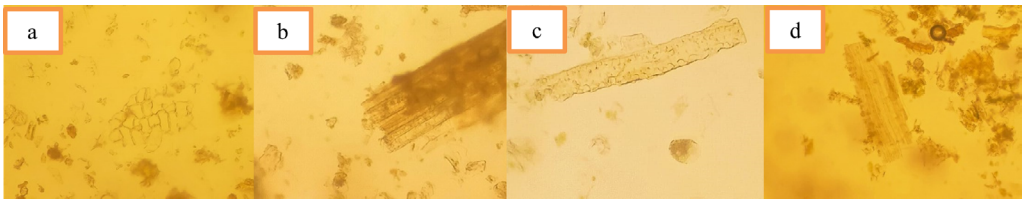

Figure 1. Microscopic results of mangosteen leaf powder sample A: (a) upper epidermis; (b) xylem fibers; (c) tracheid fibers; (d) vascular bundles with 100x magnification.

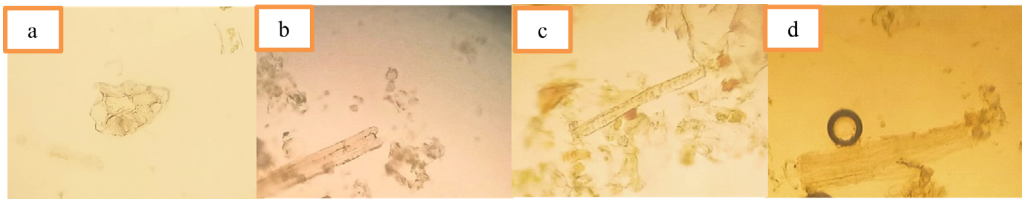

Figure 2. Microscopic results of mangosteen leaf powder sample B: (a) upper epidermis; (b) xylem fibers; (c) tracheid fibers; (d) vascular bundles with 100x magnification.

The microscopic test is one of a series of tests on simplicia quality parameters to ensure the validity of the tested material. The results showed several identifying fragments that had similar shapes to the mangosteen leaf fragments in the MMI Library. (Depkes RI, 1995). Apart from that, the validity of the material was also strengthened from the results of determinations carried out at the Jatinangor Herbarium, Plant Taxonomy Laboratory, Biology Department, FMIPA UNPAD, with identity No.38/HB/01/2021.

Microscopic testing is also an initial identification process to determine the presence of secondary metabolite content, especially the compound that is the test's target. The presence of identified fragments in plant parts indicates where the biosynthetic process of a particular class of compounds occurs. Flavonoids are often found in epidermal cells. Most of the flavonoids are collected in plant cell vacuoles even though the place of synthesis is outside the vacuole (Salisbury FB, 1995).

Table 1. The results of the test on the quality parameters of mangosteen leaves Simplicia A and B

Parameter	Sample A	Sample B
Water-soluble essence content	19.93 ± 0.04	17.46 ± 0.02
Ethanol-soluble essence content	29.46 ± 0.03	26.23 ± 0.03
Drying shrinkage	9.37 ± 0.02	9.44 ± 0.003
Water content	8 ± 0.00	6 ± 0.00
Total ash content	3.695 ± 0.007	3.83 ± 0.05
Flavonoid	+	+

Remarks: (+) identified (-) unidentified

Overall, the two samples of mangosteen leaves have fairly good simplicia quality (Table 1), indicated by small values of water content, total ash content, and drying shrinkage. Based on the Indonesian Herbal Pharmacopoeia, the water content in a simplicia should not be more than 10%. The ash content provides an overview of the internal and external mineral content from the initial process until the simplicia is formed so that it can determine the impurity level of a simplicia by metals and silicates. Meanwhile, the drying shrinkage value is the maximum content of compounds that quickly evaporate or are lost during the drying process. The drying shrinkage value is identical to the water content if the material does not contain essential oils or volatile compounds (Kementrian Kesehatan Republik Indonesia., 2011). The essence content of sample A was higher than that of sample B. This is possible because the locations and growing conditions of the two samples are different. Different growth areas will produce differences in the percentage composition of chemical compounds contained in a plant (Paramita et al.,

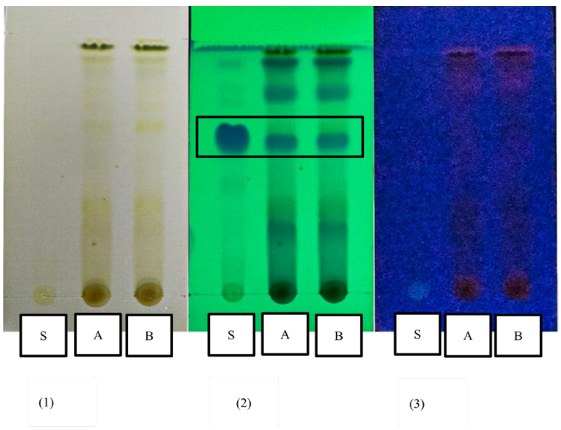

Figure 3. Chromatogram of ethyl acetate extract of mangosteen leaves using silica gel plate F254 stationary phase and chloroform-ethyl acetate mobile phase (9:1): (1) visible light; (2) UV254 nm light; (3) UV366 nm light; (S) α-mangostin standard; (A) sample A; (B) sample B

2019). For example, compounds dissolved in 96% ethanol and those dissolved in water in *Jatropha multifida* L plants originating from Kediri, Nganjuk, and Madiun have different levels (Aryantini et al., 2020). However, there are also plant compounds that are not affected by maintenance factors, for example, manggarsih plants (*Parameria laevigata* (Juss) Moldenke) whose leaves, stems and roots, both from nature and ex-situ cultivation in Banjarbaru, contain saponins, quinones, tannins, steroids, flavonoids, quinones, alkaloids and triterpenoids (Barus et al., 2019).

The main elements of xanthones are α-mangostin and α-mangostin(Rena et al., 2022; Maftucha et al., 2022; Maligan et al., 2019). Thus, a flavonoid examination is necessary to find out the α-mangostin compound from the sample. Flavonoid screening was done twice (on simplicia and leaves extract of mangosteen). The results showed that the simplicia and ethyl acetate extract of mangosteen leaves of samples A and B showed the presence of flavonoid compounds. Accordingly, the following testing was carried out: identifying the α-mangostin compound using Thin Layer Chromatography (TLC). Qualitatively, the results of examining the test samples using F254 silica gel TLC with the mobile phase chloroform-ethyl acetate (9:1) showed that samples A and B contained one spot with an Rf distance that was almost the same as that of the α-mangostin standard as seen in Figure 3.

TLC plates were analyzed using densitometry to detect Rf values. The spot was then scanned with the TLC Scanner. The densitometry method is a method for determining the levels of a substance that has been previously analyzed using TLC. The working principle of densitometry is calculating the area (AUC) and chromatogram on the TLC plate. This was also proven by the results of the densitogram on each spot, where a peak was formed on the 2-dimensional densitogram, as seen in Figure 4.

Blots scanned with the TLC Scanner show Rf 0.65 for standard and 0.62 for samples A and B. The AUC value can be determined by showing the densitogram of each thin-layer chromatography band image. A baseline is created at each peak of the resulting densitogram, generating an AUC

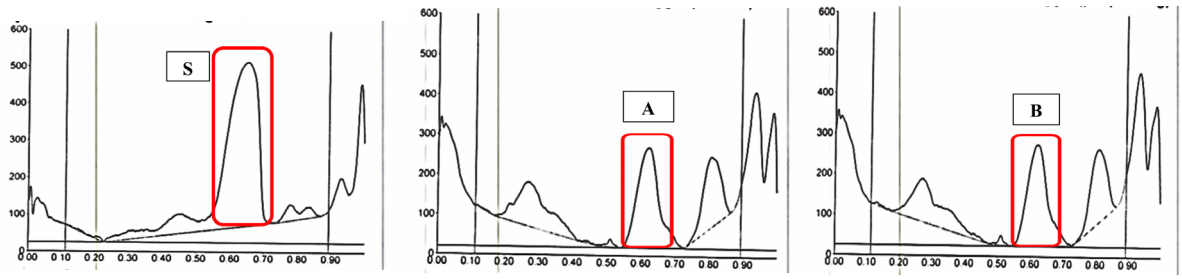

Figure 4. Standard and sample spectra results using densitometry with AUC at α 254 nm: (S) AUC of α-mangostin standard; (A) AUC of ethyl acetate extract of sample A; (B) AUC of ethyl acetate extract of sample B

Table 2. Data of α-mangostin Standard Curve

Standard series (ppm)	Area (AUC) (μg jam/mL)
25	1264.1
50	3661.4
100	5658.8
200	10423.5
250	12641.9
300	15575.7

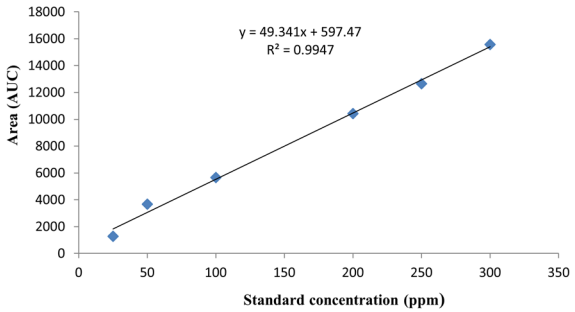

Figure 5. The standard curve between the standard concentration of α -mangostin (ppm) and area (AUC)

value automatically. The AUC value depends on the intensity of the color reflected by the image component of the band. The intensity of the color that is getting brighter results from the greater concentration of these components, producing a higher peak. The correlation coefficient shows the correlation between concentration and measurement reaction, either the Area Under Curve (AUC) or peak height (Ihsan et al., 2020). The reaction measurement results for each standard level of α-mangostin are presented in Table 2.

The curve of the relationship between α-mangostin concentration and area (AUC) can be seen in Figure 5. The x-axis is concentration, and the y-axis is area (AUC). The resulting standard curve equation is y = 49.341x + 597.47 with an r-value of 0.9973. The (r) value is close to 1, which means the concentration is directly proportional to the peak area (AUC).

Samples A and B had α-mangostin levels of 2.10 ± 0.0755% and 2.07 ± 0.0321%, respectively (Tables 3 and 4). Referring to the results obtained using the t-test, Sig (2-tailed) > 0.05, there was no significant difference between the levels of α-mangostin in sample A and sample B, meaning that the growing conditions, such as soil type, controlled or not controlled maintenance, fertilization, altitude, and tree age, did not affect the levels of α-mangostin in mangosteen leaves. Several studies have shown that external and internal plant factors have different effects on the levels of these compounds. The addition of Gandasil D fertilizer did not significantly affect chlorophyll and carotenoid content in red spinach (Manurung et al., 2020). A different result was found in the xanthorrizole compound. Temulawak contains xanthorrizol, which is known to have a strong effect as an antibacterial. Curcuma plants that grow in the highlands (around 800 meters above sea level) tend to have higher xanthorrizol content (Rahman et al., 2022). Likewise, in the babadotan plant (*Ageratum Conyzoides* L.), there were differences in the secondary metabolite content of babadotan at different altitudes. The saponin content in babadotan plants was found in the middle plains (700 m asl) but was not found

Table 3. Data on α-mangostin levels in sample A (Karangjaya)

Sample	Peak Area	Concentration (μg/mL)	%Content	Average %Content	Standard deviation
A1	11011.1	211.0543	2.11		
A2	11315.0	217.2135	2.17	2.10	0.0755
A3	10545.1	201.6098	2.02		

Table 4. Data on α-mangostin levels in sample B (Puspahiang)

Sample	Peak Area	Concentration (μg/mL)	%Content	Average %Content	Standard deviation
B1	10677.2	204.0844	2.04		
B2	10936.8	209.5484	2.09	2.07	0.0321
B3	10970.7	210.2355	2.10		

in the lowlands (320 m asl) (Katuuk et al., 2019).

CONCLUSIONS

Different growing conditions did not influence the levels of α-mangostin from ethyl acetate extract of mangosteen leaves. This is based on the results of measurements of α-mangostin levels in samples A and B, which did not differ significantly, namely 2.10% ± 0.0755 and 2.07% ± 0.0321, respectively.

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