

## Proximate Composition and Phytochemical Analysis of Malaysian *Liberica* sp. Coffee Bean and Its Pulp

Nurhuda Syahirah Ismail<sup>1</sup>, Uswatun Hasanah Zaidan<sup>1,2\*</sup>, Suhaili Shamsi<sup>1</sup>, Siti Salwa Abd Gani<sup>3</sup> and Elexson Nillian<sup>4</sup>

<sup>1</sup>Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

<sup>2</sup>Halal Products Research Institute, Universiti Putra Malaysia, Putra Infoport, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

<sup>3</sup>Department of Agriculture Technology, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

<sup>4</sup>Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 93400 Kota Samarahan, Sarawak, Malaysia

### ABSTRACT

Arabica, Robusta, and Liberica are the three main coffee species cultivated globally. Liberica coffee is a minor species, accounting for less than 1% of global cultivation. Due to favorable climatic conditions in Malaysia, Liberica coffee dominates coffee production, accounting for 73%, while Robusta makes up the remaining 27%. Nevertheless, the substantial coffee production resulted in approximately 15 million tons of discarded skin and pulp, contributing to environmental pollution. This study was conducted due to insufficient information and research on the proximate composition and phytochemical compounds of the coffee bean and pulp from *Liberica* sp. This study aims to determine the proximate composition of coffee beans and pulp extracts from *Liberica* sp. and to identify

the phytochemical composition using liquid chromatography-mass spectrometry (LC-MS) analysis. The nutritional values of carbohydrates, protein, crude fiber, crude fat, and ash were obtained using proximate analysis. Coffee beans exhibited the highest value for crude protein (11.96%) and crude fiber (11.83%), whereas coffee pulp has the highest significant value for moisture content (68.81%) and ash (7.31%). LC-MS analysis shows emmotin A and

#### ARTICLE INFO

##### Article history:

Received: 21 August 2023

Accepted: 20 November 2023

Published: 21 May 2024

DOI: <https://doi.org/10.47836/pjtas.47.2.13>

##### E-mail addresses:

hudasyahirahismail@gmail.com.my (Nurhuda Syahirah Ismail)

uswatun@upm.edu.my (Uswatun Hasanah Zaidan)

sh\_suhaili@upm.edu.my (Suhaili Shamsi)

ssalwaag@upm.edu.my (Siti Salwa Abd Gani)

nelxson@unimas.my (Elexson Nillian)

\* Corresponding author

deoxymiroestrol were the major phytochemical compounds. These findings contribute to understanding the nutritional value and phytochemical compounds of coffee beans and pulp from *Liberica* sp. that may contribute to sustainable waste management and other applications in the food and beverage industry.

*Keywords:* Coffee bean, *Liberica* sp., phytochemical composition, proximate, pulp

## INTRODUCTION

Coffee is the most demanding beverage worldwide, mostly cultivated at 600 and 2,500 m a.s.l. in Africa, South-East Asia, and Central and South America (Schenker et al., 2002). Arabica and Robusta, the main coffee species, constitute about 80 and 20% of the world's production, respectively, while *Liberica* is the least grown, with only 1% of cultivation. According to Ismail et al. (2014), the optimal growing temperature of the coffee plant in Malaysia ranges from 18 to 28°C, where Arabica is found as the minor species, while *Liberica* dominates about (73%), and Robusta (27%) are found to be the main cultivated species. *Liberica* is known for its robustness and flavor, similar to Robusta, while Arabica coffee has a sweet and caramel flavor profile.

Two primary methods are normally used for processing coffee cherries, including dry and wet. Coffee farmers often use the wet processing method to meet market demand and improve coffee production quality (Campos et al., 2020). In this approach, the coffee cherries underwent pulping, fermented, washed, and dried, with the skin and pulp removed and discarded as waste. The coffee cherries must be processed before being sold commercially. However, the widespread commercialization of coffee

production can lead to ecological damage, as the waste from coffee production can cause environmental pollution (Geremu et al., 2016). Coffee pulp is usually disposed of in large landfills without undergoing any treatments. Consequently, this issue has received greater attention in recent years as researchers have sought ways to reduce environmental pollution by finding alternative uses for waste. Despite the significant quantities produced, numerous studies have proposed strategies to reduce environmental pollution from coffee production waste.

In Malaysia, *Liberica* sp. is the main species cultivated and is native to southern Johor. The soil in Johor makes it suitable for cultivation due to the humidity, heat, and clay-like soil conditions. The coffee industry generates a significant amount of solid and liquid byproducts, approximately 15 million tons globally (Kovalcik et al., 2018), which are sources of pollution if not properly disposed of (Nabais et al., 2008). The primary source of solid byproducts in the industry was coffee pulp and ground coffee. Coffee cherries contain about 43% of pulp, and of the 15 million tons of waste generated annually, 8.5 million tons are pulp. The residues may contain bioactive compounds that have potential

value for some alternatives to reuse the waste, including fertilizers, livestock feed, compost, and mushroom cultivation, as reported by Heeger et al. (2017).

In accordance with Al-Dhabi et al. (2017), coffee beans consist of a diverse range of chemicals associated with health, including antioxidants, melanoidins, diterpenes, phenolic compounds, vitamin precursors, and xanthine. Antioxidants are substances that safeguard living tissues against free radical damage. Adam et al. (2009) have reported that free radicals are molecules produced by incomplete oxygen reduction during aerobic respiration and have been associated with oxidative stress in several illnesses, including cancer, inflammatory, cardiovascular, and neurological disorders. The coffee extract showed high antioxidant and metal-chelating properties and has gained much attention recently. In Malaysia, some previous studies have been done on *Liberica* sp. but were focused on the physical properties of coffee beans, the antioxidant activity of coffee pulp, and the physicochemical properties of coffee silver skin (Buyong & Nillian, 2023; Ismail et al., 2014; Nillian et al., 2020). However, no study is still focusing on proximate analysis and phytochemical composition of coffee beans and pulp from *Liberica* sp., thus leading to this study. Hence, this research intends to determine the proximate analysis of coffee beans and pulp from *Liberica* sp. and identify the phytochemical composition of this coffee species using LC-MS analysis.

## MATERIALS AND METHODS

### Preparation of Samples

The coffee fruits were obtained from the Jabatan Pertanian Batu Pahat, Johor. The collected fruits were thoroughly washed and separated to obtain the bean and pulp, as shown in Figures 1 (a) and (b). The bean and pulp were dried and ground into fine powder with approximately 200  $\mu\text{m}$  particle size. The fresh sample of coffee beans and pulp was used to analyze moisture. In contrast, the ground sample was used to analyze protein, crude fat, crude fiber, carbohydrate, and ash using the method described by AOAC INTERNATIONAL (1990), Chang (2003), James (1995), as well as Kirk and Sawyer (1991). Each sample was repeated thrice for each analysis.



Figure 1. (a) Coffee bean; and (b) coffee pulp after being separated from coffee fruit of *Liberica* sp.

### Proximate Analysis of Coffee Bean and Coffee Pulp

**Determination of Moisture Content.** This procedure used the gravimetric method described by AOAC INTERNATIONAL (1990). The 5 g sample was measured and oven-dried at 105°C for six days until a constant weight was obtained. It was weighed after being cooled to room

temperature at 25°C. The drying, cooling, and weighing process were repeated until stable weight was achieved. The moisture percentage was calculated based on the difference in weight between the samples before and after drying.

$$\text{Moisture (\%)} = \frac{(\text{Weight of sample before drying}) - (\text{Weight of sample after drying})}{\text{Weight of sample before drying}} \times 100\%$$

**Determination of Protein.** Kjeldahl's method was carried out according to Chang (2003). The total nitrogen content was multiplied by a factor of 6.25. In a digestion flask, 10 ml of concentrated sulfuric acid (H<sub>2</sub>SO<sub>4</sub>, R&M Chemicals, United Kingdom) was mixed with 5 g of the sample and a selenium catalyst tablet was added. Beneath the fume cupboard, the solution was subsequently heated until a clear solution was achieved. The digest was diluted in a volumetric flask to a volume of 100 ml prior to starting the analysis. In the Kjeldahl distillation apparatus, an equivalent volume of 45% sodium hydroxide (NaOH, R&M Chemicals, United Kingdom) was mixed with 10 ml of the digest, and three drops of a mixed indicator (bromocresol green/methyl red, Sigma-Aldrich, USA) were added afterward upon collection of the distillate into 10 ml of 40% boric acid (H<sub>3</sub>BO<sub>3</sub>, R&M Chemicals, United Kingdom). In total, 50 ml of distillates were collected and titrated against 0.02 N

ethylenediamine tetraacetic acid (EDTA, Sigma-Aldrich, USA), starting from a green endpoint to a deep red endpoint. A blank reagent was further digested, distilled, and titrated. The nitrogen content was calculated using the following formula:

$$\text{Nitrogen (\%)} = \frac{100\%}{w} \times \frac{N \times 14}{1000} \times \frac{Vt}{Va} \times t.b$$

where, w = Sample weight (0.5 g), N = Titrant normality (0.02 N H<sub>2</sub>SO<sub>4</sub>), Vt = Total digest volume (100 ml), Va = Volume of digest analyzed (10 ml), t = Titrate sample value, and b = Blank titer value.

**Determination of Total Ash Content.**

According to AOAC INTERNATIONAL (1990) and James (1995), this study used a furnace incinerating process, where 5 g of the sample was placed into a weighed porcelain crucible and was burnt at 550°C in a muffle furnace until completely burned to ash. The weight of the ash was estimated as a percentage of the sample weight after cooling and weighing.

$$\text{Ash (\%)} = \frac{(\text{Weight of crucible with ash}) - (\text{Weight of crucible})}{\text{Weight of sample}} \times 100\%$$

**Determination of Crude Fiber.** The crude fiber was analyzed using the procedure of James (1995), which involved refluxing a 5 g sample in 150 ml of 1.25% H<sub>2</sub>SO<sub>4</sub> solution

(R&M Chemicals, United Kingdom) for 30 min. A double cloth was used to collect the particles, which were washed with hot water in several portions. Under the same condition, it was mixed with 150 ml of 1.25% NaOH (R&M Chemicals, United Kingdom) in the flask and boiled again for another 30 min. The sample was rinsed with several parts of water and then allowed to drain until dry. It was then quantitatively transferred to a weighed crucible, where it was dried in the oven at 105°C until no further changes in weight. The sample burnt where it was, leaving only ashes, and was transferred to a muffle furnace. The difference in the samples' weight determined the crude fiber's weight.

$$\text{Crude fibre (\%)} = \frac{w_2 - w_3}{\text{Weight of sample}} \times 100\%$$

where  $w_2$  = Weight of the crucible with the sample after washing, boiling, and drying, and  $w_3$  = Weight of the crucible with a sample of ash.

**Determination of Crude Fat.** The determination of crude fat was done using a previous method by Kirk and Sawyer (1991). A gravimetric solvent extraction procedure was used where 5 g of samples were placed in the thimble after being wrapped in porous paper (Whatman filter paper). The thimble was put in a Soxhlet extractor (Gerhardt, United Kingdom) and 200 ml of petroleum ether (R&M Chemicals, United Kingdom) as solvent. The process

of solvent was heating, evaporating, and condensing in the reflux flask, where the water condenser was connected to the top part of the reflux. The oil extract was then poured into the boiling flask after the sample in the thimble was submerged in the solvent until the reflux flask was full. This process was repeated for 8 hr. The oil extracts flask was oven-dried at 60°C for 24 hr to remove the residual solvent. Once the flask was cooled to room temperature, the flask was weighed to determine the percentage of crude fat according to the formula:

$$\text{Fat (\%)} = \frac{(\text{Weight of flask with oil extracts}) - (\text{Weight of empty flask})}{\text{Weight of sample}} \times 100\%$$

**Determination of Carbohydrate.** The percentage of carbohydrates was determined by the difference of moisture, protein, ash, crude fat, and crude fiber of 100% using the following equation below:

$$\text{Carbohydrate (\%)} = 100\% - (\text{Moisture} + \text{protein} + \text{ash} + \text{crude fiber} + \text{crude fat})\%$$

### Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis of Coffee Pulp and Coffee Bean

**Sample Extraction Preparation.** Methanol was used as a solvent to extract compounds in the coffee beans and coffee pulp according to the method previously described by Redfern et al. (2014). Initially, both samples

were dried, and mortar and pestle were used to acquire powdered samples. The resulting powder was placed in a thimble and put into a Soxhlet extractor apparatus (Gerhardt, United Kingdom). A quantity of 200 ml of methanol (R&M Chemicals, United Kingdom) as solvent was added to the apparatus. It was subjected to heat with isomantle and allowed to evaporate. The evaporated solvent was poured back, and the cycle was repeated for 8 hr. Upon completion of the process, the solvent was removed, leading to a yield of approximately 2 to 3 ml of extracted plant material.

**LC-MS Analysis.** The LC-MS system, which consists of an Agilent 1290 Infinity LC system linked to Agilent 6520 Accurate-Mass Q-TOF mass spectrometer with dual ESI source (Agilent Technologies, USA), was used in this study. The instrument was run using an electrospray (ES) interface in positive ion mode. The Agilent Zorbax Eclipse XDB-C18 Analytical, 150 mm x 4.6 mm, 5 microns (P/N: 993967-902) (Thermo Fisher Scientific, USA) was utilized with a 40°C of column temperature and flow rate of 0.4 ml/min, and liquid chromatography was carried out on 3.0 µl of the sample at a concentration 1 mg/ml. The mobile phase was made up of 0.1% formic acid (R&M Chemicals, United Kingdom) in water as Solvent A, while 0.1% formic acid (R&M Chemicals, United Kingdom) in methanol (R&M Chemicals, United Kingdom) as Solvent B with a 24-min runtime was operated in the gradient mode.

The mass spectra were obtained in positive ionization mode with a range of  $m/z$  100 to 3,200. The parameters of the MS scan were the positive ion capillary voltage of 4,000 V, the voltage of the fragmentor was 125 V, and the voltage of the skimmer was 65 V. The nebulizer pressure and flow rate were adjusted to 45 psi and 10 L/min, respectively, and a temperature of 300°C drying gas was used. The software used to analyze the MS data was Agilent Masshunter Qualitative Analysis (version B.07.00, Agilent Technologies, Germany), which generated a list of potential compounds with more than 100 counts of peak-only using the molecular feature extraction (MFE) algorithm. The compounds with a relative height of more than 2.5% and an absolute height greater than 5,000 counts were included. The permitted ionic species for positive ions were hydrogen ( $H^+$ ), sodium ( $Na^+$ ), potassium ( $K^+$ ), and ammonium ( $NH_4^+$ ), whereas chloride ( $Cl^-$ ) was allowed for negative ions. With a high score indicating a closer resemblance, the software also provided a score (%) to demonstrate how closely the software matched the actual formula with the created molecular formula of the compound.

### Statistical Analysis

The obtained data were statistically analyzed using an analysis of variance (ANOVA) *t*-test, and the result was subsequently presented in mean  $\pm$  standard deviation (SD) using SPSS Statistic 27.



## RESULTS AND DISCUSSION

### Proximate Analysis of Coffee Bean and Coffee Pulp from *Liberica* sp.

The proximate composition of coffee bean and pulp extracts is shown in Table 1. The data shows that coffee bean and pulp extracts have the highest value of moisture (60.39 and 68.81%) and crude protein (11.96 and 9.07%) but the lowest value of fat content (4.28 and 0.80%). The moisture content level is important for the preservation of coffee and affects the quality of coffee (Ismail et al., 2013). In the previous study reported by Zainol et al. (2020), the moisture content of three different species of coffee beans ranged from 61.83 to 66.51%, where the moisture content of *Liberica* coffee was 61.83%. Similar research has been conducted by Rohaya et al. (2023), who reported that the moisture content of coffee pulp from Indonesia from three different species ranged from 67.61 to 79.49%, where *Arabica* coffee has the highest moisture content. The findings show that the moisture level was influenced by the conditions of the location cultivation, species variety and the processing technique (Harahap, 2017).

The ash content of coffee beans and pulp found in this study was 3.42 and 7.31%. The ash content is commonly associated with many mineral compounds, which is crucial in determining the nutritional value. Coffee represents the number of essential minerals such as magnesium (6.4 to 7.5%), potassium (6.6%), phosphorus (2.2%), sodium (2.2%), and calcium (0.7%), which play an important role in the body metabolism (Olechno et al., 2021; Sena et al., 1998). The ash content of coffee beans from this study was higher than that of three different coffee species from a previous study by Zainol et al. (2020). Meanwhile, the previous study by Rohaya et al. (2023) reported that *Liberica* coffee (1.74%) has the highest ash content than *Arabica* (0.25%) and *Robusta* (0.52%). The differences in the ash content are due to the nutrients and minerals available in the environmental climate (Oliveira et al., 2013). Due to the different roles and purposes, coffee beans serve as the energy-rich seed for plant growth, while coffee pulp provides nutrients and moisture to support the bean's development.

The results show that coffee bean has a high amount of fat compared to coffee

Table 1  
*Proximate analysis of coffee bean and coffee pulp*

Composition	Coffee pulp (w/w, %)	Coffee bean (w/w, %)
Moisture	68.81 ± 0.10	60.39 ± 1.23
Ash	7.31 ± 0.05	3.42 ± 0.02
Fat	0.80 ± 0.20	4.28 ± 0.23
Crude protein	9.07 ± 0.55	11.96 ± 0.27
Crude fiber	7.48 ± 0.06	11.83 ± 0.06
Carbohydrate	6.77 ± 0.82	8.12 ± 1.39

*Note.* Values are presented as mean ± SD

pulp, which is 4.28 and 0.80%, respectively. Wibowo et al. (2022) have stated that fat found in the coffee bean serves as a protective bean and will form a layer of oil when roasted. Fat content is one of the chemical compositions that influence the coffee flavor. This finding on the ash content of coffee pulp is aligned with the previous study by Rohaya et al. (2023), where the fat content of coffee pulp from three different species ranged from 0.30 to 1.01%. In the study, it was reported that Robusta coffee pulp has the highest amount of fat content. Meanwhile, the previous study by Zainol et al. (2020) reported that Liberica coffee has the highest fat content, which is 2.79%, comparable to Arabica with 1.38% and Robusta with 1.67%. The differences in fat content can be caused by various factors, including species variety, parts of the plants, processing methods, and storage conditions (Mussatto et al., 2011; Wibowo et al., 2022). Coffee beans from *Liberica* sp. have higher protein content than coffee pulp, which is 11.96 and 9.07%.

Protein is also one of the chemical components that influence the taste and the nutritional value. The study reported that the protein content of coffee beans from three different species ranged from 5.15 to 7.20%, with Liberica coffee having the highest protein content while Arabica coffee has the lowest value of protein content of 5.15% (Zainol et al., 2020). According to a previous study by Rohaya et al. (2023), the amount of protein ranged from 4.08 to 7.88%, where Robusta coffee pulp has the highest protein content.

This finding shows that coffee pulp from Liberica coffee can be used in animal feed such as ruminants, supported by Núñez et al. (2015).

The amount of crude fiber in this study was lower than that of coffee beans and pulp reported by the previous study. Zainol et al. (2020) reported that the crude fiber of coffee beans from three different species was 19%, with Arabica coffee having the highest amount at 19.82%. Meanwhile, the crude fiber of coffee pulp from Rohaya et al. (2023) ranged from 13.79 to 38.51%, whereas Liberica coffee has the highest amount of crude fiber. Crude fiber is a residue derived from materials containing cellulose with a small amount of lignin and pentose (Sitohang & Pandiangan, 2022). The differences in the amount of crude fiber of the coffee bean and pulp were influenced by the type of coffee species and the elevation of coffee cultivation (Rohaya et al., 2023).

The number of carbohydrates in coffee beans in this study was similar to the previous study by Zainol et al. (2020), in which the range of carbohydrates from three different species was 6.22 to 8.91%, where Liberica coffee was the highest. Carbohydrates, on the other hand, contribute to bodily metabolism by providing the requisite energy. The amount of carbohydrates in coffee beans was higher than in coffee pulp. Consequently, the coffee bean has the potential to serve as a significant reservoir of both energy and fiber essential for bodily functions (Mohd Jailani et al., 2020; Zaidan et al., 2019).



### LC-MS Analysis of Coffee Bean and Coffee Pulp

The LC-MS chromatogram shows a total of 11 compounds in both coffee bean and pulp extracts, including several phytochemicals that comprised numerous phytochemicals displaying mass that matched (5 ppm tolerance) in the database. The compound classes in both extracts dominantly belonged to phenolic acids, terpenes, coumarins, and stilbenes. As shown in Tables 2 and 3, emmotin A is the highest compound found in coffee bean and pulp extracts, as shown by peak 10 in Figure 2 and peak 8 in Figure 3. Emmotin A belongs to the terpenes compound class, and Chu et al. (2016) have reported that Arabica coffee contains a large number of terpenes containing 16-O-methyl coffee, which has been used as a marker to distinguish small and medium

coffee. According to Saleem et al. (2020), emmotin A has been identified as a primary source of natural antioxidants and enzyme inhibitor compounds, which is most likely contributing to the antioxidant properties in both coffee bean and pulp extracts. Deoxymiroestrol shows the highest number of compounds after emmotin A in the coffee bean and pulp extracts, according to Tables 2 and 3, as shown by peak 9 in Figure 2 and peak 7 in Figure 3. Deoxymiroestrol is a phytoestrogen compound class with a health benefit, including reproductive health, heart health, weight loss, hormone-dependent tumors, and immune systems (Desmawati & Sulastri, 2019). Based on the findings, Liberica coffee would be a useful alternative as animal feed because coffee beans and pulp have phytoestrogen compounds, which have beneficial effects such as stimulating

Table 2

*Bioactive compounds of coffee bean extract from Liberica sp. were identified using liquid chromatography-mass spectrometry*

Peak	Name	Formula	Difference (MFG. ppm)	RT	Vol (%)	Score (MFG)	Score (DB)	Difference (DB. ppm)
1	2-amino-3-methyl-1-butanol	C <sub>5</sub> H <sub>13</sub> NO	0.86	3.684	2.47	99.82	99.82	0.86
2	Nigerose	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	1.11	4.115	0.54	94.18	89.45	1.11
3	Scopolin	C <sub>16</sub> H <sub>18</sub> O <sub>9</sub>	-0.01	6.761	0.69	99.62	99.62	-0.01
4	Scopolin	C <sub>16</sub> H <sub>18</sub> O <sub>9</sub>	-0.15	7.361	6.46	99.84	99.84	-0.15
5	Cis-5-caffeoylquinic acid	C <sub>16</sub> H <sub>18</sub> O <sub>9</sub>	1.15	7.37	0.63	99.06	99.03	1.15
6	3-O-feruloylquinic acid	C <sub>17</sub> H <sub>20</sub> O <sub>9</sub>	1.49	7.971	4.35	98.93	98.91	1.49
7	3-O-feruloylquinic acid	C <sub>17</sub> H <sub>20</sub> O <sub>9</sub>	0.35	8.102	1.39	99.31	99.31	0.35
8	Hydroxyamobarbital	C <sub>11</sub> H <sub>18</sub> N <sub>2</sub> O <sub>4</sub>	-1.06	11.557	0.33	91.33	91.33	-1.06
9	Deoxymiroestrol	C <sub>20</sub> H <sub>22</sub> O <sub>5</sub>	0.46	11.829	9.06	99.85	99.84	0.46
10	Emmotin A	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	0.34	11.93	23.55	99.83	99.83	0.34
11	Trp Ile Lys	C <sub>23</sub> H <sub>35</sub> N <sub>5</sub> O <sub>4</sub>	0.02	12.589	0.63	95.11	95.11	0.02

*Note.* MFG = Molecular Formula Generator; RT = Retention Time; Vol = Volume; DB = Database

**Table 3**  
*Bioactive compounds of coffee pulp extract from Liberica sp. were identified using liquid chromatography-mass spectrometry*

Peak	Name	Formula	Difference (MFG. ppm)	RT	Vol (%)	Score (MFG)	Score (DB)	Difference (DB. ppm)
1	D-glucoside	C <sub>7</sub> H <sub>14</sub> O <sub>6</sub>	1.63	3.78	3.82	98.51	97.64	1.62
2	Quinic acid	C <sub>7</sub> H <sub>12</sub> O <sub>6</sub>	-0.46	4.048	4.39	98.51	98.51	-0.46
3	(2S,3S)-2,3-Dihydro-2,3-dihydroxybenzoate	C <sub>7</sub> H <sub>8</sub> O <sub>4</sub>	1.65	4.05	0.23	91.07	91.06	1.66
4	Penicilic acid	C <sub>8</sub> H <sub>10</sub> O <sub>4</sub>	1.70	5.446	0.28	97.47	97.47	1.70
5	L-galactose	C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>	-1.84	6.882	0.56	95.76	95.76	-1.84
6	Lucuminic acid	C <sub>19</sub> H <sub>26</sub> O <sub>12</sub>	0.52	7.995	0.17	99.04	99.03	0.52
7	Deoxymiroestrol	C <sub>20</sub> H <sub>22</sub> O <sub>5</sub>	0.77	11.833	6.68	99.72	99.71	0.77
8	Emmotin A	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	1.37	11.934	25.63	99.39	99.36	1.37
9	Nonoxynol-9	C <sub>33</sub> H <sub>60</sub> O <sub>10</sub>	0.38	12.539	0.51	99.10	99.09	0.38
10	Digitoxigenin	C <sub>23</sub> H <sub>34</sub> O <sub>4</sub>	1.69	12.577	0.42	95.66	95.66	1.69
11	Trp Ile Lys	C <sub>23</sub> H <sub>35</sub> N <sub>5</sub> O <sub>4</sub>	-0.69	12.595	0.98	99.29	99.28	-0.69

Note. MFG = Molecular Formula Generator; RT = Retention Time; Vol = Volume; DB = Database

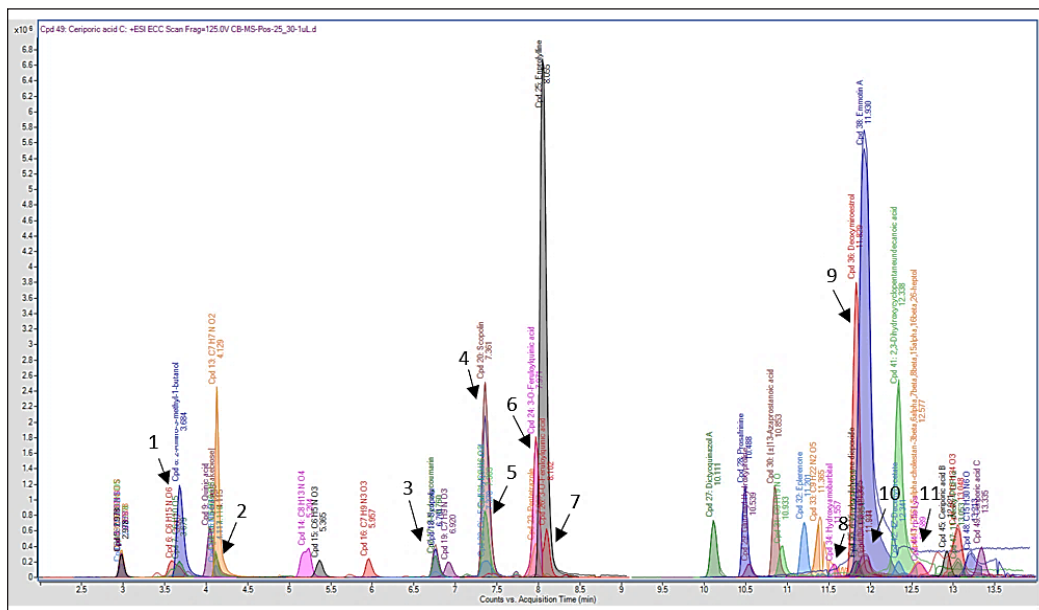


Figure 2. Total ion chromatogram from coffee bean extract of *Liberica* sp.

growth rate and increasing weight gain in livestock (Pace et al., 2006).

Based on Table 2, scopolin was a major compound in the coffee bean extracts,

as shown by peaks 3 and 4 in Figure 2. Scopolin is a derivative of coumarins, which plays an important role in being present in high concentrations in several dietary

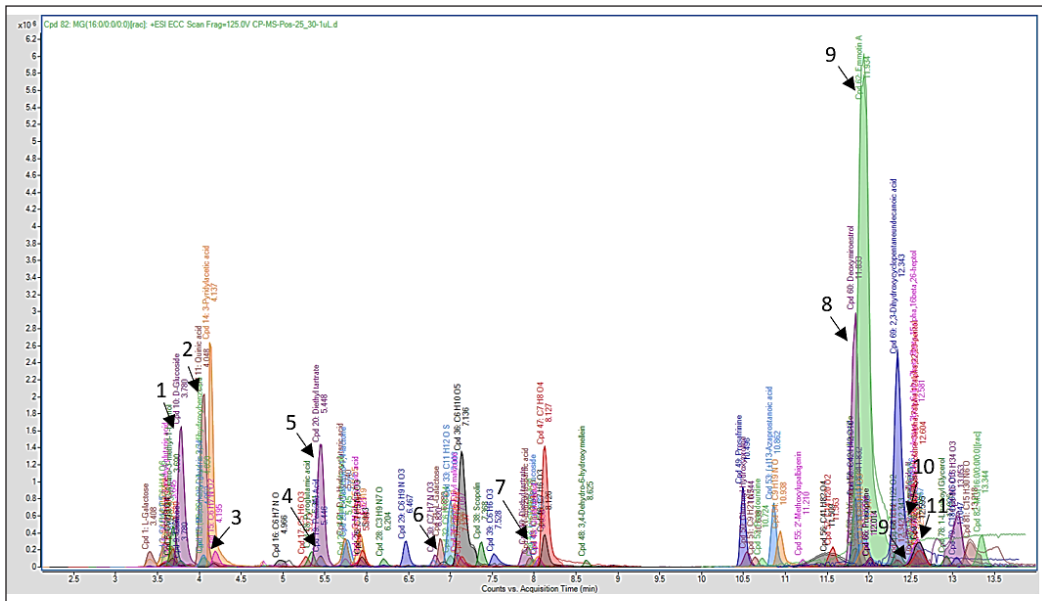


Figure 3. Total ion chromatogram from coffee pulp extract of *Liberica* sp.

plant species. Coumarin is a phytochemical compound tested for pharmacological properties such as anti-inflammatory, antioxidant, antimicrobial, antidepressant, neuroprotective, or antitumoral effects (Srikrishna et al., 2018). According to this finding, coffee beans from *Liberica* can be used as a nutraceutical product as coumarins were found in some widely used foods such as tea and coffee (Lončar et al., 2020). According to Table 2, coffee bean extract from *Liberica* has chlorogenic acids, which are *cis*-5-caffeoylquinic acid and 3-*O*-feruloylquinic acid, as shown in Figure 2 by peaks 5, 6 and 7. Chlorogenic acid is the main phenolic compound, which has the biological functions of lowering blood lipid, antioxidant, and antibacterial (Asamenew et al., 2019).

Table 3 shows that coffee pulp has a D-glucoside bioactive compound, as shown

by peak 1 in Figure 3. The glucoside is a glycoside which is chemically derived from glucose. This finding was aligned with the previous study by Murthy et al. (2012), which reported that Arabica coffee pulp also has glucoside compounds. The findings of this study align with the previous research by Yulianti et al. (2023), which focuses on green beans in Arabica coffee and possesses reported similar compound classes. To be hypothesized, coffee bean and pulp extracts may contain bioactive compounds similar to those of the same species. Different coffee bean and pulp compounds resulted from their specialized roles, biochemical processes, and environmental interaction.

Using coffee beans and pulp from *Liberica* sp. as a food ingredient is essential to characterize these compounds. In this aspect, the application of chromatographic techniques capable of differentiating the

bioactive compounds in the methanolic extracts of coffee bean and pulp from *Liberica* sp. positive ionization modes was performed with a quadrupole time-of-flight mass spectrometry (Q-TOF-MS) coupled to LC system equipped with dual electrospray ionization source (ESI) of methanolic extracts.

## CONCLUSION

The proximate and phytochemical compounds of coffee bean and pulp extracts provide valuable insights into these two components' distinct composition and functional roles within the coffee fruit. Based on the findings, coffee beans exhibited high contents of crude protein, crude fiber, carbohydrates, and fats, emphasizing the beans' energy reserves. In contrast, coffee pulp shows a high content of moisture and ash as the supportive nutritional role for the growth of the bean. On the other hand, LC-MS analysis determines that emmotin A and deoxymiroestrol are the major phytochemical compounds in coffee beans and pulp, contributing to coffee's overall taste, aroma, and nutritional value. Overall, these findings enhance the understanding of the nutritional value and phytochemical compounds in coffee beans and pulp from *Liberica* sp., which could be beneficial for use as a food ingredient or other applications in the food and beverages industry, contributing to sustainable waste management.

## ACKNOWLEDGEMENTS

The author expresses appreciation and gratitude to the Food and Microbial

Technology Laboratory (FAMTech), Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia (UPM), Halal Products Research Institute of UPM, Monash Universiti Malaysia, and the Faculty of Medicine and Health Science of UPM for supplying facilities to carry out this study.

## REFERENCES

- Adam, M., Dobiáš, P., Eisner, A., & Ventura, K. (2009). Extraction of antioxidants from plants using ultrasonic methods and their antioxidant capacity. *Journal of Separation Science*, *32*(2), 288-294. <https://doi.org/10.1002/jssc.200800543>
- Al-Dhabi, N. A., Ponmurugan, K., & Jeganathan, P. M. (2017). Development and validation of ultrasound-assisted solid-liquid extraction of phenolic compounds from waste spent coffee grounds. *Ultrasonics Sonochemistry*, *34*, 206-213. <https://doi.org/10.1016/j.ultsonch.2016.05.005>
- AOAC INTERNATIONAL. (1990). *Official methods of analysis of the Association of Official Analytical Chemists* (15 ed.). AOAC INTERNATIONAL.
- Asamenew, G., Kim, H.-W., Lee, M.-K., Lee, S.-H., Lee, S., Cha, Y.-S., Lee, S. H., Yoo, S. M., & Kim, J.-B. (2019). Comprehensive characterization of hydroxycinnamoyl derivatives in green and roasted coffee beans: A new group of methyl hydroxycinnamoyl quinate. *Food Chemistry: X*, *2*, 100033. <https://doi.org/10.1016/j.fochx.2019.100033>
- Buyong, N. L., & Nillian, E. (2023). Physiochemical properties of Sarawak's adapted *Liberica* coffee silver skin utilizing varying solvents. *Food Science and Nutrition*, *11*(10), 6052-6059. <https://doi.org/10.1002/fsn3.3541>

- Campos, G. A. F., Sagu, S. T., Celis, P. S., & Rawel, H. M. (2020). Comparison of batch and continuous wet-processing of coffee: Changes in the main compounds in beans, by-products and wastewater. *Foods*, 9(8), 1135. <https://doi.org/10.3390/foods9081135>
- Chang, S. K. C. (2003). Protein analysis. In S. S. Nielsen (Ed.), *Food analysis* (pp. 133-146). Springer. [https://doi.org/10.1007/978-1-4419-1478-1\\_9](https://doi.org/10.1007/978-1-4419-1478-1_9)
- Chu, R., Wan, L.-S., Peng, X.-R., Yu, M.-Y., Zhang, Z.-R., Zhou, L., Li, Z.-R., & Qiu, M.-H. (2016). Characterization of new *ent*-kaurane diterpenoids of Yunnan Arabica coffee beans. *Natural Products and Bioprospecting*, 6, 217-223. <https://doi.org/10.1007/s13659-016-0099-1>
- Desmawati, D., & Sulastri, D. (2019). Phytoestrogens and their health effect. *Open Access Macedonian Journal of Medical Sciences*, 7(3), 495-499. <https://doi.org/10.3889/oamjms.2019.086>
- Geremu, M., Tola, Y. B., & Sualeh, A. (2016). Extraction and determination of total polyphenols and antioxidant capacity of red coffee (*Coffea arabica* L.) pulp of wet processing plants. *Chemical and Biological Technologies in Agriculture*, 3, 25. <https://doi.org/10.1186/s40538-016-0077-1>
- Harahap, M. R. (2017). Identifikasi daging buah kopi Robusta (*Coffea robusta*) berasal dari provinsi Aceh [Identification of the pulp of Robusta coffee (*Coffea robusta*) originating from Aceh province]. *Elkawanie: Journal of Islamic Science and Technology*, 3(2), 201-210. <https://doi.org/10.22373/ekw.v3i2.2770>
- Heeger, A., Kosińska-Cagnazzo, A., Cantergiani, E., & Andlauer, W. (2017). Bioactives of coffee cherry pulp and its utilisation for production of Cascara beverage. *Food Chemistry*, 221, 969-975. <https://doi.org/10.1016/j.foodchem.2016.11.067>
- Ismail, I., Anuar, M. S., & Shamsudin, R. (2013). Effect on the physico-chemical properties of Liberica green coffee beans under ambient storage. *International Food Research Journal*, 20(1), 255-264.
- Ismail, I., Anuar, M. S., & Shamsudin, R. (2014). Physical properties of Liberica coffee (*Coffea liberica*) berries and beans. *Pertanika Journal of Science and Technology*, 22(1), 65-79.
- James, C. S. (1995). *Analytical chemistry of foods*. Blackie Academic & Professional.
- Kirk, R. S., & Sawyer, R. (1991). *Pearson's composition and analysis of foods* (9<sup>th</sup> ed.). Longman.
- Kovalcik, A., Obruca, S., & Marova, I. (2018). Valorization of spent coffee grounds: A review. *Food and Bioprocess Technology*, 110, 104-119. <https://doi.org/10.1016/j.fbp.2018.05.002>
- Lončar, M., Jakovljević, M., Šubarić, D., Pavlić, M., Služek, B. V., Cindrić, I., & Molnar, M. (2020). Coumarins in food and methods of their determination. *Foods*, 9(5), 645. <https://doi.org/10.3390/foods9050645>
- Mohd Jailani, F. N. A., Zaidan, U. H., Abdul Rahim, M. B. H., Abd Gani, S. S., & Halmi, M. I. E. (2020). Evaluation of constituents and physicochemical properties of Malaysian underutilized *Ziziphus mauritiana* (Bidara) for nutraceutical potential. *International Journal of Fruit Science*, 20(3), 394-402. <https://doi.org/10.1080/15538362.2019.1641458>
- Murthy, P. S., Manjunatha, M. R., Sulochannama, G., & Naidu, M. M. (2012). Extraction, characterization, and bioactivity of coffee anthocyanins. *European Journal of Biological Sciences*, 4(1), 13-19.
- Mussatto, S. I., Ballesteros, L. F., Martins, S., & Teixeira, J. A. (2011). Extraction of antioxidant phenolic compounds from spent coffee grounds. *Separation and Purification Technology*, 83, 173-179. <https://doi.org/10.1016/j.seppur.2011.09.036>

- Nabais, J. M. V., Nunes, P., Carrott, P. J., Carrott, M. L. R., García, A. M., & Díaz-Díez, M. A. (2008). Production of activated carbons from coffee endocarp by CO<sub>2</sub> and steam activation. *Fuel Processing Technology*, 89(3), 262-268. <https://doi.org/10.1016/j.fuproc.2007.11.030>
- Nillian, E., Ismail, N. S., Boli, M. E., Buyong, N. L., Sng, N. N., Adeni, D. S. A., & Hussini, A. A. S. A. (2020). The feasibility study of physicochemical properties of Sarawak *Liberica* sp. coffee pulp. *Pertanika Journal of Tropical Agricultural Science*, 43(4), 477-490. <https://doi.org/10.47836/pjtas.43.4.05>
- Núñez, A. C., Rentería, I. D. C. D., Villagómez, M. Á. L., Elorza, P., Martínez, C. E. M. S., Pulido, S. A. A., & Rojas-Ronquillo, R. (2015). The use of coffee pulp as a potential alternative supplement in ruminant diets. *Journal of Agriculture, Science and Technology A*, 5(3), 214-217. <https://doi.org/10.17265/2161-6256/2015.03.010>
- Olechno, E., Puścion-Jakubik, A., Socha, K., & Zujko, M. E. (2021). Coffee brews: Are they a source of macroelements in human nutrition? *Foods*, 10(6), 1328. <https://doi.org/10.3390/foods10061328>
- Oliveira, P. D., Borém, F. M., Isquierdo, E. P., da Silva Giomo, G., de Lima, R. R., & Cardoso, R. A. (2013). Physiological aspects of coffee beans, processed and dried through different methods, associated with sensory quality. *Coffee Science*, 8(2), 211-220.
- Pace, V., Carbone, K., Spirito, F., Iacurto, M., Terzano, M. G., Verna, M., Vincenti, F., & Settineri, D. (2006). The effects of subterranean clover phytoestrogens on sheep growth, reproduction and carcass characteristics. *Meat Science*, 74(4), 616-622. <https://doi.org/10.1016/j.meatsci.2006.05.006>
- Redfern, J., Kinninmonth, M., Burdass, D., & Verran, J. (2014). Using Soxhlet ethanol extraction to produce and test plant material (essential oils) for their antimicrobial properties. *Journal of Microbiology and Biology Education*, 15(1), 45-46. <https://doi.org/10.1128%2Fjmb.e.v15i1.656>
- Rohaya, S., Anwar, S. H., Amhar, A. B., Sutriana, A., & Muzaifa, M. (2023). Antioxidant activity and physicochemical composition of coffee pulp obtained from three coffee varieties in Aceh, Indonesia. In *IOP Conference Series: Earth and Environmental Science* (Vol. 1182, No. 1, p. 012063). IOP Publishing. <https://doi.org/10.1088/1755-1315/1182/1/012063>
- Saleem, H., Sarfraz, M., Khan, K. M., Anwar, M. A., Zengin, G., Ahmad, I., Khan, S.-U., Mahomoodally, M. F., & Ahemad, N. (2020). UHPLC-MS phytochemical profiling, biological propensities, and *in-silico* studies of *Alhagi maurorum* roots: A medicinal herb with multifunctional properties. *Drug Development and Industrial Pharmacy*, 46(5), 861-868. <https://doi.org/10.1080/03639045.2020.1762199>
- Schenker, S., Heinemann, C., Huber, M., Pompizzi, R., Perren, R., & Escher, R. (2002). Impact of roasting conditions on the formation of aroma compounds in coffee beans. *Journal of Food Science*, 67(1), 60-66. <https://doi.org/10.1111/j.1365-2621.2002.tb11359.x>
- Sena, L. P., Vanderjagt, D. J., Rivera, C., Tsin, A. T. C., Muhamadu, I., Mahamadou, O., Millson, M., Pastuszyn, A., & Glew, R. H. (1998). Analysis of nutritional components of eight famine foods of the Republic of Niger. *Plant Foods for Human Nutrition*, 52, 17-30. <https://doi.org/10.1023/a:1008010009170>
- Sitohang, A., & Pandiangan, M. (2022). Formulasi limbah kulit Ari kopi pembuatan pellet ikan [Formulation of Ari coffee skin waste for makin fish pellets]. *Jurnal Riset Teknologi Pangan Dan Hasil Pertanian (RETIPA)*, 3(1), 1-12. <https://doi.org/10.54367/retipa.v3i1.2240>
- Srikrishna, D., Godugu, C., & Dubey, P. K. (2018). A review on pharmacological properties



- of coumarins. *Mini Reviews in Medicinal Chemistry*, 18(2), 113-141. <https://doi.org/10.2174/1389557516666160801094919>
- Wibowo, N. A., Djufry, F., Syafaruddin., Iffah, T., & Dani. (2022). The quality of Arabica coffee beans evaluation at various processing in Luwu Regency South Sulawesi, Indonesia. In *IOP Conference Series: Earth and Environmental Science* (Vol. 1038, No. 1, p. 012068). IOP Publishing. <https://doi.org/10.1088/1755-1315/1038/1/012068>
- Yulianti, Y., Adawiyah, D. R., Herawati, D., Indrasti, D., & Andarwulan, N. (2023). Detection of markers in green beans and roasted beans of Kalosi-Enrekang Arabica coffee with different postharvest processing using LC-MS/MS. *International Journal of Food Science*, 2023, 6696808. <https://doi.org/10.1155/2023/6696808>
- Zaidan, U. H., Ab Karim, N., Ahmad, S., Abd Ghani, S. S., & Halmi, M. I. E. (2019). Nutraceutical evaluation and antioxidant potential of red kidney bean (*Phaseolus vulgaris*) and chickpea (*Cicer arietenum*) seed coats. *Asian Journal of Agriculture and Biology*, 7(1), 19-26.
- Zainol, M. K., Mohd Subri, I., Zamri, A. I., Mohd Zin, Z., Faisal, A., & Mamat, H. (2020). Antioxidative properties and proximate analysis of spent coffee ground (SCG) extracted using ultrasonic-methanol assisted technique as a potential functional food ingredient. *Food Research*, 4(3), 636-644. [https://doi.org/10.26656/fr.2017.4\(3\).358](https://doi.org/10.26656/fr.2017.4(3).358)