



Total Phenolic, Flavonoid, and LC-MS Analysis of the Ethanolic Extract of Matoa (*Pometia pinnata*) Leaves from Kudus, Central Java, Indonesia

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Abstract

Matoa (*Pometia pinnata*) is a plant used as a floral identity in Indonesia, especially in Papua. Local people use boiled water from matoa leaves, which is believed to treat hypertension. Matoa leaves are traditionally used as a therapeutic agent for burns and wounds in Indonesia. Phytochemical screening of matoa leaf extract contains secondary metabolite compounds, including flavonoids, steroids, tannins, and saponins. So far, many of these activities have been reported from the polyphenol group. This research aimed to identify the phytochemical composition of matoa leaves from Kudus in the ethanolic extract using LC-MS. The total phenolic content of ethanol extract using the Folin Ciocalteu method was 35.689 ± 0.726 mg GAE/g extract. The total flavonoid content of ethanol extract using the $AlCl_3$ colorimetric method was 1.384 ± 0.012 mg QE/g extract. Twelve compounds could be identified in the ethanol extract of matoa leaves. The phenolic compounds were vanillin, p-hydroxybenzaldehyde, p-Coumaroyl glycolic acid, syringic acid, gallic acid, phenol, and vanillic acid. The flavonoid compounds were identified as epigallocatechin and apigenin -7-O-diglucuronide. The organic compound was identified as jasmonic acid. The aromatic compound was identified as benzene. Besides, tannin compounds were also identified. The major compounds in matoa leaves from Kudus, Central Java, are vanillin, phenol, and benzene.

1. Introduction

Matoa (*Pometia pinnata*) is a member of the Sapindaceae family, which is widespread in the Asia Pacific, including Malaysia, Indonesia, Philippines, Papua New Guinea, Solomon Islands, Fiji, and Tonga [1]. This plant is used as a floral identity in Indonesia, especially in Papua [2]. Phytochemical screening of matoa leaf extract contains secondary metabolite compounds, including flavonoids, steroids, tannins, and saponins [3]. Matoa leaves are traditionally used as a therapeutic agent for burns and wounds in Indonesia. Local people use boiled water from matoa leaves, which is believed to treat hypertension [4].

Research has been carried out on matoa leaf extract containing flavonoid compounds by observing the

hypertension profile in the Angiotensin Receptor Blocker class. The results showed that the percentage reduction in blood volume of mice given the best matoa leaf extract was 300 mg/kgBW [5]. Apart from that, the research results on matoa leaf extract showed a correlation or positive relationship between antioxidant and antidiabetic activity, where the higher the antioxidant activity, the higher the potential for inhibition of the α -amylase enzyme [6]. The potential for antioxidant activity is classified as strong, as reported that the ethanol extract of matoa leaves has an IC_{50} value of 11.75 ppm [7] and 9.20 ppm [8]. The study reported that the IC_{50} value for the ethanol extract was higher at 1.403 ppm compared to the n-hexane extract at 306.49 ppm and the ethyl acetate extract at 261.07 ppm [9].

Two rhamnoside flavonol compounds were isolated from the ethyl acetate fraction of methanol extract of matoa leaves from Padang, West Sumatera, Indonesia, using the chromatography method, and their structures were identified as kaempferol-3-O-rhamnoside and quercetin-3-O-rhamnoside [10]. In the same study, other metabolites were also isolated, such as kaempferol-3-O-glucoside (astragalol), quercetin-3-O-glucoside (isoquercetin), kaempferol-3-O-glucosyl-(1→4)-rhamnoside (nicotiflorin), quercetin-3-O-glucosyl-(1→4)-rhamnoside (rutin), kaempferol, and quercetin. Isolation of the active compounds from a leaf extract of *Pometia pinnata* was collected from the Hat-Yai District, Songkhla Province, Thailand, resulted in the isolation of active compound, identified as proanthocyanidin A2, epicatechin, kaempferol-3-O-rhamnoside, quercetin-3-O-rhamnoside, 1-O-palmitoyl-3-O- $[\alpha$ -D-galactopyranosyl-(1,6)- β -D-galactopyranosyl]-sn-glycerol, stigmasterol-3-O-glucoside, and 3-O- α -L-arabinofuranosyl-(1→3)- $[\alpha$ -L-rhamnopyranosyl-(1→2)]- α -L-arabinopyranosil hederagenin [11].

The different environmental conditions can dramatically influence the production of secondary metabolites [12]. Environmental factors such as seasonal changes in climate, quality of soil (nutritive, moisture content, and porosity), ecological factors, as well as the possibility for hybridization between closely related species, even within one species, can have an impact on metabolite production of the higher plant [13]. Therefore, this research aims to identify the composition of compounds in the ethanol extract of matoa leaves from Kudus, Central Java using LC-MS characterization.

2. Experimental

The matoa plant (*Pometia pinnata*) determination test was conducted in the Ecology and Biosystems Laboratory, Biology Department, Faculty of Science and Mathematics, Diponegoro University. The research was comprised of sample preparation, extraction, and LC-MS characterization. LC-MS characterization was carried out at the Integrated Laboratory of Diponegoro University.

2.1. Equipment and Materials

The tools used in the research were beaker (Herma), measuring cup (Herma), measuring flask (Herma), spatulas, stirring rod, dropper pipette, micropipette (Lichen), microcuvette (Quartz), 40 mesh sieve, dehydrator cabinet, analytical balance (mettler, JL 602-G/L), centrifuge, vials, 330 mL jar, and rotary shaker.

The research materials were matoa leaves collected from Kota District, Kudus Regency, Central Java. The matoa leaves utilized were fresh (mature) leaves, initially subjected to drying in a dehydrator cupboard. Other materials included in the study were distilled water, aquabides, ethanol, methanol, Tween 80 solution, quercetin, gallic acid, folin ciocalteu, CH₃COONa, AlCl₃, and Na₂CO₃.

2.2. Material Preparation

The matoa leaves used were fresh. Plant materials were determined at the Ecology and Biosystems

Laboratory, Biology Department, Faculty of Science and Mathematics, Diponegoro University. Determination key: 1b-2b-3b-4b-6b-7b-9b-10b-11b-12b-13b-14a-15b- (Goal 9. Dispersed compound Leaves plants)-109b-119b-120a-121b-122b- (Family 69 Sapindaceae) - (Genus *Pometia*) - (*Pometia pinnata*). Subsequently, the matoa leaves were cleaned and dried in a dehydrator cupboard and then ground into powder. The powder was then sieved using a 40-mesh size.

2.3. Extraction of Matoa Leaves using Ethanol

The extraction process was based on Wen *et al.* [14] with slight modifications. Matoa leaves were extracted using ethanol in a ratio of 1:5 (v/v) by maceration with 70% ethanol. Then, the mixture was shaken using a shaker for 1 hour. The sample was centrifuged to obtain a filtrate. The filtrate obtained was evaporated using a rotary evaporator.

2.4. Determination of Total Phenolics

Total phenolics were determined using the Folin Ciocalteu method [15] with slight modifications. Half a milliliter of the extract was placed into a vial and mixed with 2.5 mL of aquabides. Subsequently, 2.5 mL of Folin Ciocalteu reagent was added, and the mixture was shaken and incubated for 15 minutes. Two mL of sodium carbonate was added and mixed, then incubated for 30 minutes in a dark room. Methanol was used as a blank, while gallic acid was used as a test standard and treated the same as the sample extract. The blank consisted of all reagents and solvents without sample solution. The standard gallic acid calibration curve was used to determine the content. The results were given as mg gallic acid equivalents (GAE)/g of extract.

2.5. Determination of Total Flavonoid

Total flavonoids were determined using the AlCl₃ colorimetric method [16]. One mL of extract was mixed with 3 mL of methanol, 0.2 mL of AlCl₃, 0.2 mL of CH₃COONa, and aquabides added up to 10 mL. After that, the mixture was shaken and incubated in a dark room for 30 minutes. Methanol was used as a blank, and quercetin was used as a test standard, treated the same as the sample extract. The blank consisted of all reagents and solvents without sample solution. The standard quercetin calibration curve was used to determine the content. The results were as mg quercetin equivalents (QE)/g of extract.

2.6. LC-MS Characterization

Liquid Chromatography/Mass Spectroscopy (LC/MS) analysis was performed according to the method [17] with slight modifications. ESI (Electrospray ionization) has become a powerful ion source capable of interacting with LC and demonstrated its application to several important classes of biological molecules [18]. The matoa leaf extract was stirred, filtered with a 0.22 μ m microfilter, and injected into the LC-MS system for metabolite analysis. Each sample was analyzed twice using LC-MS. An Advison C18 column (2.1 mm \times 50 mm, 1.8 μ m) was kept at 20°C with a 0.3 mL/min flow rate. The mobile phase was consisted of solvent A (0.1% formic acid in

water) and solvent B (0.1% formic acid in methanol). The gradient solvent system was eluted with 90% A→100% B 20 (0–10 min), 100% B (11–16 min), and 90% A (16–20 min) to equilibrate before the next injection. The conditions for the mass spectrometer were set as follows: ionization mode employed ESI in positive polarity, the mass spectrometry (MS) scan range covered m/z 50–1800, the capillary temperature was maintained at 250°C, source gas temperature was set to 200°C, and the capillary voltage was adjusted to 180 V.

3. Results and Discussion

3.1. Extraction of Matoa Leaves

Samples were extracted utilizing the maceration method or solid-liquid extraction due to its simplicity, ease, and absence of heating. The avoidance of heat is crucial, as it can potentially damage or diminish the levels of compounds present in the extract. Ethanol was selected as the solvent for extraction due to its universal and polar nature. Ethanol's ability to penetrate plant cell walls makes it advantageous for facilitating rapid and extensive diffusion of bioactive compounds, surpassing the capabilities of water solvents [19]. According to Pandey and Tripathi [20], these active compounds include polyphenols, flavonoids, tannins, terpenoids, alkaloids, sterols, and polyacetylene. Ethanol can also attract some non-polar phenolic compounds, producing higher total phenol content than water [19]. The plant materials and resulting extract are depicted in Figure 1.

3.2. Total Phenolic and Flavonoid Content

The total phenolics were determined using the Folin Ciocalteu method. Total phenol using the Folin–Ciocalteu reagent is based on measuring the total antioxidant capacity in a sample based on electron transfer, which reduces the Folin–Ciocalteu reagent from an antioxidant source to form a blue chromophore. This blue color indicates a broad light absorption with a maximum wavelength of 765 nm [21]. The determination of total flavonoids was carried out using the $AlCl_3$ colorimetric method. The principle of this method is based on the addition of $AlCl_3$, which forms a stable acid complex with the C-4 ketone group, along with the C-3 or C-5 hydroxyl groups of flavone and flavonoids. $AlCl_3$ forms stable acid complexes with orthodihydroxyl groups on rings A or B [22]. Total phenolic and flavonoid content can be seen in Table 1.



Figure 1. Preparation of dried matoa leaves and its extract: (a) matoa trees in Kota District, Kudus Regency, Central Java, Indonesia, (b) fresh matoa leaves, (c) dry powder of matoa leaves (simplicia), and (d) ethanol extract of matoa leaves

Table 1. Total phenolic and flavonoid content of matoa leaves ethanol extract

Sample	Total phenolic content (mg GAE/g extract)	Total flavonoid content (mg QE/g extract)
Ethanol extract of <i>Pometia pinnata</i>	35.689 ± 0.726	1.384 ± 0.012

Based on Table 1, the total phenolic content of the ethanol extract of *Pometia pinnata* is 35.689 ± 0.726 mgGAE/ gram of extract. The total flavonoid content of the ethanol extract of *Pometia pinnata* is 1.384 ± 0.012 mgGAE/gram of extract. In a previous study, the total phenolic and flavonoid contents of *Pometia pinnata* reflux extract with 96% ethanol solvent were 197.21 ± 0.21 mg GAE/g and 28.73 ± 0.07 mg QE/g [21]. Different methods result in different total phenolics and flavonoids. The reflux method using 96% ethanol produces higher total phenolics and flavonoids than the maceration method using 70% ethanol. The total phenolic content is expressed as the equivalent mass of gallic acid [15]. The examination data of the gallic acid standard solution yielded a calibration curve with the regression equation $y = 0.0068x - 0.0099$ and an R^2 value of 0.9987.

Phenolic compounds present in plants are a big secondary metabolite and are very important. It is found that phenolic compounds have many health benefits and potential importance in fruit maturation and food preservation [3]. The phenolics are composed of one or more aromatic rings bearing one or more hydroxyl groups and are, therefore, potentially able to quench free radicals by forming stabilized phenoxyl radicals [22].

The total flavonoid content is expressed in mg QE/g extract. The maximum wavelength produced is 431 nm. From the examination data of the quercetin standard solution, a calibration curve was obtained with the regression equation $y = 0.0087x - 0.004$, with an R^2 value of 0.9992.

3.3. LC-MS Characterization Analysis

LC-MS characterization aims to determine compounds identified in the ethanol extract of matoa leaves. The working principle of LC-MS is the separation of sample components based on differences in polarity, and then the charged ions will be detected by a mass spectrometer detector. The mass spectrometry (MS) spectrum can be seen in Figures 2 and 3. Based on Figures 2 and 3, identification data of metabolite compounds from ethanol extract of matoa leaves using LC-MS can be seen in Table 2.

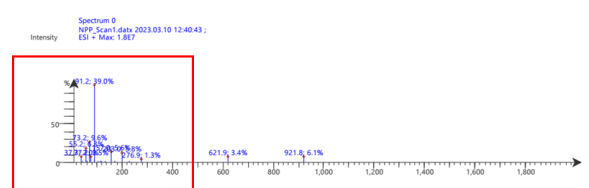


Figure 2. MS spectrum of matoa leaves ethanol extract

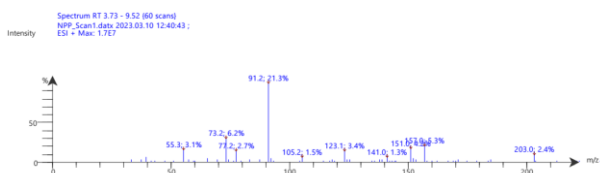


Figure 3. The magnification of the MS spectrum of the ethanol extract of matoa leaves

Table 2 shows the compounds identified are tentative based on molecular mass data from several existing literature studies. LC-MS analysis was conducted using the Advion LC/MS Single Quadrupole instrument with the ESI method. The sample that has been separated in LC is then ionized at the ion source, where precursor ions will be produced as a result of separating the ions based on their *m/z*. Based on the LC-MS data in Table 2, it shows that there is a change in the composition of metabolite compounds in ethanol extract of matoa leaves. There were 12 compounds that could be identified in the ethanol extract of matoa leaf samples.

Liquid chromatography-tandem mass ionization electrospray spectrometry (LC/(+)ESI-MS) produces $[M+H]^+$ or *m/z*. The ethanol extract of matoa leaves sample yielded *m/z* of 150.9, 123.1, 219, 200, 171.1, 91.2, and 170.1 were allegedly identified as phenolic compounds, vanillin, *p*-hydroxybenzaldehyde, *p*-Coumaroyl glycolic acid, syringic acid, gallic acid, phenol, and vanillic acid, respectively. The sample yielded *m/z* of 306 and 621.9, identified as flavonoid compounds that are epigallocatechin and apigenin -7-O-diglucuronide. The organic compound was identified as jasmonic acid with *m/z* = 210. The aromatic compound is also identified as benzene with *m/z* = 77.2. Besides, the sample yielded *m/z* of 922, which was allegedly identified as tannin.

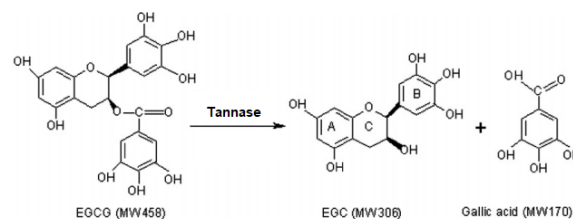


Figure 4. Reaction of epigallocatechin gallate (EGCG) by tannase, producing epigallocatechin (EGC) and gallic acid [23]

The ethanol extract of matoa leaves sample showed the presence of the epigallocatechin compound, which is thought to be degraded by the plant’s tannase enzyme. Tannase is an important biotechnological enzyme distributed throughout plants, animals, and microbes [24].

Compounds suspected to be syringic acid and gallic acid from the ethanol extract of matoa leaves were identified. The compound suspected to be apigenin-7-O-diglucuronide, which is included in the flavonoid compound group, appeared in the ethanol extract of matoa leaves sample. The compound identified with *m/z* = 922 is considered a tannin group compound, which is not yet specifically known. A compound suspected of *p*-hydroxybenzaldehyde was identified in the ethanol of the sample of matoa leaves. The compound suspected extract to be *p*-Coumaroyl glycolic acid, which is included in the cinnamic acid group. This compound is a class of compounds known as coumaric acid esters, which are aromatic compounds containing ester derivatives of coumaric acid.

Table 2. Results of compound identification in ethanol extract of matoa leaves

Compound name	Class	Molecular weight (<i>m/z</i>)		Peak area (%)
		Theoretical	Experiment	
Epigallocatechin	Flavonoid	306.267	306	2×10 ³
Vanillin	Phenolic	152.147	150.9	6.4×10 ¹⁰
<i>p</i> -hydroxybenzaldehyde	Phenolic acid	122.123	123.1	3.4
<i>p</i> -Coumaroyl glycolic acid	Phenolic acid	222.194	219	1.6×10 ²
Syringic acid	Phenolic acid	198.173	200	3.1×10 ³
Gallic acid	Phenolic acid	170.12	171.1	6.5×10 ⁶
Phenol	Phenolic	94.11	91.2	5.2×10 ⁹
Jasmonic acid	Organic compound	210.27	210	1.2×10 ⁵
Vanillic acid	Phenolic acid	168.147	170.1	3.3×10 ⁷
Tannin	Tannin	-	922	6.1
Apigenin 7-O-diglucuronide	Flavonoid	622.485	621.9	3.4
Benzene	Aromatic	78.11	77.2	7.6×10 ⁹

Previous studies showed that matoa leaf ethanol extract using LC-MS/MS characterization contained quercetin, kaempferol, and procyanidin [7]. The compound content in matoa leaves were collected from the environment of Limau Manis, Padang, West Sumatera, Indonesia in June 2017 identified and resulted kaempferol-3-O-rhamnoside, quercetin-3-O-rhamnoside, kaempferol-3-O-glucoside (astragalins), quercetin-3-O-glucoside (isoquercetin), kaempferol-3-O-glucosyl-(1→4) rhamnoside (nicotiflorin), quercetin-3-O-glucosyl-(1→4) rhamnoside (rutin), kaempferol, and quercetin [10]. Various distributions of the compounds investigated in different plant samples originate from different populations of a single species. The different environmental conditions can dramatically influence the production of secondary metabolites [12]. Environmental factors such as seasonal changes in climate, quality of soil (nutritive, moisture content, and porosity), ecological factors, and the possibility for hybridization between closely related species all have an impact on the metabolite production of the higher plants [13].

4. Conclusion

The total phenolic content of ethanol extract of matoa 35.689 ± 0.726 mg GAE/g extract. The total flavonoid content of ethanol extract of matoa is 1.384 ± 0.012 mg QE/g extract. Liquid chromatography-tandem mass ionization electrospray spectrometry (LC/(+ESI-MS) produces [M+H]⁺ or m/z. There were 12 compounds that could be identified in the ethanol extract of matoa leaf samples. The phenolic compounds were identified as vanillin, p-hydroxybenzaldehyde, p-Coumaroyl glycolic acid, syringic acid, gallic acid, phenol, and vanillic acid. The flavonoid compounds were identified as epigallocatechin and apigenin -7-O-diglucuronide. The organic compound was identified as jasmonic acid. The aromatic compound was identified as benzene. Besides, the tannin compounds were also identified. The major compounds in matoa leaves from Kudus, Central Java, are vanillin, phenol, and benzene.

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