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Effect of regional variation on the total flavonoid level of ethanol extract of mangosteen (*Garcinia mangostana*) peels

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ABSTRACT

Background: Currently, traditional medicines have been widely used by the public. One of them is mangosteen peel. Extract of mangosteen peel contains alkaloids, glycosides, steroids, flavonoids, polyphenols, and tannins. In order to ensure the quality of the extract so that its chemical content can be guaranteed, it is necessary to standardize the quality of the extract that consists of specific parameters.

Objective: This study aims to determine the specific parameters using Thin-Layer Chromatography (TLC) and total flavonoid level of the ethanol extract of mangosteen peel from Kalimantan, Java, and Sumatra.

Methods: This research was an explorative study. Extraction technique used was maceration with 70% ethanol solvent. The tested specific parameters, namely the identity of the extract with TLC and total flavonoid level, were determined afterward using visible spectrophotometry with the AlCl3 reagent.

Results: The result of the qualitative test with TLC showed that the extract of mangosteen peel contains flavonoids, terpenoids, and anthraquinones. Total flavonoid level of ethanol extract of mangonsteen peel from Kalimantan, Java, and Sumatra were (0.301 ± 0.009); (0.398 ± 0.015); (0,747 ± 0,010) mg QE/g extract, respectively.

Conclusion: Extract of mangosteen peel contains flavonoids, terpenoids, and anthraquinones. Total flavonoid level of ethanol extract of mangonsteen peel from Kalimantan, Java, and Sumatra were (0.301 ± 0.009); (0.398 ± 0.015); (0,747 ± 0,010) mg QE/g of extract, respectively.

Latar Belakang: *Saat ini obat tradisional telah banyak digunakan oleh masyarakat. Salah satunya adalah kulit buah manggis. Ekstrak kulit buah manggis diketahui mengandung alkaloid, glikosida, steroid, flavonoid, polifenol dan tanin. Dalam rangka menjamin mutu ekstrak agar kadar kandungan kimia ekstrak dapat terjamin maka perlu dilakukan standardisasi mutu ekstrak yang terdiri atas paramater spesifik.*

Tujuan: *Penelitian ini bertujuan untuk mengetahui parameter spesifik dengan uji Kromatografi Lapis Tipis (KLT) dan kadar flavonoid total ekstrak etanol kulit buah manggis dari Kalimantan, Jawa, dan Sumatra.*

Metode: *Penelitian ini termasuk jenis penelitian eksploratif. Teknik ekstraksi yang digunakan adalah maserasi dengan pelarut etanol 70% yang kemudian diuji parameter spesifik yaitu identitas ekstrak dengan uji KLT dan kadar flavonoid total yang ditetapkan secara spektrofotometri visibel dengan pereaksi $AlCl_3$.*

Hasil: *Hasil penelitian uji kualitatif dengan KLT menunjukkan bahwa ekstrak kulit buah manggis mengandung senyawa flavonoid, terpenoid, dan antrakuinon; kadar flavonoid total berturut-turut dari*

Kalimantan, Jawa, dan Sumatra adalah : (0,301 ± 0,009); (0,398 ± 0,015); (0,747 ± 0,010) mgQE/g ekstrak.

Kesimpulan: *Ekstrak etanol kulit buah manggis (Garcinia mangostana L.) mengandung flavonoid, antrakuinon, dan terpenoid. Kadar flavonoid total ekstrak etanol kulit buah manggis dari Kalimanatan, Jawa, dan Sumatra secara berturut-turut adalah (0,301 ± 0,009); (0,398 ± 0,015); dan(0,747 ± 0,010) mg QE/g ekstrak.*

INTRODUCTION

Nowadays, traditional medicines have been widely used in public. One of them is mangosteen peel. Mangosteen plant (*Garcinia mangostana* L.) grows in tropical regions and has high adaptability.[1] In Indonesia, mangosteen plant grows in lowland areas up to 600 meters from sea level.[2] Part of this plant that is often used as medicine is the peel of its fruit. Ethanol extract of mangosteen peel is known to contain alkaloids, glycosides, steroids, flavonoids, polyphenols, and tannins that are responsible for its pharmacological activity.[3,4]

In order to ensure the quality of the extract so that its chemical content can be guaranteed, it is necessary to standardize the quality of the extract that consists of specific parameters.[5] Standardization of plant extract is one of the important stages in the development of native medicine in Indonesia that has more than 3000 types of medicinal plant with more than 1000 of these plants utilized in the industry of traditional medicine.[6]

One of the factors that affects the quality of an extract is the location of origin of the plant, meaning the environment in which the plant interacts in the form of energy (weather, temperature, and light).[7] Mangosteen plant grows in almost every province in Indonesia.[8] Until now, the standardization of simplicia and peel extract of mangosteen (*Garcinia mangostana* L.) is still incomplete. One of the quality parameters of an extract chemically is the active compound content of the extract. Therefore, it is necessary to determine specific parameters of content test using TLC and total flavonoid level of ethanol extract of mangosteen peel based on the variation of its original region.[9]

Flavonoids are one of the most common groups of secondary metabolite compounds found in plant tissues. Flavonoids belong to the phenolic class with the chemical structure C6-C3-C6. The skeleton consists of one aromatic ring A, one aromatic ring B, and an oxygen-containing heterocyclic middle ring (Figure 1).[10]

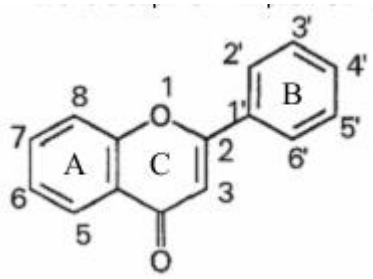


Figure 1. Flavonoid structure[11]

Identification methods for flavonoid are strongly related to its molecular structure. If there is no interfering tissue, plant tissue can be tested by steaming it with ammonia vapor. Yellow color indicates the presence of flavonoids that form quinoid compounds.[12,13] Determination of total flavonoid levels based on the general standard parameters for medicinal plant extract from the Ministry of Health of the Republic of Indonesia is done using visible-light spectrophotometric method.[7] This method involves the formation of a complex between flavonoids and AlCl3 that forms a stable complex with the keto group C4 and the hydroxyl groups of C3 or C5 in flavones and flavonols. These potentials are represented by their hydroxyl groups which are capable of capturing free radicals.[14]

Spectrophotometry is a method of measurement based on the interaction between atoms or molecules and electromagnetic

radiation, based on the fact that chemical substances selectively scatter, absorb, and emit electromagnetic energy at different wavelengths. In most laboratories, the wavelength used for measurement invisible light is 400-700 nm.[15]

METHODS

Materials

The main materials used in this study was mangosteen peel powder (*Garcinia mangostana* L.) from Pekalongan (Central Java), Pekanbaru (Sumatra), and Martapura (Kalimantan), as well as 70% technical ethanol (Brataco) as extracting liquid. TLC test was carried out using stationary phase of F254 silica gel (Merck), the mobile phase of n-butanol, acetic acid, water, ethyl acetate, chloroform (JT. Baker), and 10% $AlCl_3$ spray reagent, 10% KOH reagent, 10% H_2SO_4 reagent. The chemicals used in the assay were ethanol p.a (Merck), standard quercetin (Sigma), 2% $AlCl_3.6H_2O$ solution (Merck), and aquadest (Brataco Chemica).

Tools

Tools used in this study include a set of maseration tools, 20, 30 and 50 Mesh sieves, rotary evaporator (Heidolph), vacuum, analytical scales, glassware (Pyrex), Halogen Moisture Analyzer (Mettler Toledo), UV-Vis Spectrophotometer (Pharmaspec 1700, SHIMADZU), hot plate (KIA), a set of tools for ELT elution, UV_{254} and UV_{366} lamps, and spray tools.

Preparation of Mangosteen Peel Powder and Ethanol Extract of Mangosteen Fruit Peel

Mangosteen peel is cut into small pieces, dried in an oven at 50°C, mashed then the powder was sieved using Sieving Machine with a multilevel sieve until powder with the size of 30/50 mesh is obtained. Furthermore, mangosteen peel powder was weighed to 200 grams, poured into 1000 ml beaker glass and maserated using 400 ml of 70% ethanol, then the mixture was stirred using the electric mixer for 6 hours at 300 rpm. The maserate was evaporated afterward with a Rotary Evaporator at a temperature of 60°C until viscous extract was obtained. The viscosity of the extract is marked by the vanishing of the smell of ethanol while the extract can still be poured. The viscous extract was used for the TLC test and the determination test of total flavonoid level.

Qualitative Test Using TLC

Qualitative test to determine flavonoid level was performed by dissolving the extract with ethanol, stationary phase of F_{254} silica gel, mobile phase of n-butanol : acetic acid : water (3:1:1). The elution results were sprayed with 10% $AlCl_3$ spray reagent. Qualitative test to determine anthraquinone level was performed by dissolving the extract with ethanol, stationary phase of F_{254} silica gel, and the mobile phase of methanol : ethyl acetate : water (2,7:20:2). The elution results were sprayed with 10% KOH solution spray reagent. Qualitative test to determine terpenoid level was performed by dissolving the extract with ethanol, stationary phase of F_{254} silica gel, and mobile phase of chloroform : methanol (1:3). The elution results were sprayed with 10% H_2SO_4 solution spray reagent.

Quantitative Test of Total Flavonoid with Standard Quercetin

Determination of Operating Time (OT): 1.0 ml of 2% $AlCl_3$ was added to 15 µg/ml of standard solution of quercetin, then its absorbance was measured at the start of the addition of $AlCl_3$ 2% until a stable absorbance time was reached, at a wavelength of 440 Nm for 90 minutes, as mentioned in the literature.[16]

Determination of Wavelength of Maximum Absorbance: 1.0 ml of 2% AlCl3 was added to 15 µg/ml of the standard solution of quercetin, then the mixture was left until OT time before its absorbance was measured at a range of wavelengths between 300-600 nm.

Preparation of the Solution for Quercetin Standard Curve: 0.2 mg/ml quercetin parent solution was prepared in series of concentrations of 5, 7, 9, 11, 13, 15, 17, 19 µg/ml. Each of these solutions was taken at a volume of 1.0 ml and put into test tubes, then 1.0 ml of $AlCl_3$ 2% was added. Measurement of the solution was performed at operating time and at a wavelength of maximum absorbance.

Determination of Total Flavonoid Level: Ethanol extract of mangosteen peel was

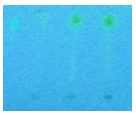
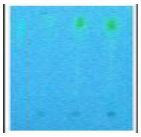
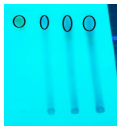
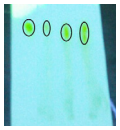
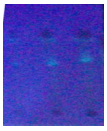
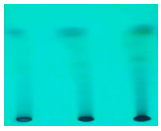
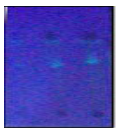
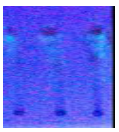
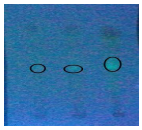
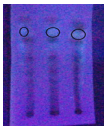
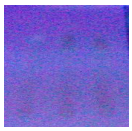
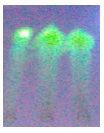
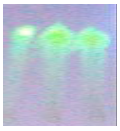
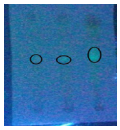
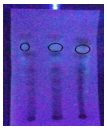
weighed to 50.0 mg and mixed with ethanol p.a to 10.0 ml. A volume of 1.0 ml was taken from the solution than 1.0 ml AlCl3 2% was added. The mixture was left at operating time before its absorbance was read at a wavelength of maximum absorbance.

RESULTS

TLC Test Results

TLC test was performed to confirm the results obtained from phytochemical screening. Because it served as a confirmation, the TLC test was only performed for groups of compounds that showed positive results. TLC test performed in this

Table 1. TLC Test of Flavonoid, Anthraquinon, and Terpenoid Compounds from Kalimantan, Java, and Sumatra

Compound/ detection	Region of origin	Before spraying	After spraying	Annotations
Flavonoid Sprayed using AlCl3 reagent, in visible light	Kalimantan			Yellow fluorescence in UV_{254}.[17] Flavonoid (+)
	Sumatra			
	Jawa			
Anthraquinone Sprayed using 10% KOH solution reagent	Kalimantan			Yellow fluorescence spots were found in UV_{366}.[18] Anthraquinone (+)
	Sumatra			
	Jawa			
Terpenoid Sprayed using 10% H_2SO_4	Kalimantan			Green fluorescence in UV_{366}.[19] Terpenoid (+)
	Sumatra			
	Jawa			

experiment included flavonoid, anthraquinone, and terpenoid compounds (Table 1).

Determination of Total Flavonoid Level
Determination of Operating Time (OT)

Determination of operating time is meant to establish the appropriate time to measure the absorbance of the extract solution that was supposed to be performed when the absorbance was stable. Results of this study showed that OT was established at the 22nd-23rd minute when the absorbance was between 0.497-0.5002.

Determination of Wavelength of Maximum Absorbance

According to Ganjar and Rohman (2007), wavelength of maximum absorbance is determined to obtain maximum results in the determination of content level. The theoretical wavelength of maximum absorbance for total flavonoid compounds is 440 nm. Results of this study showed that the wavelength of maximum absorbance was at 430.60 nm with an absorbance of 0.531.

Formulation of Quercetin Standard Curve

The formulation of standard curve is meant to determine the correlation between quercetin concentration and absorbance. The results of standard curve calculation are shown in Table 2.

Table 2. Data of Absorbance of Standard Quercetin at Different Concentrations

No	Concentration (mg/ml)	Absorbance
1	0,007	0,204
2	0,011	0,298
3	0,013	0,352
4	0,015	0,449
5	0,017	0,504
6	0,019	0,664

Based on the data in Table I, the correlation coefficient (R) is 0.9730 with the equation: y = 3.6757x - 0.091, and table r 0.8114 (n = 6 and p = 95%). It can be concluded that arithmetic r is greater than table r so that the line equation obtained shows a significant relationship

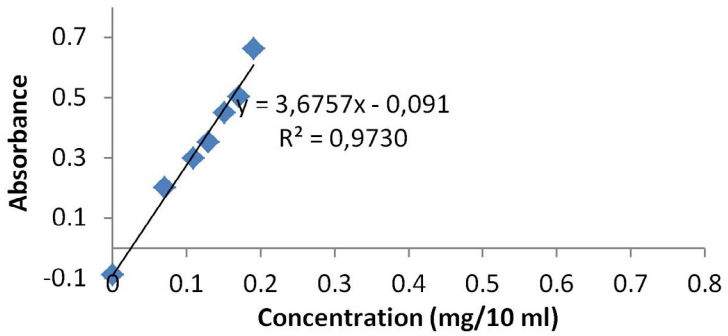


Figure 2. Graph of Correlation between Concentration and Absorbance of Quercetin Solution

between the concentration of standard quercetin solution and absorbance (Figure 2).

Determination of Total Flavonoid Level

Determination of total flavonoid level in the sample was done by diluting the viscous extract with ethanol p.a. Afterward, 2% $AlCl_3$ reagent was added to form a yellow solution. This color formation occurs because of the formation of a complex between flavonoids and $AlCl_3$ so that the determination of the levels can be performed using a visible spectrophotometric method. According to Mabry et al. (1970), the reaction between $AlCl_3$ and hydroxy groups forms a complex that is unstable in the acidic atmosphere, whereas the reaction between $AlCl_3$ and the hydroxy carbonyl group forms a complex that is stable even with the addition of acid.[21] The complex-forming reaction between flavonoids and $AlCl_3$ can be seen in Figure 3. Table III presents the results of the determination of flavonoid level. The mean diagrams of total flavonoid levels from Kalimantan, Java, and

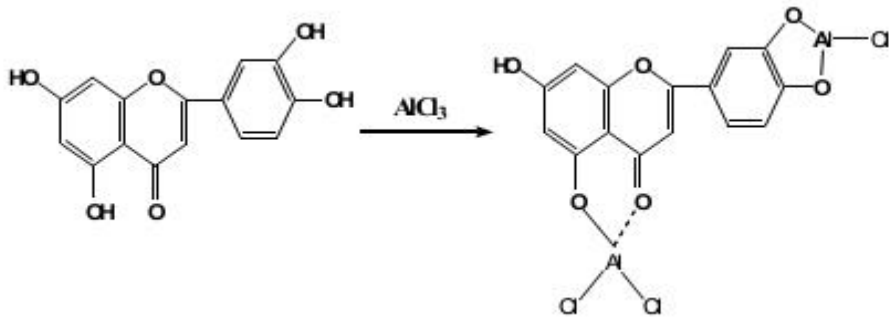


Figure 3. Complex-forming reaction between flavonoid and aluminum

Table 3. Total Flavonoid Level of the Extract of Mangosteen Peel Based on Varying Regions of Origin

Region of origin	Replica-tion	Weight of extract (mg)	Absorbance	Total flavonoid level (mg QE/g of extract)	((ū) ± LE) mg QE/g of extract CV %
Sumatra	1	50.1	0.514	0.764	
	2	50.1	0.524	0.778	0.747 ± 0.010
	3	49.9	0.507	0.756	
	4	50.2	0.503	0.746	3,96
	5	49.9	0.501	0.748	
	6	50.4	0.468	0.692	
Java	1	50,2	0,673	0,414	0,398 ± 0,015
	2	50,1	0,639	0,396	
	3	50,1	0,633	0,393	2,76
	4	50,0	0,623	0,388	
Kalimantan	1	50,2	0,499	0,319	
	2	50,1	0,439	0,288	0,301 ± 0,009
	3	50,4	0,468	0,301	
	4	50,1	0,496	0,318	3,95
	5	50,5	0,489	0,312	
	6	49,7	0,481	0,313	

Annotation: ū = Average price of total flavonoid level; LE = Limit of Error; QE = Quercetin Equivalent

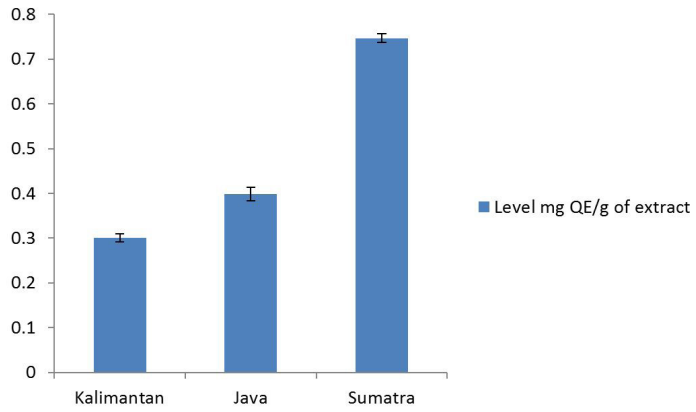


Figure 4. Diagrams of average total flavonoid levels of Kalimantan, Java, and Sumatra

Sumatra are presented in Figure 4.

DISCUSSIONS

Extract of mangosteen peel contains flavonoids, terpenoids, and anthraquinones. The highest level of flavonoid was obtained from the mangosteen peel of Sumatra at (0.747 ± 0.010) mg QE/g of extract, followed by Java at (0.398 ± 0.015) mg QE/g of extract, and the smallest was from Kalimantan at (0.301 ± 0.009) mg QE/g of extract. From the price of CV, the smallest was of Java at 2.76%. CV price shows the homogeneity of the results obtained. Differences in flavonoid levels are influenced by the growing areas of the mangosteen plant. Differences in geographical location of the plant, as well as climate change, environmental conditions, cultivation mode, harvest time, and post-harvest processing may affect the variation of secondary metabolite content of a plant.[22]

CONCLUSIONS

Ethanol extract of mangosteen peel (*Garcinia mangostana* L.) contains flavonoids, athraquinones, and terpenoids. Total flavonoid level of ethanol extract of mangonsteen peel from Kalimantan, Java, and Sumatra were (0.301 ± 0.009); (0.398 ± 0.015); (0,747 ± 0,010) mg QE/g of extract, respectively.

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