Food Science and Technology 8(4): 79-86, 2020
DOI: 10.13189/fst.2020.080402 http://www.hrpub.org

Characterization and Properties of Gedi (*Abelmoschus Manihot L.*) Leaf Extract with Liquid Chromatography Mass Spectrometry Using Quadrupole Time-of-Flight Technology (LCMS-QToF)

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Received October 18, 2020; Revised November 21, 2020; Accepted December 20, 2020

Cite This Paper in the following Citation Styles

(a): *[1] Tri Yuni Hendrawati, Afra Nuraini, Rusnia Junita Hakim, Nurul Hidayati Fithriyah , "Characterization and Properties of Gedi (Abelmoschus Manihot L.) Leaf Extract with Liquid Chromatography Mass Spectrometry Using Quadrupole Time-of-Flight Technology (LCMS-QToF)," Food Science and Technology, Vol. 8, No. 4, pp. 79-86, 2020. DOI: 10.13189/fst.2020.080402.*

(b): *Tri Yuni Hendrawati, Afra Nuraini, Rusnia Junita Hakim, Nurul Hidayati Fithriyah (2020). Characterization and Properties of Gedi (Abelmoschus Manihot L.) Leaf Extract with Liquid Chromatography Mass Spectrometry Using Quadrupole Time-of-Flight Technology (LCMS-QToF). Food Science and Technology, 8(4), 79-86. DOI: 10.13189/fst.2020.080402.*

Abstract Gedi (*Abelmoschus Manihot L.*) is a tropical plant in the Malvaceae family, one of the groups of plants in the herbal community. Gedi plants are known to contain flavonoids, hormones, alkaloids, tannins and phenolic compounds. An ultrasonic extraction tool is used in the extraction process of Gedi leaves. The objective of the study is to know the yield and analyze Gedi leaf extract, including proximate analysis and active substance components with LCMS-QTof. The material used is Gedi leaves from Manado. Ultrasonic extraction was carried out with a dry Gedi leaf ratio and Ethanol 1:40 (m/v) with a time of 30 minutes. The concentration of the extract is with a rotary evaporator temperature of 50-60°C. The yield of the extract of Gedi leaf is 9.86 %. Study of the proximity of dried Gedi leaf powder revealed 27.135% protein, 41.675% carbohydrate, 329 kcal energy, 11.235% water and 13.83% ash material. Meanwhile for proximity products, the Gedi leaf extract contains 0.635% protein, 1.27% carbohydrate, 9.26% total energy, 7.1% water, and 0.635% ash. The active compounds in the Gedi extract have been analyzed using LCMS. Trigonelline, Gentiatibetine, Harman, Periplocoside C, and Biotin were detected using the positive-ionization mode, whereas Chloramphenicol was detected with negative-ionization mode.

Keywords Gedi Leaf, Extraction, Ultrasonic, Antioxidant, LCMS-QToF

1. Introduction

Gedi (*Abelmoschus Manihot L.*) is a tropical plant of the Malvaceae family and is classified as a medicinal herbal plant [1-3]. A. Manihot may be used as an alternative medicine to lower blood glucose levels and blood pressure and to act as an anti-inflammatory, antioxidant and antidepressant [4-8]. It is known to contain flavonoids, steroids, tannins and phenols. The study conducted by Pine et al. (2010) showed that Gedi leaves had relatively high concentrations of flavonoids (23-41 %) which made it a good candidate as a source of antioxidants [5]. Tannin is a compound of polyphenols[7].

This plant also contains isoquercitrin, hyperoxide, hibifolin, quercetin-3'-0-glucoside, quercetin and

isorhamnetin, which have antidepressant effects [9]. In the meantime the flowers contain myricetin, cannabiscitrin, myricetin-3-0-beta-D-glucopyranoside, glycerol monopalmitate, 2,4-dihydroxy benzoate, guanosine, adenosine, maleic acid, heptatria contanoic, 1-triacontanol, tetracosane, beta-sitosterol, and beta-sitosterol-3-0-beta-D-glucoside, which have anti-diabetic and anti-inflammatory effects [10]. Trigonelline Gentiatibetine, Harman, Periplocoside C, Biotin and Chloramphenicol may be detected in Gedi leaves (**Figure 1**).

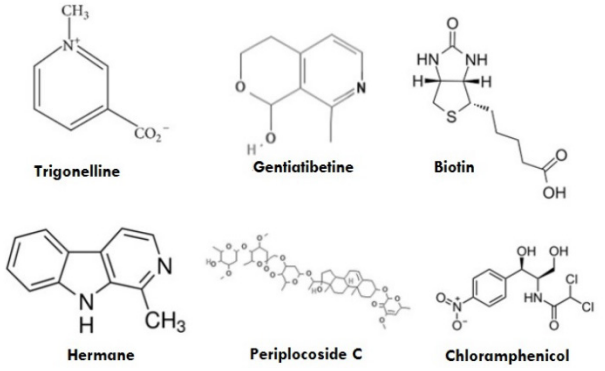


Figure 1. Structures of Compounds extracted from Gedi Leaf [9]

Flavonoid compounds have many important functions for health, including reducing the risk of cardiovascular disease, blood pressure, atherosclerosis, and as an antioxidant [11]. Agglycono flavonoids are found in plants with different structural shapes. Each structure contains carbon atoms in the base nucleus arranged in the form of configurations C6-C3-C6, i.e. two aromatic rings connected by a unit of three carbons that may or may not form a third ring [12]. All flavonoid variants are interrelated due to the same bio-synthesis path of the mat seed and the acetate-malonate flow. Flavonoids in plants are generally bound as glycosides, both O-glycosides and C-glycosides [13,14].

Flavonoids in vegetables are secondary metabolites utilized for health and clotting ingredients which are major contributors to its functional capacity as an antioxidant [15]. In addition to acting as an antioxidant, flavonoids can also modulate cell signaling pathways and their effects can be marked on cell function by altering proteins and fat phosphorylation and modulating genetic expression [16, 17].

The high flavonoid content of Gedi leaves has encouraged efforts to exploit this ability by creating more efficient extraction methods. Conventional methods present disadvantages, such as long extraction time, copious solvent and minimal extraction yield. The optimization of phytochemical extraction can be accomplished with the use of ultrasonic extraction. Ultrasonic extraction takes a shorter time compared to thermal and traditional processes. In addition, it is healthy and provides a higher volume of crude yield. Ultrasonic extraction happens at lower temperatures and is thus an effective method of isolating bioactive compounds that are vulnerable to high thermal exposure [18]. Extraction of Feluric Acid from the rice bran using ultrasonic ethanol methods [19, 26].

The purposes of study was to calculate yields and analyze compounds extracted from Gedi leaf extraction using the proximate and LCMS-QToF ultrasonic methods.

2. Materials and Methods

The study lasted for four months in the Physics-Chemistry Laboratorium, Chemical Engineering Department, Engineering Faculty, Universitas Muhammadiyah Jakarta, and Testing Laboratorium of Saraswati Indo Genentech Company. The materials consisted of Gedi leaves from Menado and ethanol. The equipment employed comprised of 60-mesh siever, ultrasound sonicator, oven, glass bottles, filter paper, glass Beakers, hotplates, digital scales, graduated cylinders, Erlenmeyer flasks, and thermometer. The analyses of active compounds extracted from Gedi leaves were conducted using LCMS/MS-QTOF supported by UNIFI software containing a mass spectrum library of natural active compounds compiled by Waters^{TM} [20].

The research stages were the production of Gedi leaf powder followed by the production of Gedi leaf extract. To generate Gedi leaf powder, leaves were washed, reduced into smaller pieces, dried at 70^{o}C, and ground. The size reduction was intended to increase the surface area and, in turn, increase the reaction rate of Gedi leaves and solvent. To obtain a uniform grain size of powder, the ground Gedi leaves were sieved with a 60-mesh sieve. The extraction stage started with mixing the Gedi leaf powder with ethanol, as the solvent, at the ratio of 1:40 (m:v). The resulting solution underwent ultrasonic extraction for 30 minutes in a sonicator with the wavelength set at 40 kHz. The sonicated solution was condensed in a rotary vacuum evaporator at 50-60 ^{o}C. The yield was calculated by comparing the final weight at the end of the rotary vacuum evaporator step to the initial weight of Gedi leaf powder.

To determine the characteristic properties of Gedi leaf powder and extract, proximate and LCMS QToF analyses were conducted. Proximate analysis is employed to group the components found in food based on their chemical compositions and functions [21]. A procedure has been developed to specifically characterize the compounds in Gedi leaves, that are intended to be isolated and purified (**Table 1**). Trigonelline, Gentiatibetine, and Harman are alkaloid compounds, Periplocoside C is a steroid, Biotin is vitamin B7, and Chloramphenicol is an antioxidant.

The filtrate was analyzed with reversed-phase mass spectrometry liquid chromatography using UV-Vis (254 nm) and mass spectrometry detection. Samples were run

through symmetric columns, C 18 5 um, 4.6 mm × 150 mm, with linear gradient elution using water-acetonitrile as the mobile phase at the rate of 1.0 ml/minute. This method was utilized to determine the compounds found in Gedi leaf extract. LCMS-QToF detects compounds in the specific form of M + H, and therefore, there were additional molecules in the M + 1.

Table 1. Names and Molecular Mass of Known Compounds extracted from Gedi Leaf

Name of Compound	Molecular Formula	Molecular Mass (g/mol)
Trigonelline	$C_7H_7NO_2$	137.14
Gentiatibetine	$C_9H_{11}NO_2$	165.19
Harman	$C_{12}H_{10}N_2$	182.22
Periplocoside C	$C_{49}H_{76}O_{16}$	921.10
Biotin	$C_{10}H_{16}N_2O_3S$	244.31
Chloramphenicol	$C_{11}H_{12}Cl_2N_2O_5$	323.132

3. Results and Discussion

3.1. The Yield of Gedi Leaf Extract

The yield in this study, from the previously described procedure (**Figure 1**), was 0.6%. Based on study by [1], reported a yield of 6.33%, in which they extracted Gedi leaves using maceration in a sequential solvent of n-hexane, acetone, and methanol. The greater yield from our study might have been a result of a higher content of polar compounds than that of non-polar in Gedi leaves. It also reported that proteins and carbohydrates in Gedi leaf extract made up 9.6% and 17.76% respectively, compared to 6.05% of fats [1]. It, therefore, could be expected that Gedi leaf extraction using ethanol would result in higher yields than that using sequential solvents.

Figure 2. The ultrasonic extraction process of Gedi leaf

3.2. Proximate Analysis

The proximate analysis produced results that are presented in **Table 2**. For comparison, previous proximate analyses of Gedi mucilage showed contents of 14.66% proteins, 35.38% carbohydrates, 10.46% water content, and 38.8% ash content [22].

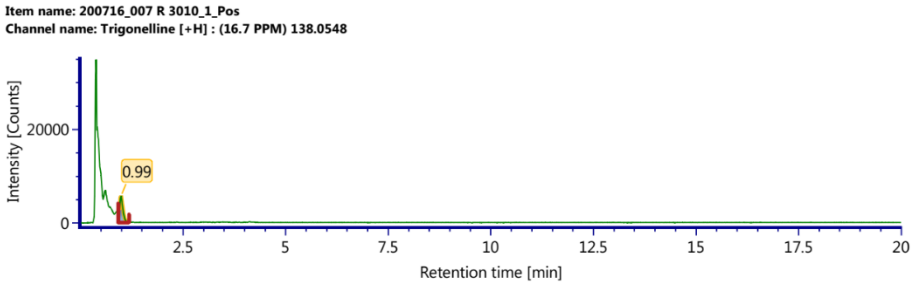
Table 2. Proximate Analysis of Gedi Leaf Powder and Extract

Parameter	Unit	Results of Gedi Leaf Powder	Results of Gedi Leaf Extract
Protein	%	27.135	0.635
Carbohydrate	%	41.675	1.27
Total Energy	kcal. 100 g	329.5	9.26
Water content	%	11.235	17.1
Total Fat	%	6.095	< 0.02
Energy and Fat	kcal. 100 g	54.5	0
Ash Content	%	13.83	0.635

Ash content is the parameter to demonstrate the number of minerals (inorganic substances) contained in an object or product. The inorganic compounds that can be found include Calcium, Potassium, Phosphorus, Iron, Magnesium, and so on [23]. The ash content in Gedi leaves (13.83%) is similar to that in Red Betel leaves (14.33%), which is higher than that in the leaves of *Orthosiphon aristatus* (locally known as Kumis Kucing, 7.89%). These plants are used in herbal medicine [24]. These figures showed that Gedi and Red Betel leaves contain a higher level of minerals than do Kumis Kucing leaves. In this study, carbohydrates constituted the largest group found in Gedi leaf extract (**Table 2**). Comparable to plant leaves in general, the types of carbohydrates found in Gedi leaves most likely consist of crude fibers, such as lignin and pectin. The second-largest component was proteins. Gedi leaves contained higher protein content (27.135%) than the leaves of Salam (Indonesian Bay Leaf, *Syzygium polyanthum*), which was previously determined to be 7.61% and also known to be used to treat Diabetes Mellitus [25]. The component with the lowest amount was fats (6.095%). This figure is smaller than 8.36%, which was the number of fats found in Salam leaves [26].

3.3. LCMS-QToF Analysis of Gedi Leaf Extract

LCMS-QToF is a method used to separate certain compounds or mixtures based on their polarities as well as to determine the molecular masses of those compounds or mixtures. The results of LCMS-QToF analyses of all samples in positive-ionization modes are presented in **Figure 3 to 7**, whereas those in negative-ionization modes are in **Figure 8**.



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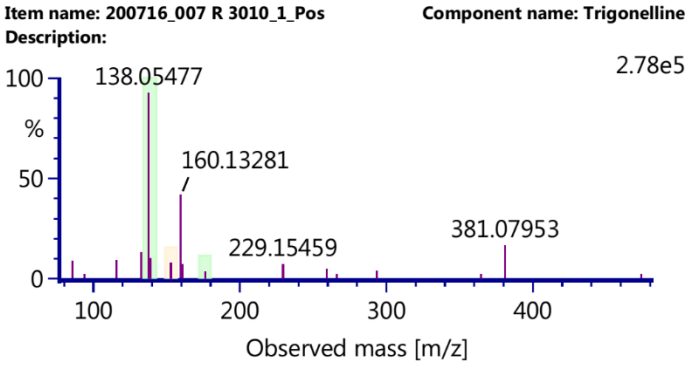

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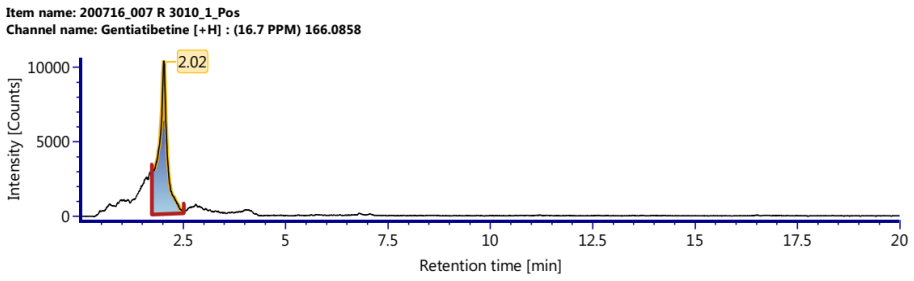
Figure 3. Results LCMS-QToF for Trigonelline: (a) extracted Chromatograms, (b) mass spectral data



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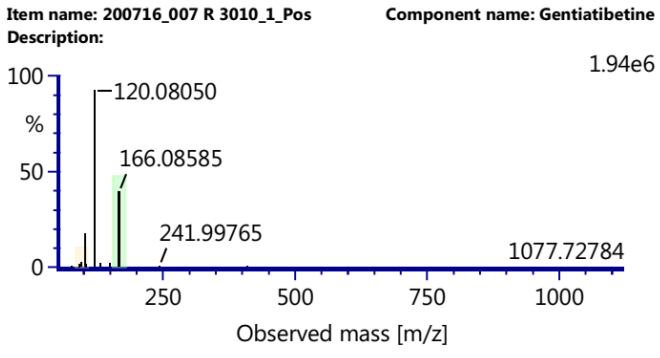

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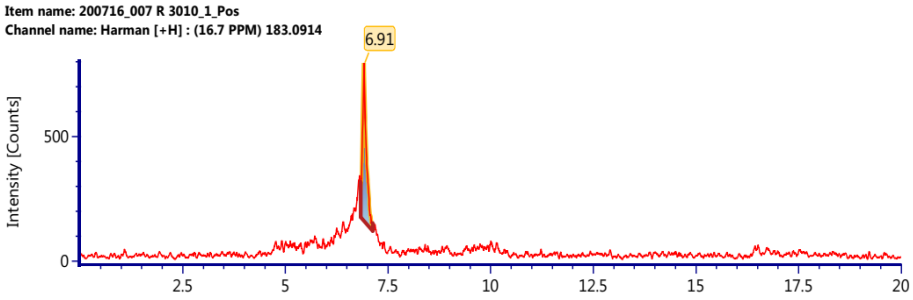
Figure 4. Results LCMS-QToF for Gentiatibetine: (a) extracted Chromatograms, (b) mass spectral data



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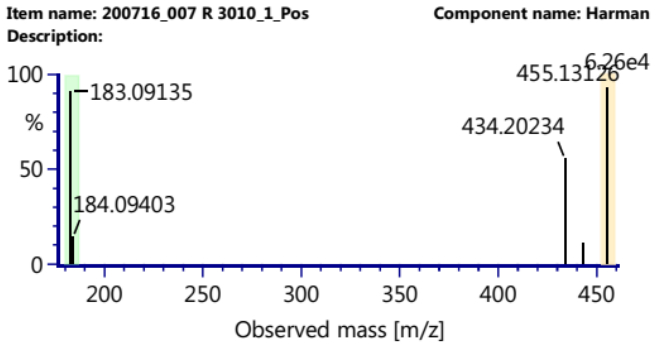

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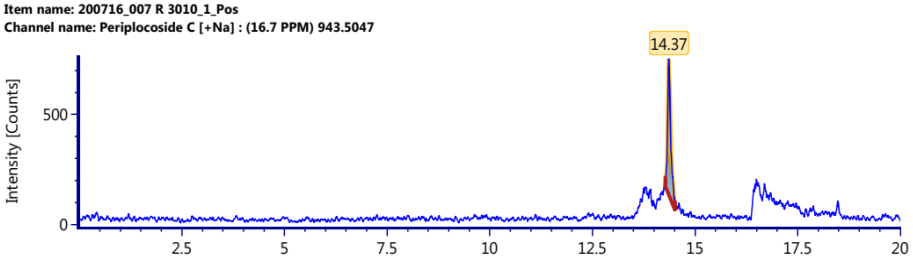
Figure 5. Results LCMS-QToF for Harman: (a) extracted Chromatograms, (b) mass spectral data



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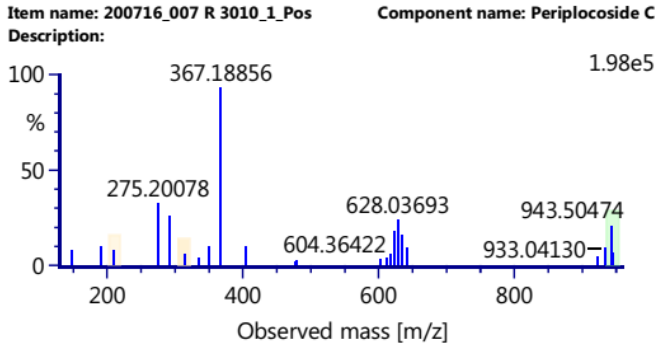

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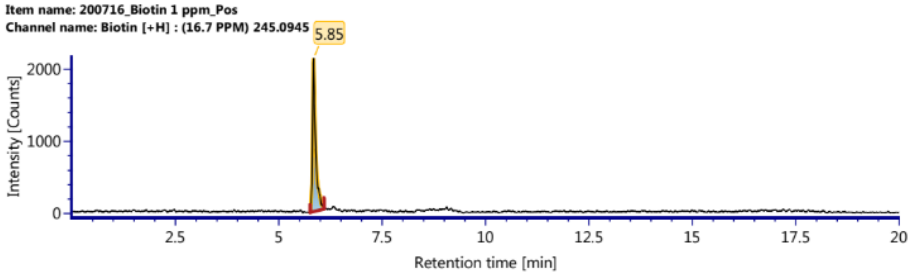
Figure 6. Results LCMS-QToF for Periplocoside C: (a) extracted Chromatograms, (b) mass spectral data



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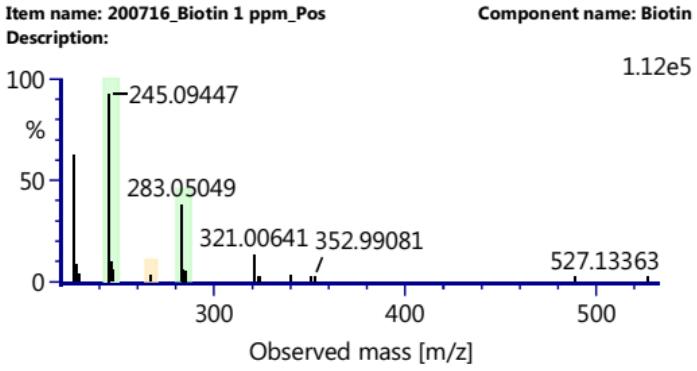

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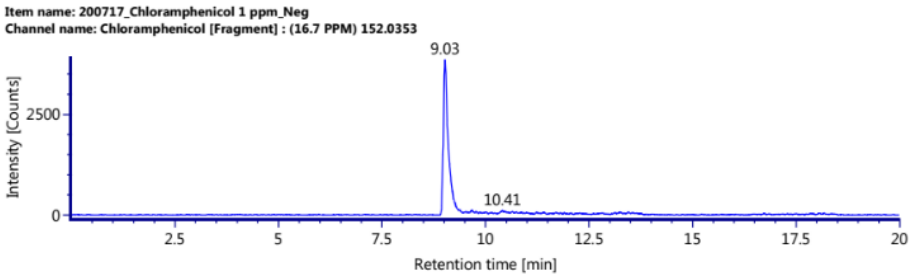
Figure 7. Results LCMS-QToF for Biotin: (a) extracted Chromatograms, (b) mass spectral data



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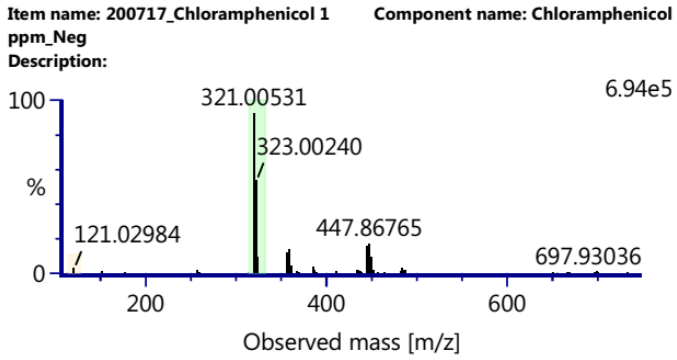

(b)

Figure 8. Results LCMS-QToF for Chloramphenicol: (a) extracted Chromatograms, (b) mass spectral data

Trigonelline was the earliest compound to be detected at 0.99 minutes because it is the smallest compound of the group, and therefore, exhibits the lowest retention time (**Figure 3**). **Figure 4 to 7** shown the following compounds detected based on the increasing retention times, which were Gentiatibetine (2.03 min), Harman (6.92 min), Periplocoside C (14.37 min), and Biotin (5.85 min). Trigonelline, Gentiatibetine, and Harman are alkaloids known as natural blood pressure-lowering agents, sedatives, and antispasmodic. Periplocoside C is a

quercetin glycosidic compound and known as a glycosidic antioxidant, and Biotin is also known as Vitamin B7 and needed for the syntheses of hair, nail, and dermis. Chloramphenicol was the only compound detected with a negative-ionization mode and exhibited a retention time of 9.03 minutes (**Figure 8**). This compound is a known antibiotic against various bacterial infections.

4. Conclusions

The yield of Gedi leaf extraction using ethanol as a solvent at the ratio of 1:40 (m/v) and ultra-sonication for 30 minutes was 9.86 %. Proximate analyses of Gedi leaf powder produced the values 27.135 % proteins, 41.675 % carbohydrates, 329 kcal total energy, 11.235 % water content, and 13.83% ash content. Meanwhile, the values from the proximate analyses of Gedi leaf extract were 0.635% proteins, 1.27% carbohydrates, 9.26% total energy, 17.1% water content, and 0.635% ash content. The active compounds in Gedi leaf extract were detected and characterized using LCMS-QToF. In these analyses, compound detections were specifically formatted to M + H, and therefore, additional molecules were present in M + 1 ions. Trigonelline, Gentiatibetine, Harman, Periplocoside C, and Biotin were detected using the positive-ionization mode, whereas Chloramphenicol was detected with negative-ionization mode.

Acknowledgments

We would like to thank the Ministry of Research and Technology/National Research and Innovation Agency for financial support of this work through a research grant for the year 2020. We would also like to express gratitude towards LPPM and the Department of Master in Chemical Engineering, Engineering Faculty, Universitas Muhammadiyah Jakarta for all the facilities and support provided throughout the research.

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