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| RESEARCH ARTICLE

GC-MS and LC-MS/MS Analysis of *Bouea macrophylla* Fruit Juice

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| ABSTRACT
Bouea macrophylla Griff is a member of the Anacardiaceae family, which has edible fruits. Due to the high nutritional value, appealing taste, and attractive color, the fruit juice of *B. macrophylla* has the potential to be developed as a functional beverage. The present study was conducted to determine the phytochemical profile of *B. macrophylla* fruit juice using GC-MS and LC-MS/MS. GC-MS analysis was performed with the Agilent Technologies 7890 Gas Chromatograph with automated sampling and tandem Agilent 5975 Mass Selective Detector and peak analysis with the Chemistation data system. LC-MS/MS QTOF analysis was performed with UPLC Waters I Class and tandem MS/MS Xevo G2S QTOF with UNIFI software. GC-MS analysis identified five compounds with a quality match of 90% and above, namely 3-pentadecylphenol (31.68%), 5,5'-(oxybis(methylene)]-bis-2-furancarboxy-aldehyde (5.67%), 5-(hydroxymethyl)-2-furancarboxy-aldehyde (23.51%), 4H-pyran-4-one, 2,3-dihydro-3,5-dihydro-6-methyl (5.00%), and n-hexadecanoic acid (4.71%). Five major compounds were identified for the first time in *B. macrophylla* fruit juice by LC-MS/MS analysis, i.e., (E)-hexadecyl-ferulate, digupigan A, eburicoic acid, hydroginkgolinic acid, and terminalic acid. The health benefit of some of these detected phytochemicals supports the use of juice as the main ingredient for functional food.

| KEYWORDS
Bouea macrophylla, fruit juice, GC-MS, LC-MS/MS, phytochemicals

| ARTICLE INFORMATION
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1. Introduction
B. macrophylla Griff. or plum mango is a member of the Anacardiaceae family, which has edible fruits. This plant is native to Indonesia and some tropical Asia countries, such as Malaysia, Thailand, Southern China, Myanmar, and Andaman Island. In Indonesia, this plant is thrives in the Island of Sumatra, Java, Kalimantan and Maluku (Harsono, 2017; Priyadi et al., 2010). The distribution of *B. macrophylla* in Sumatra includes West Sumatra, Palembang, Lampung, Bangka Belitung, Medan, Jambi and Aceh. On the island of Java, this fruit can be found in Banten and West Java. In Kalimantan, *B. macrophylla* can be found in West Kalimantan and South Kalimantan. Meanwhile, Ambon City is the city of distribution of *B. macrophylla* fruit on the island of Maluku (Harsono et al., 2016, 2018). *B. macrophylla* fruit has many common names within the native habitat in Indonesia, such as gandaria (Java), jatake (Sunda and Banten), remieu (Gayo), barania (Dayak Ngaju) (Harsono, 2017), merinya (Aceh), haramania (Padang Bolak), kundang (Bengkulu, Riau), raman (Pekanbaru dan Lampung), gondorio (Palembang), gandaria (Bogor, Ambon and Java) and ramania (Kalimantan) (Harsono et al., 2016).

B. macrophylla fruit is round to oval shape with purple seeds. The size, color, and taste of this fruit depend on the cultivars. Generally, *B. macrophylla* fruit has a fruit size ranging from 2,5-5,0 cm. The unripe fruit usually has green color, without aroma, and has a sour taste. Ripe fruit has yellow to orange skin and flesh with a sweet to sour taste, a juicy texture, and a pleasant aroma (Harsono, 2017; Rajan & Bhat, 2017). Several cultivars of *B. macrophylla* have been found in Kalimantan, including ramania pipit, ramania hintalu, ramania tembaga, and ramania harang. Ramania pipit has an orange, round to oval shape, and sweet taste fruit, ramania hintalu has a large, yellow and round fruit shape, and sweet to sour taste, ramania tembaga and ramania harang have yellow fruit skin color with black spots, is smaller in size and has a sweet-sour taste (Harsono, 2017).

B. macrophylla, both unripe and ripe widely used in Indonesia. The unripe fruit is used as an additive and flavoring in "sambal", "rojak," salads, and pickles. Ripe fruit is consumed as juice, syrup, or jam (Harsono, 2017; Rajan & Bhat, 2017).

B. macrophylla fruit has been proven to have antioxidant activity that is beneficial for human health (Sukalingam, 2018). Therefore it is considered potential raw material for functional beverages. However, the analysis of secondary metabolites in *B. macrophylla* fruit juice is limited. Therefore, this study was conducted to determine the secondary metabolites found in *B. macrophylla* fruit juice using liquid chromatography/mass spectrometry (LC-MS/MS) and gas chromatography-mass spectrometry (GC-MS) analysis using an untargeted approach.

2. Literature Review

2.1 Botanical description

The genus Bouea is part of the family Anacardiaceae. Bouea is a genus that has 3 species. The species are *Bouea macrophylla* Griff., *Bouea oppositifolia* (Roxb.) Adelb. and *Bouea poilanei* Evr. B. poilanei is another species of Bouea that has red fruit reported from Trang, Bom, Vietnam. *B. macrophylla* and *B. oppositifolia* are Bouea species that are distributed in the tropics, one of which is Indonesia. *B. macrophylla* and *B. oppositifolia* were distinguished based on the shape and size of the fruit, leaf size, and bud size. Both species have the same characteristics, namely opposite leaf bases, while the distinguishing feature is the size of the leaves and bud that grow at the base of the leaves (Harsono et al., 2016; Harsono, 2017; Harsono et al., 2018).

The unripe *B. macrophylla* fruit has a light green to dark green color, a neutral aroma, and a sour taste. Ripe fruit has a yellow to orange color, is juicy, and has a sweet taste mixed with sour with a pleasant aroma. Its pleasant aroma resembles the aroma of turpentine (Rajan et al., 2014; Rajan & Bhat, 2017). *B. macrophylla* fruit is a type of stone fruit that is slightly round to oval in shape. The size of the fruit is very diverse. The variety of fruit sizes, colors, and flavors of *B. macrophylla* fruit varies depending on the local identity of each region. Fruit size ranges from 2.5 to 5.0 cm. This fruit has purple seeds that are a bit hard (Rajan et al., 2014; Tanasale, 2011).

2.2 Distribution and habitat

B. macrophylla is a tropical plant that grows on light and fertile soil. This plant grows wild in the lowlands at an altitude of 300 meters above sea level (Rifai, 1991). *B. macrophylla* in Ambon City, Hunuth Village, Baguala District, can adapt, grow, and develop well at an altitude of 1-36 meters above sea level (MASL) (Taihuttu, 2013). The cultivation of *B. macrophylla* also illustrates that this plant can grow well at an altitude of about 5-850 MASL (Tanasale, 2011).

B. macrophylla is a plant that is suitable to grow in areas that have a flat topography (0-3%). However, some research results show that B. macrophylla can grow, develop and reproduce well in slightly sloping or wavy topography (8-15%) to slightly steep (30-45%) (Taihuttu, 2013).

B. macrophylla is a native plant of Indonesia. This plant is distributed in several regions in Asia, such as peninsular Malaysia, Thailand, South China, Indochina, Myanmar, and the Andaman Islands. In Indonesia, this plant can be found in several areas, such as Sumatra, Java, Kalimantan, and Maluku. *B. macrophylla* spreads from the coast to the highlands. Some of the growth centers of *B. macrophylla* include Carita Beach (West Java), Sampit, Banjarmasin (Kalimantan), Padang Bolak (North Sumatra), Long Strait (Riau), and Ambon City (Harsono, 2017; Harsono et al., 2018).

Distribution of *B. macrophylla* on the island of Maluku, apart from being found in Ambon City, these plants are also found in coastal areas such as Poka-Rumah Tiga, Hunuth, and Hative Besar. In choppy and hilly areas, it can be found in Soya Village, Urimessing Village, Suli, Waai, and Telaga Kodok. In addition, it is also found in the areas of Saparua Island and West Seram, Central Maluku Regency (Tanasale, 2011).

2.3 Nutritional content

B. macrophylla, both unripe and ripe, are edible fruits that can be consumed. According to Tanasale's research, 2011, fruit every 100 g of the fruit contains 85 g of water, 12 mg of protein, 600 mg of fiber, 230 mg of ash, 6 mg of calcium, 10.8 mg of phosphorus, 0.31 mg of iron, 0.043 mg of carotene, 0.031 mg thiamine, 0.025 mg riboflavin, 0.286 mg niacin, and 75 mg vitamins. The content of vitamins A and C from *B. macrophylla* fruit occupies the second position after mango and cashew (Tanasale, 2011). Rajan & Bhat (2014) stated that unripe and ripe *B. macrophylla* contained proximate, mineral, essential amino acids, and non-essential amino acids with different nutritional content. The nutritional content of unripe fruit is mostly higher than that of ripe ones.

2.4 Phytochemical content

B. macrophylla has a pleasant aroma. This aroma is influenced by the cultivar, fruit maturity, and ecology in the area of plant cultivation. The cause of the pleasant aroma of *B. macrophylla* is the content of volatile compounds contained in it. Volatile compounds produced from *B. macrophylla* that have been reported include terpene hydrocarbons, aldehydes, ketones, esters, acids, and their chemical compounds. These compounds are responsible for the aroma possessed by *B. macrophylla*. The different aroma of unripe fruit and ripe fruit is influenced by the difference in the amount of these compounds (Rajan & Bhat, 2017).

B. macrophylla also contains secondary metabolites that cause antioxidant activity. The content of secondary metabolites includes alkaloids, anthraquinones, saponins, phenols, tannins, phenolics, flavonoids, flavonols, anthocyanins, and ascorbic acid. The percentage of each metabolite compound in *B. macrophylla* varies depending on the solvent used and the level of ripeness of the fruit (Rajan & Bhat, 2016; Sukalingam, 2018).
Chemical compounds related to secondary metabolites such as alkaloids, terpenoids, phenol groups, flavonoids, quinones, tannins, and saponins are widely found in plants and have the potential to be developed by researchers in the search for medicinal raw materials (Lolaen et al., 2013; Rajan & Bhat, 2016; Sulaiman & Ooi, 2014).

Secondary metabolites are precursors for the presence of antioxidant abilities in *B. macrophylla*. This ability varies depending on the solvent used and the level of ripeness of the fruit. The percentage of antioxidant activity in ripe fruit using water as a solvent was 83%, while the unripe fruit had a percentage of 82%, with the highest concentration of 50 g/mL. Different results were obtained when other solvents were used, such as methanol extract of ripe fruit with a percentage of 82% and unripe fruit with a percentage of 70% (Sukalingam, 2018).

The antioxidant ability is still detected even though the fruit has gone through the processing process. The antioxidant activity of *B. macrophylla,* which has been processed into juice, is known to have an IC50 value of 36.3 mg/ml. This value was obtained using the DPPH (1,1-diphenyl2picrylhydrazyl) radical absorption method(Lolaen et al., 2013). According to Sulaiman & Ooi (2014), the antioxidant content of *B. macrophylla* juice was 258.17 ± 4.25 g VCEAC/g sample using the DPPH method and 133.31 ± 0.70 g TEAC/g sample using the FRAP (Ferric Reducing Antioxidant Power) method.

B. macrophylla, which is processed into juice, has been proven to contain antioxidants. The potential of *B. macrophylla* fruit juice is also supported by the cooling, refreshing, detoxifying, and diuretic properties of *B. macrophylla* fruit, so the processed *B. macrophylla* fruit into juice is preferred. Fruit juice can be used as a therapeutic drug to lower blood cholesterol levels as well as treat dysentery, diarrhea, and diabetes. This is because *B. macrophylla* fruit juice is rich in vitamins and phenolics, which are known to play a major role as antioxidants and glucosidase inhibitors. The total phenol content in *B. macrophylla* fruit juice was 372.35 ± 1.43m g GAE/g sample, and vitamin C of 156.29 ± 3.72h g AAE/g sample (Sulaiman & Ooi, 2014).

3. Methodology

3.1 Plant materials

Freshly harvested, ripe fruits were collected from a local farm in South Paringin District, Balangan Regency, South Kalimantan Province, Indonesia. Specimens were identified at Herbarium Bogoriense, Research Center for Biology, National Research and Innovation Agency, Indonesia No: B-212/V/DI.05.07/10/2021.

3.2 Chemicals and reagents

The solutions and reagents used include standard biotin 1000 mg/L, Chloramphenicol 1000 mg/L, methanol, dan aquabides.

3.3 Preparation of B. macrophylla fruit juice

B. macrophylla fruit was washed with water and separated from the seeds. Fruit flesh was processed into juice with a slow-juicer without the addition of water. The juice was placed in a glass beaker, covered with aluminum foil, and stored in the freezer at -17ºC. When frozen, the juice was freeze-dried in an automatic lyophilizer at -46ºC for 174 hours.

3.4 Gas chromatography-mass spectrometry (GC-MS) analysis of B. macrophylla fruit juice

GC-MS analysis was carried out at the Regional Health Laboratory of DKI Jakarta, Indonesia. 5 grams of lyophilized *B. macrophylla* fruit juice was dissolved in 25 mL methanol and macerated. 10 mL of the sample was pipetted into a plastic tube and dehydrated in an oven at 60ºC for 1 hour. After drying, the sample was reconstituted with the remaining 200 mL of extract. The sample was then injected into the Agilent Technologies 7890 GC-MS instrument equipped with an automatic Agilent injector, an Agilent 5975 mass spectrometer detector, and an HP Ultra 2 capillary column 30 m X 0.20 mm X 0.11 µm (length x inside diameter x film thickness). The carrier gas is helium constant column mode using a 1,2 mL/min flow. The initial temperature was 80ºC for 0 minutes, then increased at a rate of 3ºC/min to 150ºC stables for 1 minute, and finally increased by 20ºC/min to 280ºC, which was stable for 26 minutes. The injector temperature, ion temperature, interface temperature, and quadropole temperature in scan mode were set at 250ºC, 230ºC, 280ºC, and 140ºC, respectively. The electron impact ionization mode on the instrument was 70 eV. The structure of the reported compounds was assessed by comparing the fragmentation patterns obtained with 1:8 splits. Chromatogram and relative percentage of compounds were used in the final analysis.

3.5 Liquid chromatography-mass spectrometry (LC-MS/MS) analysis of B. macrophylla fruit juice

LC-MS/MS analysis was carried out at the Saraswanti Indo Genetech, Bogor, Indonesia. The standard solution for LC-MS/MS was prepared by making a solution of 1 mg/L biotin and 1 ppm chloramphenicol. The standard solution was made in a 25 mL volumetric flask. The remaining volume was made with aquabides to 25 mL. Sample preparation was carried out by weighing 0,5 mg of lyophilized *B. macrophylla* fruit juice, put into a 10 mL volumetric flask, and extracted with methanol for 30 minutes. The remaining volume was made up of aqubides to 10 mL and homogenized. The solution was filtered with a 0,22 µm PTFE filter membrane.

LC-MS/MS QTOF was performed using UPLC Waters I Class and tandem MS/MS Xevo G2S QTOF with UNIFI software. Peaks were identified from a mass spectrum library of natural active substances from the Waters database (Qiao et al., 2013). The LC instrument setup has a C18 column with column temperature and autosampler temperature of 40ºC and 15ºC, respectively, with an injection volume of 10 µL. The mobile phases were: A (0,1% formic acid in acetonitrile) and B (0,1% formic acid in aquabides) with a Gradient 0,6 mL/min wild column. The instrument's tandem MS/MS setting was set to Tof MSE operating mode, ESI (-)/ESI(+) ionization mode, and an acquisition range of 59-1200 Da.

4. Results and Discussion

GC-MS analysis of dried *B. macrophylla* fruit juice resulted in a chromatogram, as shown in Figure 1. Peaks were identified by matching the MS spectrum with the adjusted database for Balitro. To our knowledge, the phytochemical content of *B. macrophylla* fruit juice investigated by GC-MS was carried out for the first time in this study. The results of this GC-MS analysis revealed the content of volatile compounds contained in *B. macrophylla* fruit juice.

From Table 1, there are 5 compounds identified with quality more or equal to 90%. Quality is a marker of the level of similarity of compounds in the sample with compounds in the library. The expected quality in order to approach the similarity of compounds in the library is ≥90%. The higher the match quality indicates that the compound has high quality or the same as the compound in the GC-MS instrument library. The 5 compounds are 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one (5,00%), 5-(hydroxymethyl)- 2-furancarboxaldehyde (23,51%), n-hexadecanoic acid (4,71%), 5,5'-(oxybis(methylene)]bis-2-furancarboxaldehyde (5,67%), dan 3-pentadecylphenol (31,68%).

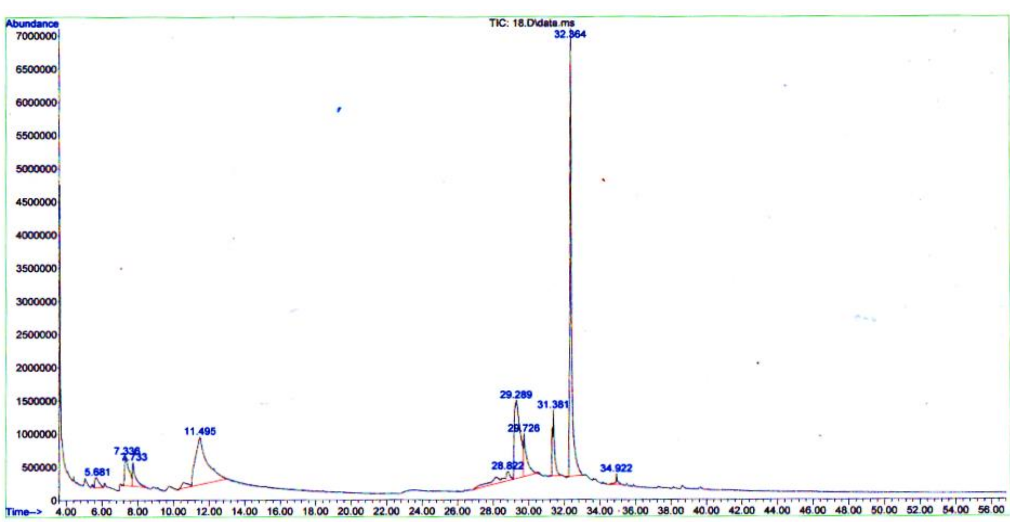


Figure 1. Gas chromatogram of *Bouea macrophylla* fruit juice

Table 1. Volatile compounds in *Bouea macrophylla* fruit juice identified with GC-MS analysis

RT (min)	Quality	Compounds	Content (%)
5.683	50	1H-Imidazole-4-carboxylic acid, methyl ester	1.62
7.338	95	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	5.00
7.731	58	Nicotinyl Alchohol	3.03
11.496	90	2-FURANCARBOXALDEHYDE,5-(HYDROXYMETHYL)-	23.51
28.824	95	n-Hexadecanoic acid	4.71
29.286	72	2-2-FURANCARBOXALDEHYDE,5,5’-(OXYBIS (METHYLENE)] BIS-	18.20
29.727	90	2-FURANCARBOXALDEHYDE,5,5’-(OXYBIS (METHYLENE)] BIS-	5.67
31.382	80	Phenol, 3-pentadecyl	5.78
32.361	95	3-PENTADECYLPHENOL	31.68

Keterangan:
RT: Retention time
The detected compounds have a quality match of ≥90%
The data obtained is compared to the library database on instrument

Of the five compounds, the highest content in *B. macrophylla* fruit juice is 3-pentadecylphenol (phenol, 3-pentadecyl or PDP), which is 31.68% with an RT (Retention time) of 32.361 minutes and a quality match of 95. This appears as the highest peak found in the chromatogram (Figure 1), which is the percentage of the content of these compounds. This compound is an alkylphenol, a phenolic lipid which is exhibits anti-inflammatory, antipyretic, antibacterial, fungicidal, and cytotoxic activity. PDP has also been found in rose apple fruit (*Syzygium jambos*) (Cieślik-Boczula & Koll, 2009; Devakumar et al., 2016). Fruits are generally a source of phenolic compounds and other bioactive phytochemicals (Rosa et al., 2018).

5-(hydroxymethyl)-furancarboxaldehyde, better known as 5-hydroxymethyl-2furaldehyde (5-HMF), is another compound identified in *B. macrophylla* fruit juice with a percentage of 23.51%. This compound is an Amadori-reaction product compound formed during the Maillard reaction (non-enzymatic browning). This reaction occurs because of the reaction between carbohydrates, especially reducing sugars with free amine groups from amino acids or proteins or from other compounds (ALjahdali & Carbonero, 2019). *B. macrophylla* fruit is a fruit that contains carbohydrates and proteins, so it is possible to find 5-HMF compounds because of the possibility of the Maillard reaction (Rajan et al., 2014; Tanasale, 2011). The presence of Amadori compounds in dried fruits is common. This also adds to the reason for the presence of the compound 5-HMF in the dried fruit juice of *B. macrophylla* (Luo et al., 2021; Sanz et al., 2001). This compound has been evaluated for activity in the prevention and treatment of hypoxia, anemia, and sickle cell disease (Lucas et al., 2019; Shapla et al., 2018; Woyke et al., 2021; Zheng et al., 2019).

The next compound is 2-Furancarboxaldehyde, 5.5'-(methylene)] bis- or 5.5'(oxy-bis(methylene))bis-2-furfural (OBMF). The presence of OBMF in *B. macrophylla* fruit juice is probably due to OBMF formed from the etherification of two 5-HMF molecules. OBMF has a role in the synthesis of heterocyclic ligands and antiviral precursors of hepatitis (Mliki & Trabelsi, 2015).

2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one (DDMP) is a powerful antioxidant compound. Antioxidant activity is demonstrated through its excellent free radical scavenging ability (Chen et al., 2021; Yu et al., 2013). This antioxidant activity of DDMP can explain the antioxidant ability of *B. macrophylla* fruit juice (Lolaen et al., 2013; Sulaiman & Ooi, 2014).

The fifth is the compound n-hexadecanoic acid or better known as palmitic acid (PA). PA is the most common saturated fatty acid found in plants (Zhukov, 2015). The presence of AP in *B. macrophylla* fruit was also reported by Rajan & Bhat (2017) with an analysis using GC-MS. This compound is associated with the anticancer and anti-inflammatory cytotoxic activity of a substance (Aparna et al., 2012; Othman et al., 2015; Ravi & Krishnan, 2016).

Chromatograms of *B. macrophylla* fruit juice as results of the analysis with LC-MS/MS QTOF are shown in Figures 8 and 9, and the identification of phytochemical content of *B. macrophylla* fruit juice analyzed by LC-MS QTOF is shown in Table 2. The results of the analysis showed the presence of 5 compounds from the phenol group, tannins, triterpenoids, and organic acids.

From Figures 2 and Figure 3, analysis of LC-MS/MS QTOF in positive and negative ionization mode, it can be seen that in positive ESI mode with ionization technique produces molecular ions ([M+H]+ no compounds are confirmed. In negative ESI mode with the ionization technique to produce molecular ions ([M+H]- confirmed the presence of 5 compounds, including 1 phenolic, 1 tannin, 1 triterpenoid, and 2 organic acid type compounds. Detailed information can be seen in Table 2.

Table 2. Chemical composition of *Bouea macrophylla* fruit juice by LC-MS/MS QTOF

Compounds	ESI Mode	RT (min)	Molecular Formula	Compound Group	Response	Result
(E)-Hexadecyl-ferulate	(-)	18.00	$C_6H_{42}O_4$	Organic acid	12119	Positive
Digupigan A	(-)	4.19	$C_{18}H_{26}O_{12}$	Phenol	580	Positive
Eburicoic acid	(-)	18.22	$C_{31}H_5O_3$	Triterpenoid	435	Positive
Hydroginkgolinic acid	(-)	17.67	$C_{21}H_{34}O_3$	Organic acid	12499	Positive
Terminalic acid	(-)	17.00	$C_{20}H_{28}O_3$	Tannin	581	Positive

Note :

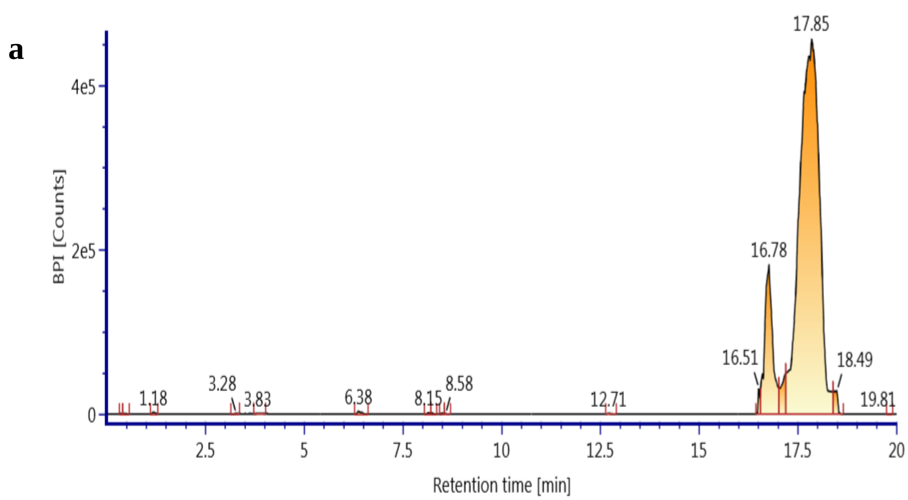
1. Mass error ≤ 5 ppm
2. Isotope match MZ RMS PPM ≤ 6 ppm & Isotope match MZ RMS % ≤ 10 %
3. Intensity/Response ≥ 300
4. Fragment match ≥ 1 mass fragment
5. Positive, if points 1 to 4 are meet
6. Low abundance if 3 criteria meet and Intensity/Response < 300

The chromatogram shows that there are several peaks from the results of the LC-MS/MS QTOF analysis. Based on the chromatogram, the highest peak was owned by the compound hydroginkgolinic acid. This compound is a compound of organic acid that was isolated for the first time from *Ginkgo biloba* L. (Feng-Yung et al., 1962; D. Wang et al., 2018; Zhou et al., 2012). According to Sun et al. (2020), this compound is a potential source of anticancer drugs for ovarian cancer. This compound also has the potential as a therapeutic agent for improving asthma (Clyne et al., 2020).

(E)-Hexadecyl-ferulate with molecular formula $C_6H_{42}O$ is another compound. This compound can also be seen from the high peak of the compound on the chromatogram. This compound is an organic acid commonly found in fruits. Organic acids act as the main nutrients and flavor components in fruit juices which contribute to the main content of soluble solids in fruit juices (Li et al., 2020).

Research related to terminalic acid compounds is still lacking until now. Terminalic acid is a new compound from the tannin class found in the traditional Chinese medicinal plant Terminalia chebula (Liu et al., 1998). This compound was also found in Hedyotis diffusa and Hedyotis corymbosa plants with the same mass spectrometer, namely QTOF-MS, and with UNIFI data processing software (Y. Wang et al., 2018). The tannins found in apple juice have been shown to improve lipid profiles (Asmawati et al., 2021).

Digupigan A is a compound from a phenol group compound that can also be found in Lycium plants such as stems and fruits of *L. chinense* and *L. barbarum* fruit. Plants of the genus Lycium have been widely used in traditional Chinese medicine and functional foods (Hui Chen, 2019; Qian et al., 2017). In addition to the Lycium plant, this compound is also found in the seeds of *Artocarpus heterophyllus* (Olalere et al., 2020). Phenol groups have been widely studied to treat metabolic syndromes such as hypercholesterolemia and atherosclerosis (Castro-Barquero et al., 2020; Sinaga et al., 2021).



b

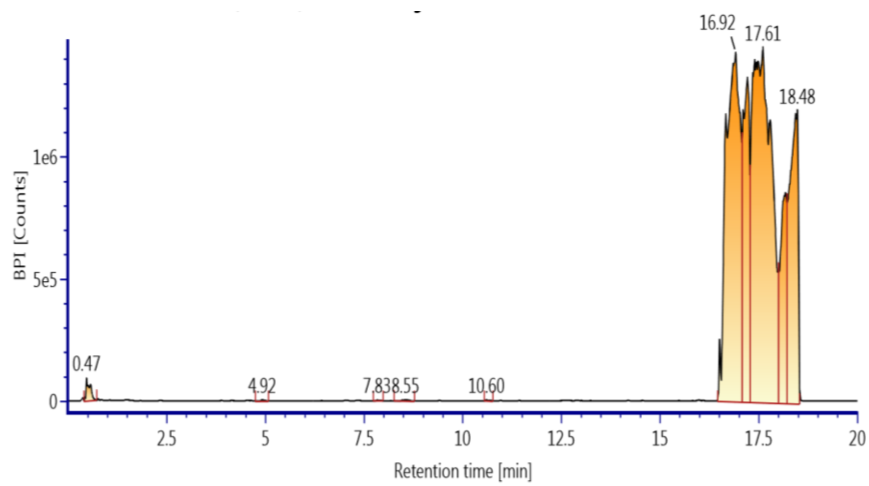


Figure 2. Liquid chromatogram of *Bouea macrophylla* fruit juice ESI positive (+) mode.
(a) Blank chromatogram; (b) *Bouea macrophylla* fruit juice chromatogram

a

b

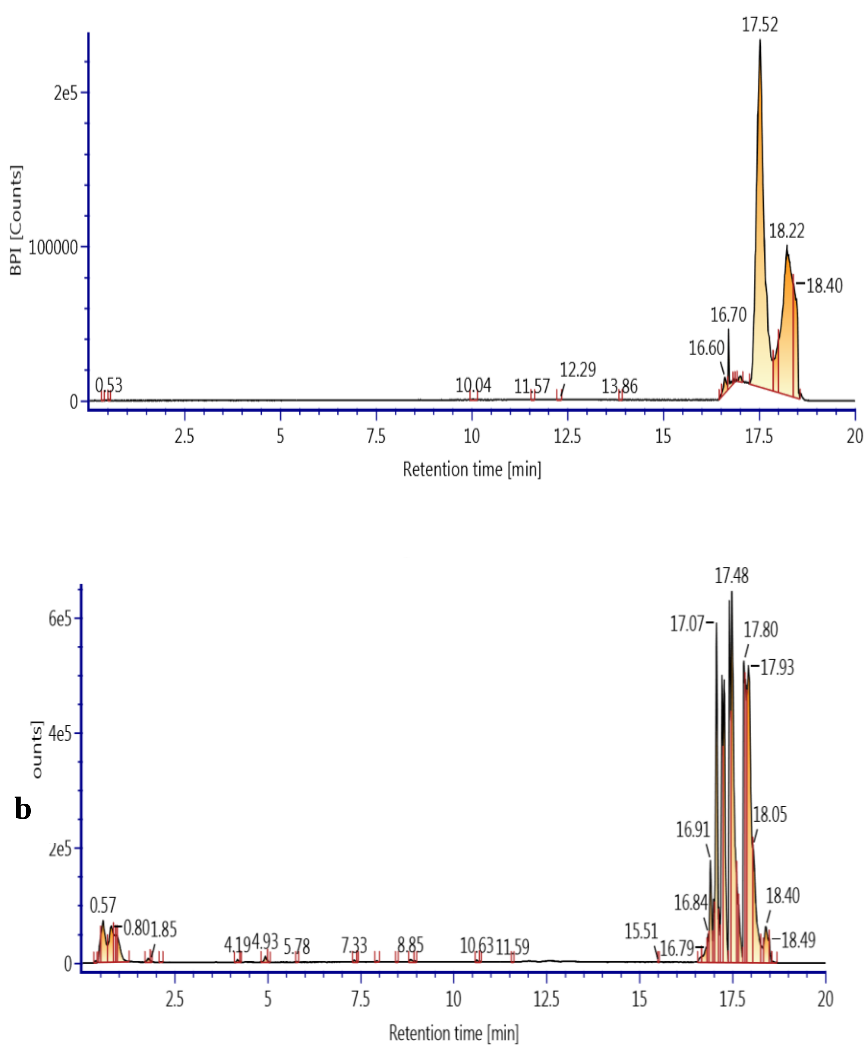


Figure 3. Liquid chromatogram of *Bouea macrophylla* fruit juice ESI negative (-) mode.
(a) Blank chromatogram; (b) *Bouea macrophylla* fruit juice chromatogram

Eburuicoic acid compound with the molecular formula C31H5O3 is a compound from the triterpenoid group. Eburicoic acid compounds are found in many Antrodia mushrooms. Eburicoic acid isolated from *Antrodia camphorata* had antidiabetic and antihyperlipidemic effects in rats fed a high-fat diet. A. camphorate itself is a medicinal mushroom endemic to Taiwan that has been used to treat various diseases such as poisoning, inflammation to cancer. The content of secondary metabolites that play a role in the therapeutic activity of the fungus is terpenoids (Kuang et al., 2021; Lin et al., 2017).

Eburicoic acid is a compound that comes from the terpenoid group. Terpenoid compounds are also found in some fruit juices, such as guava juice, papaya juice, banana juice, apple juice, and pineapple juice. Giving some of these fruit juices to mice fed a high-fat diet managed to improve lipid profiles (Tijjani et al., 2020). This compound is also found in another type of Taiwanese medicinal mushroom, namely *Antrodia cinnamomea,* which has hepatoprotective, anti-inflammatory, anti-hepatitis B, and anticancer activities. Eburicoic acid from *A. cinannamomea* has significant liver anticancer activity by reducing the viability of Hep 3B cells within 24 hours (Su et al., 2012). The anti-inflammatory effect is produced by Erucic acid isolated from Antrodia camphorate (Deng et al., 2013).

5. Conclusion

This article is the first report related to the phytochemical screening of *B. macrophylla* fruit juice by GC-MS and LC-MS/MS analysis. *B. macrophylla* fruit juice contains five compounds with quality more or equal to 90% by GC-MS analysis : 3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one (5,00%), 5-(hydroxymethyl)- 2-furancarboxaldehyde (23,51%), n-hexadecanoic acid (4,71%), 5,5'-(oxybis(methylene)]bis-2-furancarboxaldehyde (5,67%), and 3-pentadecylphenol (31,68%). Phytochemical screening by LC-MS/MS identified five compounds, namely (E)-Hexadecyl-ferulate, Digupigan A, Eburicoic acid, Hydroginkgolic acid, and Terminalic acid. The detected phytochemical content shows that *B. macrophylla* fruit juice has antioxidant activity that can be used as a functional food that is beneficial for health.

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Conflicts of Interest: The authors declare no conflict of interest.

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