Journal of Sustainability Science and Management
Volume 20 Number 12, December 2025: 2638-2655
eISSN: 2672-7226

PHYTOCHEMICAL ANALYSIS OF COFFEE BY-PRODUCTS: SARAWAK LIBERICA COFFEE SILVERSKIN AND COFFEE LEAVES

ELEXSON NILLIAN[1]*, BRYAN VOON LI QI[1], NICK LAURENCE BUYONG[1], WENNY SUNARHARUM[2] AND TUNJUNG MAHATMANTO[2]

[1]*Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia.*
[2]*Faculty of Agricultural Technology, Universitas Brawijaya, 65145 Malang, East Java, Indonesia.*

**Corresponding author: nelexson@unimas.my*
http://doi.org/10.46754/jssm.2025.12.005
Submitted: 3 October 2024 *Revised: 18 May 2025* *Accepted: 28 May 2025* *Published: 15 December 2025*

Abstract: Coffee Silverskin (CS) and the leaves are by-products generated during the harvesting of green coffee beans. CS and the leaves obtained from the major Liberica species cultivated in Sarawak, Malaysia, remain underexplored. This study investigated the bioactive compound composition of Sarawak Liberica coffee silverskin and leaf extracts using gas chromatography coupled with mass spectrometry (GC-MS). Phytochemical analysis was conducted on the extracts of both CS and leaves. The GC-MS analysis revealed the presence of 41 chemical compounds, with four major compounds identified: 5-hydroxymethylfurfural (5-HMF), D-allose, 1,6-anhydro-β-D-glucofuranose, and caffeine in the CS extract. The results indicated that the percentage of caffeine content in the CS extracts showed no significant difference ($p < 0.05$) between ethanol (1.55 ± 0.47%), methanol (1.77 ± 0.38%), and water (1.92 ± 0.65%). Additionally, the total phenolic content (TPC) of Liberica coffee leaves was measured at 35.76 ± 2.12 mg GAE/g extract, while the total flavonoid content (TFC) was 605.85 ± 8.20 mg QE/g extract. The antioxidant activity of Liberica coffee leaves was recorded at 54.37 ± 0.10%. The substantial presence of bioactive compounds suggests potential applications in food and pharmaceutical formulations.

Keywords: Gas Chromatography-Mass Spectroscopy (GC-MS), UV-VIS spectrophotometer, total phenolic content, total flavonoids content, antioxidant activities.

Introduction

In Malaysia, 73% of coffee species grown are Liberica, while 27% are Robusta, as the optimum temperature for both species to grow is within the range of 18 to 28°C (Ismail *et al.*, 2014). Liberica coffee (*Coffea liberica*) is a type of coffee that has less commercial value and usually grows in lowland areas with a warm tropical climate, such as Liberia, Surinam, and Malaysia (Lim, 2013). Malaysia, as one of the countries that cultivates Liberica coffee and plays an important role in supplying a variety of coffee in the market, has gained many benefits. The continuous growth of the coffee industry in Malaysia has led to an increasing interest in sustainable agricultural practices, focusing on the potential uses of coffee by-products. Parts of coffee plants, such as flowers, leaves, coffee cherries, branches, and wood, can be classified as by-products during coffee production. The roasting of green beans also produces coffee silverskin (CS), which is typically discarded as waste.

For every 10 million tonnes of coffee produced, approximately 400 thousand tons of CS are generated. Although CS constitutes only 4% (w/w) of the coffee cherry, the amount produced—tens of thousands of tonnes per year—makes CS a worthy by-product to investigate (Martuscelli *et al.*, 2021; Nolasco *et al.*, 2022). Nevertheless, more than 50% of the coffee cherry component is discarded as waste, including the silverskin, as the market usually only focuses on the beans themselves (Hejna *et al.*, 2021). However, CS is a rich source of antioxidant constituents due to the presence of phenolic, flavonoid, and alkaloid compounds that work synergistically (Alnsour *et al.*, 2022), as well as bioactive compounds such as fibre, min-

erals, and caffeine (Bessada *et al.*, 2018a). Most studies have been conducted on CS from both Arabica and Robusta coffee. CS from Arabica and Robusta has high phenolic and antioxidant content but low toxic mineral levels (Martuscelli *et al.*, 2021). CS from Liberica recorded the highest total phenolic content (TPC) when extracted using methanol (15.24 ± 0.65 mg GAE/g) and the highest total flavonoid content (TFC) when extracted using ethanol (Buyong & Nillian, 2023). Furthermore, the amount of caffeine is a vital factor in determining coffee quality, and caffeine can provide health benefits for humans (Wanyika *et al.*, 2010). UV-Vis spectrophotometry, which is fast and efficient, is a suitable method for determining caffeine content (Tesfatye *et al.*, 2024).

Other than the coffee beans used to make coffee drinks, the leaves of the coffee tree can also be utilised as a beverage. People in West Sumatra, particularly in the Tanah Datar area, have been using leftover coffee leaves from Robusta coffee (*Coffea canephora*) to make a customary drink known as "Kawa Daun", which translates to "brewing water from coffee leaves" (Defri *et al.*, 2021). The increasing interest in coffee leaves has sparked a surge in research, as they are said to have more beneficial qualities than coffee bean beverages. According to Chen (2018), coffee leaves contain a greater phenolic content compared to coffee beans, which is linked to antioxidant qualities and possible health advantages. Thus, the application of Microwave-Assisted Extraction (MAE) will be effective in removing bioactive substances from a variety of natural sources. MAE is a recommended option for extracting bioactive substances, as it has several advantages over traditional extraction techniques. MAE often leads to higher yields and purities of bioactive compounds compared to traditional methods.

Therefore, this study aims to investigate the bioactive compounds present in the extracts of CS and leaves using gas chromatography coupled with mass spectrometry, and further investigate the caffeine content of the CS extract, alongside phytochemical analysis of the leaves. The analysis of the potential bioactive compound composition of CS and leaves as beneficial sources of substrate may help create a new stream of revenue while assisting in the proper management of waste into a value-added product.

Materials and Methods

Sample Preparation

The CS was obtained from Reka Jaya Plantation Sdn Bhd after undergoing a roasting process. The CS was first ground using a pestle and mortar to increase the surface area of the sample and maximise the compound extracted when in contact with the solvents. The CS was then extracted using the Soxhlet apparatus with organic solvents, ethanol and methanol, based on a study by Nillian *et al.* (2023). Ten grams of the ground CS was inserted into a thimble (33 mm × 118 mm, Whatman, GE Healthcare Life Sciences) in 200 mL of solvent at 78-80°C for 8 hours in a Soxhlet extractor. The extract was evaporated using a rotary evaporator (IKA RV8), then weighed and transferred to an amber vial for storage at a temperature of 4°C before proceeding to analysis. The dried sample was resuspended in a GC-MS vial with dichloromethane (DCM) to create a concentration of 15 mg/mL for analysis.

Determination of Caffeine Content via GC-MS

Gas Chromatography- Mass Spectrometry (GC-MS)

Based on the procedure performed by Samling *et al.* (2021), the Shimadzu GC-MS QP2010 Plus (Shimadzu, Japan) was used for the GC-MS analysis. A 30 m × 0.25 mm I.D., 0.25 mm film thickness, BPX-5 fused-silica capillary column containing 5% phenyl polysilphenylene-siloxane (Trajan Scientific and Medical, Australia) was employed. The scan mass range was set to 28–400 m/z, with an electron impact ionisation energy of 70 eV and an interface temperature fixed at 250°C. The oven was preheated to 40°C for 1 minute, then raised to 220°C at a rate of 5°C per minute, resulting in a total run time of

37 minutes. Helium gas was employed as the carrier, with a flow rate of 1 mL/min, and both the injector and detector were set at 250°C. An aliquot of 1 µL extract sample was injected, while a splitting ratio of 20:1 was applied. The chemical constituents of the CS extract were identified by comparing their mass spectra with those in the National Institute of Standards and Technology 2017 (NIST-17) mass spectral library, which is incorporated in the data system to ascertain their names, molecular weights, and structures.

Determination of Caffeine Content

The standard curve was created by calibrating standards across a known concentration range of 25 - 100 µg/mL of caffeine (1,3,7-trimethylxanthine) solution. By plotting the peak area against the caffeine concentration on the chromatogram, the slope and y-intercept of the calibration curve were calculated (Itodo *et al.*, 2010). An acceptable linearity was achieved, with a correlation coefficient (R^2) of 0.97. The equation for the calibration curve was y = 31903x – 253622, where the y value represents the intercept and the x value represents the slope of the curve. The caffeine content was reported in mg/g on a dry basis, along with the percentage of caffeine content per extract concentration. The percentage of caffeine content was calculated as follows:

$$Caffeine\% = \frac{C_e}{M} \times 100 \quad \text{Equation (1)}$$

where C_e is the CS extract caffeine mass (mg), M is the dry mass of the CS extract (mg).

Determination of Caffeine Content via UV-VIS Spectrophotometer

Sample Preparation

The sample was prepared according to the method described by Vuletić *et al.* (2021), with minor modifications to certain steps. Specifically, 2 g of CS was dissolved in 20 mL of distilled water and allowed to boil (< 100°C) for approximately 10 minutes while stirring at 300 rpm. Afterward, the solution was filtered using a strainer to remove the ground CS and separate the filtrate. The filtrates were then subjected to centrifugation at 350 rpm for 15 minutes (Benchmark Scientific, LC-8). The clear supernatant from the filtrates was carefully removed and transferred into a round-bottom flask. Next, the CS aqueous extract was dried using a rotary evaporator (IKA RV8) to obtain the dry mass of the extract. The dried sample was then re-suspended in distilled water for subsequent analysis.

Caffeine Extraction

The CS extract was then transferred into a separatory funnel for caffeine separation and extraction (Vuletić *et al.*, 2021). Two grams of sodium bicarbonate were added to the extract in the funnel, and a 1:1 (v/v) ratio of extract to chloroform ($CHCl_3$) was used as the separating agent for the caffeine compound. The mixed solution was shaken gently and inverted two to three times to maximise the contact between the chloroform and the extract, thereby obtaining the maximum amount of caffeine from the extract. After allowing the mixture to rest for a few minutes, the bottom layer of the separation was removed carefully to avoid disturbing the top layer. An aliquot of 100 µL was taken from the separated bottom layer and mixed with 10 mL of chloroform before reading with a UV-VIS Spectrophotometer at a wavelength of 274 nm (Shimadzu UV-1900i, Shimadzu Corp, Japan).

Calibration Curve

A standard curve was plotted using caffeine standard (1,3,7-Trimethylxanthine). In brief, 10 mg of caffeine was diluted in 100 mL of chloroform to create a stock concentration of 100 ppm. The stock solution was then diluted in a manner of 2-fold dilution to produce concentrations ranging from 3.125 to 50 ppm. The absorbance across this range was measured using a UV-VIS Spectrophotometer at 274 nm (Shimadzu UV-1900i, Shimadzu Corp, Japan). A calibration curve was plotted with the concentrations of caffeine (Merck, Germany) standard against the absorbance

values, resulting in a linearity equation of y = 0.0002x + 0.0081, with a correlation coefficient (R^2) of 0.93. The caffeine content is reported as mg/g of caffeine on a dry basis of CS, as well as the percentage of caffeine content based on the extract concentration. The percentage caffeine content was calculated using Equation 1.

Sample Preparation

Liberica leaves were obtained from a coffee plantation in Tarat, Serian. The coffee leaf samples were washed with distilled water to remove dirt and other contaminants. The samples were left to air-dry in an oven for 8 hours at 70°C (Defri *et al.*, 2021). The dried coffee leaf samples were blended into a fine powder and stored in an airtight container. The samples were kept in a dark, moisture-free cabinet.

Microwave-Assisted Extraction (MAE) Method of Coffee Leaves Sample

The extraction of coffee leaf samples was performed using methanol as the solvent. The MAE method was adapted based on Wong and Nillian (2023). The sample-to-solvent ratio used for the extraction was 1:100. In a conical flask, 1.0 g of ground coffee leaf samples was soaked in 100 mL of 50% (v/v) methanol. The mixture was heated at the highest temperature in a microwave for 180 seconds (three minutes). After cooling to room temperature, the mixture was centrifuged twice at 5000 rpm and 4°C for 10 minutes using a SciLab® centrifuge. Subsequently, a Liquid-Liquid Extraction (LLE) of the Liberica coffee leaf sample with hexane was conducted to obtain the volatile compounds. The hexane containing the volatile compounds was then analysed using the Shimadzu GC-MS QP2010 Plus (Shimadzu, Japan) for the GC-MS analysis. Total phenolic content, total flavonoid content, and antioxidant activity were analysed using the Shimadzu UV-1900i ultraviolet-visible spectrophotometer.

Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) was determined using the Folin-Ciocalteu method, with a few modifications. A final concentration of leaf extract (1 mg/mL) was obtained by resuspending the dried methanolic extract in 50% (v/v) methanol in a fume hood. Then, 1 mL of this solution was mixed with 0.5 mL of stock Folin-Ciocalteu's reagent (10% w/v) and 0.5 mL of a 7.5% (w/v) sodium bicarbonate (NaHCO3) solution. After vortexing, the mixture was agitated constantly and allowed to stand at room temperature for half an hour (Wong & Nillian, 2023). A gallic acid solution (0.02–0.1 mg/mL in methanol) was used to prepare a standard curve. The absorbance was measured with a spectrophotometer at 765 nm. The total phenolic content was calculated and expressed as mg of Gallic Acid Equivalents (GAE) per gram of dry weight of the sample, using the calibration curve (Wong & Nillian, 2023).

Determination of Total Flavonoid Content (TFC)

The aluminium chloride colourimetric technique was utilised to ascertain the total flavonoid content (TFC) in coffee leaves. A 0.4 mL aliquot of extract was mixed with 0.3 mL of 5% (w/v) sodium nitrite (NaNO2). The mixture was allowed to stand for 5 minutes, after which 0.3 mL of 10% (w/v) aluminium chloride (AlCl3) was added. Following a shaking process, the mixture was allowed to stand before adding 2.0 mL of 1 M sodium hydroxide (NaOH) and 4.0 mL of distilled water. An ultraviolet-visible spectrophotometer was used to measure the absorbance at 510 nm after the test tube had been vortexed (Wong & Nillian, 2023). The quercetin stock solution was modified from Ismail *et al.* (2020) to serve as the standard flavonoid for this investigation. Quercetin solution (0.02–0.1 mg/mL in methanol) was used to prepare a standard curve. The total flavonoid content was calculated and expressed as mg of quercetin (QE) per gram of dry weight of the sample using the calibration curve (Ismail *et al.*, 2020).

Determination of Antioxidant Activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) method was used to measure the antioxidant activity of both coffee samples. The antioxidant activity of the Liberica coffee leaf sample was determined using the percentage of DPPH inhibition, which slightly modified the approach employed by Zielińska *et al.* (2017) and Abduh *et al.* (2023). A combination of three mL of Liberica coffee leaf extract and three mL of a 0.1 mM DPPH solution was prepared. After homogenisation, the mixtures were left to rest for thirty minutes at room temperature. After this period, the absorbance of the mixtures was measured using a spectrophotometer at 517 nm. The antioxidant activity of the coffee samples was then calculated using the formula below.

$$Percentage\ of\ inhibition = \left[1 - \left(\frac{Abs_{Sample}}{Abs_{Control}}\right)\right] \times 100$$

Data Analysis

The data collected were analysed using Excel for Microsoft Office 365, version 2023. The total phenolic content, total flavonoid content, and percentage of inhibition to demonstrate antioxidant activity were collected in triplicate to obtain the mean and distribution of data deviation. A one-way ANOVA test was performed on the data to assess the significance of the caffeine content using different extraction methods.

Results and Discussions

Bioactive Compounds Composition of CS

The results of the GC-MS analysis identified 30 chemical compounds based on comparisons with the NIST-17 GC-MS library. The active compounds, along with their area percentages (%), are presented in Table 1 for both the ethanol and methanol extracts. Additionally, four major compounds from the screening are listed in Table 2, along with their Molecular Formulas (MF), Molecular Weights (MW), and peak areas. In this study, these four identified compounds are the most abundant in the CS extracts.

The current study shows that the CS extract predominantly contains four different compounds: 5-hydroxymethylfurfural (5-HMF), D-allose, 1,6-anhydro-β-D-glucofuranose, and caffeine. The total ion chromatograms in Figures 1 and Figure 2 indicate that the methanol extract profiles more caffeine compounds than the ethanol extract. This difference is attributed to the higher polarity of methanol compared to ethanol, which attracts more polar compounds such as caffeine (an alkaloid).

The compound 5-HMF is one of the most highly utilised compounds in the food industry, particularly as a quality marker (Martins *et al.*, 2022). It is most commonly found in food products due to the thermal treatments applied to them, such as the roasting of coffee beans. These thermal treatments are performed not only to increase food safety and enhance the shelf life of food products but also to alter their properties. This includes improving or removing undesirable smells, inducing changes in colour, enhancing taste, and improving flavour, as seen in coffee beans (Iriondo-DeHond *et al.*, 2019).

Overall, the thermal treatment applied to food products like coffee beans aims to improve consumer acceptability, whether in terms of physical appearance or overall taste. 5-HMF is produced during the Maillard reaction or caramelisation of food products, both of which involve the application of heat. For vegetables and fruits, the 5-HMF content is higher compared to other food products due to their high natural sugar and amino acid content (Mathew *et al.*, 2018). When stored under long durations and low moisture conditions, this level increases (Martins *et al.*, 2022).

The utilisation of 5-HMF varies across industries, as the compound acts as an intermediate for producing more beneficial compounds, such as 2,5-dimethylfuran (2,5-DMF), through simple chemical reactions like the oxidation and reduction of the side groups and furan ring in the 5-HMF structure. Beneficial compounds like 2,5-DMF have garnered interest

Table 1: Bioactive compounds composition of Sarawak Liberica coffee silverskin

Classification	Compound Name	Area (%)	
		Ethanol Extract	Methanol Extract
Aldehydes	Furfural	9.51	6.17
	5-methylfurfural	0.91	0.48
	1-ethyl-1H-Pyrazole-4-carboxaldehyde	2.20	1.81
	5-Hydroxymethylfurfural	14.87	10.96
Lactone	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP)	1.39	1.20
Ketones	Levoglucosenone	1.36	0.72
	6-Methyl-2-Heptanol, acetate	0.43	n.d
	2-Pentadecanone, 6,10,14-trimethyl-	0.36	n.d
Carboxylic Acids and Esters	Succinic acid, but-3-yn-2-yl tetrahydrofurfuryl ester	1.03	n.d
	n-Hexadecanoic acid	1.92	2.21
	Octadecanoic acid	0.49	0.64
	Undec-10-ynoic acid, tetradecyl ester	0.45	n.d
	Eicosanoic acid	0.48	0.53
	Decanedioic acid,bis(2-ethylhexyl) ester	1.07	0.56
	Bis(2-ethylhexyl) phthalate (DEHP)	0.51	n.d
	5-Hydroxymethyl-2-furancarboxylic acid	n.d	0.43
	Succinic acid, 3-methylbut-2-yl 2-methylbut	n.d	0.78
	Dibutyl phthalate	n.d	0.63
	10(E),12(Z)-Conjugated linoleic acid	n.d	0.51
	9-Octadecenoic acid, (E)-	n.d	0.92
Carbohydrates	1,4:3,6-Dianhydro-α-d-glucopyranose	2.48	2.31
	D-Gluco-heptulosan	0.90	n.d
	D-allose	28.02	22.17
	1,5-Anhydro-d-altritol	0.47	n.d
	1,6-Anhydro-β-D-glucofuranose	13.54	8.43
	Rhamnitol, 1-O-octyl-	0.68	n.d
	3-O-Methyl-d-glucose	1.32	n.d
	Sucrose	n.d	0.76
	1,6-Anhydro-β-D-Glucopyranose	n.d	0.54
	α-D-Glucopyranose, 4-O-β-D-galactopyranosyl	n.d	0.53
	Methyl-α-D-Galactopyranoside	n.d	1.12
Sterols	β-Sitosterol	0.70	0.38
	β-Sitosterol acetate	0.54	n.d
	(3β)-Cholesta-4,6-dien-3-ol	0.89	0.56
Alkene	1-Decosene	0.43	0.53
	D-Limonene	n.d	0.79

Alkane	3-Ethyl-3-methylheptane	n.d	0.51
	1,1,2,2-tetrachloroethane	n.d	2.25
Amide	Hexadecanamide	0.97	n.d
Alkaloid	Caffeine	12.06	29.77
Phenol	Phenol, 3,5-bis(1,1-dimethyl ethyl)-	n.d	0.81

Table 2: Major compounds in the extracts of Sarawak Liberica coffee silverskin

No.	Name	MF	MW	Peak area (%)	
				Ethanol	Methanol
1	5-Hydroxymethylfurfural	$C_6H_6O_3$	126	14.87	10.96
2	D-allose	$C_6H_{12}O_6$	180	28.02	22.17
3	1,6-Anhydro-β-D-glucofuranose	$C_6H_{10}O_5$	162	13.54	8.43
4	Caffeine	$C_8H_{10}N_4O_2$	194	12.06	29.77

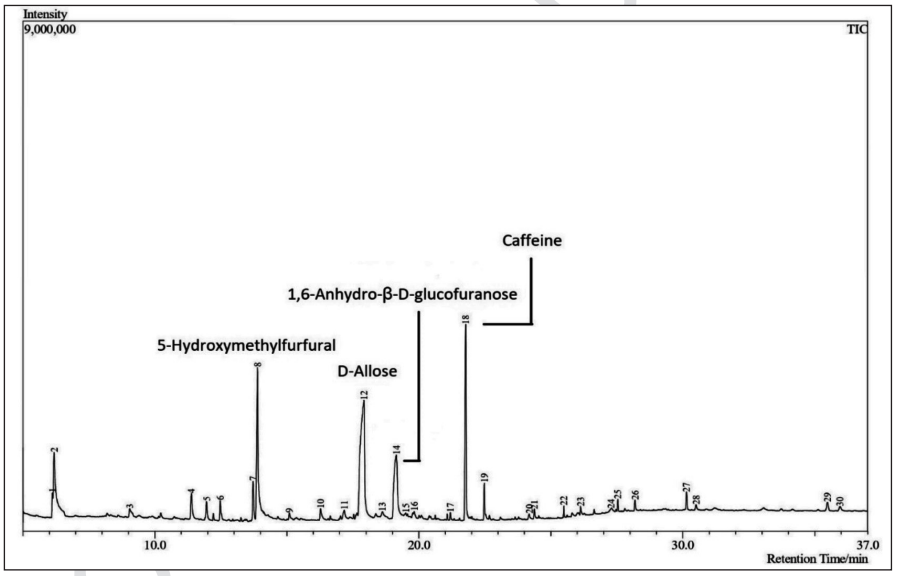

Figure 1: Chromatogram of ethanol extract of Sarawak Liberica coffee silverskin

due to their potential as alternatives to gasoline and diesel as biofuels. This process is achievable via the catalytic hydrogenolysis of 5-HMF. 2,5-DMF is known for its high octane number, high boiling point, low miscibility with water, high energy density, and low volatility, making it a promising biofuel candidate (Wang *et al.*, 2018).

In the current study, the CS displayed a relatively large peak area of 5-HMF at 14.87% and 10.96% for ethanol and methanol extracts, respectively, at a retention time of 13.8 minutes. This may correlate with the intensity of heat used during the roasting of Liberica coffee beans and the high acidity of the environment, resulting in significant levels of 5-HMF (Wang *et al.*, 2018). However, the level of 5-HMF is suspected to follow a bell-shaped curve rather than a linear increasing pattern, as a study by

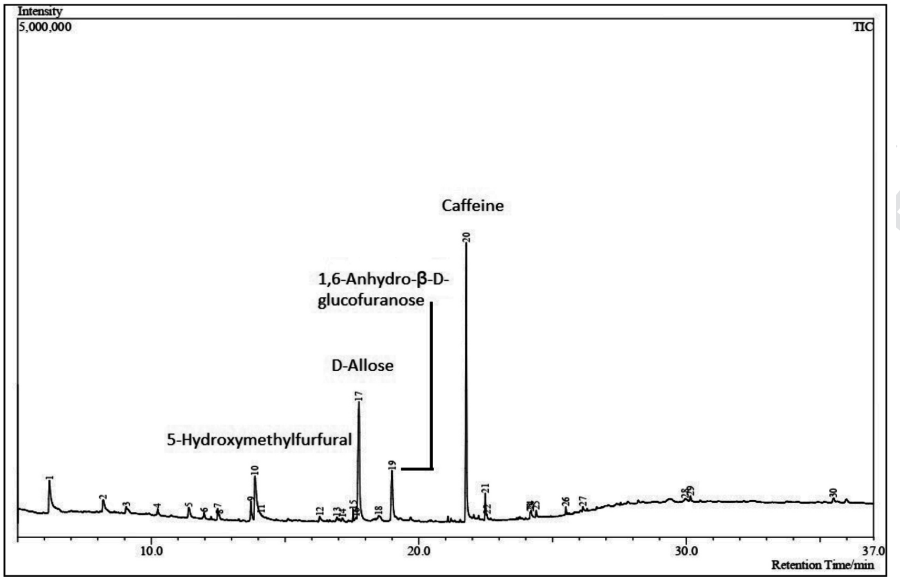

Figure 2: Chromatogram of methanol extract of Sarawak Liberica coffee silverskin

Diviš *et al.* (2019) showed that the highest levels of the compound were found at medium roasting temperatures and times for Arabica and Robusta coffee beans, rather than at the highest temperatures and times tested.

On the other hand, the compound D-allose is considered a rare functioning reducing sugar in nature, as it is very scarce and has high production costs if produced synthetically rather than being derived from natural sources (Choi *et al.*, 2021). The studies on rare sugar monosaccharides have been limited due to scarce methods for major production in the industry. The derivative of D-allose, known as D-allulose, is classified as a third-generation sweetener, providing only 80% of the sweetness relative to first-generation table sugar. This compound has been studied for its anti-cancer, antioxidant, anti-hypertensive, and anti-inflammatory properties (Choi *et al.*, 2021).

However, due to its low calorie content and non-toxic properties, the compound D-allose has multiple potential physiological applications, including as a food additive, in clinical therapy, and within the medical field. Additionally, as a reducing sugar, the compound is involved in the Maillard reaction during the thermal treatment of food products, which enhances the flavour, taste, and visual appearance of food. The long-term effect of incorporating this compound into food is to assist with weight loss, thereby benefiting patients with diabetes and hypertension complications. Currently, D-allose is used in surgery and organ transplantation as an immunosuppressive agent to reduce tissue damage during operations, thereby increasing the likelihood of success (US patent no. 5620960A, 1997) (Chen *et al.*, 2018).

In plants, D-allose is recognised as a critical compound involved in the plant immunity defence mechanism, acting as a regulator of the reactivity and accumulation of reactive oxygen species (ROS) (Zhang *et al.*, 2020). While D-allose has been studied for its growth inhibitory effects in plants, the research also supports its potential as a candidate for disease-resistant agents in agriculture (Kano *et al.*, 2010).

During the roasting of coffee, thermal treatment of the coffee beans leads to the degradation of carbohydrates, producing monosaccharides such as D-allose. In a study by Kim *et al.* (2021) on Arabica coffee beans, a reduction of glucose content by approximately 80% was detected, alongside an increase in the

formation of furans. This process also resulted in the accumulation of D-allose through the isomerisation of D-glucose to D-fructose, then to D-allulose, and finally to D-allose (Chen *et al.*, 2018). Although there are currently no studies on the monosaccharides of coffee silverskin (CS), the relationship between coffee beans and CS is considered proportional, as the phytochemicals in both are regarded as similar (Gottstein *et al.*, 2021).

CS is known to contain high levels of insoluble polysaccharides such as hemicellulose and cellulose. In CS, the cellulose content (60%) is more abundant than hemicellulose, which aids in the production of enzymes such as amylase and fructooligosaccharides (FOS) by various bacteria and moulds (Martuscelli *et al.*, 2021; Nolasco *et al.*, 2022). As CS undergoes thermal treatment during the roasting of the beans, the high temperatures, combined with low or no oxygen, will naturally induce the pyrolysis of cellulose in CS. This process produces Levoglucosan (LG) (1,6-Anhydro-β-D-glucopyranose), which is in high demand in the chemical industries for producing surfactants, propellants, plastics, resins, and in the pharmaceutical industry (Junior *et al.*, 2020).

The isomer of this beneficial compound, 1,6-Anhydro-β-D-glucofuranose, is identified as one of the major compounds detected in this study. LG is a six-membered ring, while 1,6-Anhydro-β-D-glucofuranose is a five-membered ring cyclic structure. In terms of molecular structure, both share a similar C6H10O5 arrangement but have different aldehyde attachments on the hydroxyl group, resulting in two distinct cyclic forms. D-glucofuranose is a rare monosaccharide compared to D-glucopyranose due to an equilibrium factor that favours the production of the more stable six-membered ring cyclic compound. During the hemiacetal formation of D-glucose, the majority of isomers produced are D-glucopyranose (99%), while D-glucofuranose constitutes only a minority (1%) (Ashenhurst, 2023).

Determination of Caffeine Content

In this study, the major compound screened from the CS *via* GC-MS is caffeine, along with three other compounds (Table 2). The concentration of caffeine in the extracted products was calculated using the equation of the straight line from the standard curve graphs constructed in Figures 3 (a) and (b).

The caffeine content in the CS of the ethanol, methanol, and water extracts was determined to be 23.3 ± 7.12 mg/g, 26.86 ± 5.77 mg/g, and 19.23 ± 6.51 mg/g, respectively, based on the sample dry weight. The percentages of caffeine content are 1.55 ± 0.47%, 1.77 ± 0.38%, and 1.92 ± 0.65%, respectively (Table 3), when calculated as shown in Equation (1). The results support the null hypothesis, indicating that there are no significant differences ($p > 0.05$) among the mean caffeine content of the CS extracts. Coffee beverages are known to contain high amounts of caffeine, making them one of the main sources for people worldwide to obtain their daily dose of the stimulant (Faudone *et al.*, 2021).

This current study showed that the caffeine content of Sarawak Liberica coffee silverskin is higher compared to the findings by Martuscelli *et al.* (2021), which reported 17.45 ± 0.69 mg/g of caffeine. However, that study was performed on the coffee silverskin (CS) of Arabica mixed with Robusta varieties, thus demonstrating that Liberica CS has a higher caffeine content compared to the major varieties of the coffee family. Moreover, this study indicated that the caffeine content of Liberica CS is higher than that of the Robusta variety, which was found to be within the range of 6.76 ± 0.05 to 12.15 ± 0.11 mg/g (Bessada *et al.*, 2018b). The Arabica variety also exhibited a lower caffeine content compared to the current study, with only 10.0 ± 1.1 mg/g of caffeine detected in the study by Bresciani *et al.* (2014), and 12.43 ± 0.12 mg/g reported by Xuan *et al.* (2019).

Despite this, the current study's findings are lower than those of Zengin *et al.* (2020), which reported a caffeine content of 41.88 ±

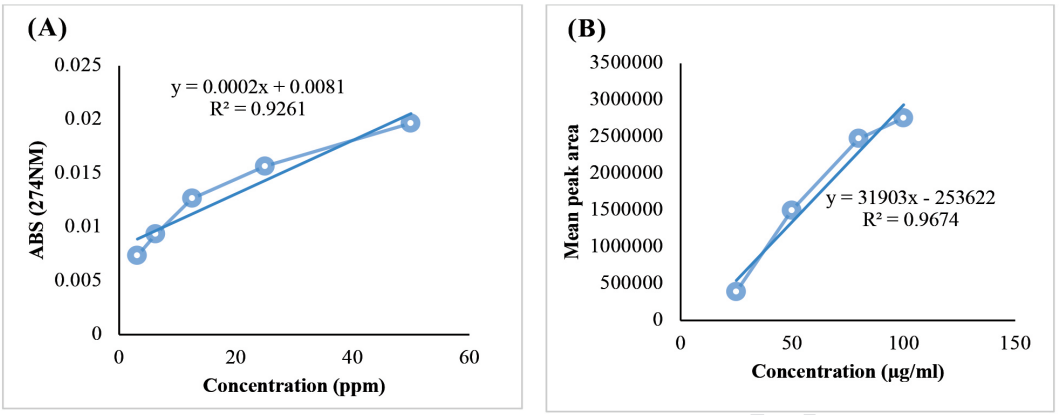

Figure 3: Caffeine standard curve via UV-VIS Spectrophotometer (a); Caffeine standard curve *via* GC-MS (b)

Table 3: Caffeine content of Sarawak Liberica coffee silverskin

Extract	Caffeine Content (mg/g)	Caffeine Content on Dry Basis (%)
Ethanol	23.30 ± 7.12	1.55 ± 0.47
Methanol	26.86 ± 5.77	1.77 ± 0.38
Water	19.23 ± 6.51	1.92 ± 0.65

Note: All data were performed in triplicate and expressed in mean ± standard deviation. Absent of significant detonation "*" indicates $p > 0.05$.

2.36 mg/g. Additionally, the study conducted in 2019 reported a higher caffeine content of 32.7 ± 1.0 mg/g (Wen *et al.*, 2019). Furthermore, the study reported by Nzekoue *et al.* (2020) also shows a high caffeine content in the Arabica variety, ranging from 1.00 to 3.59%, compared to the current study, which found caffeine levels as high as 1.55 ± 0.47 to 1.92 ± 0.65% in the Liberica CS extracts.

Bioactive Compounds Composition of Liberica Coffee Leaves

The sample was extracted using a ratio of 1:100 (sample:solvent), with methanol as the solvent. The sample then underwent liquid-liquid extraction (LLE) using hexane as a solvent to isolate the volatile compounds for GC-MS analysis. A total of 36 chemical compounds were identified based on comparisons with the NIST-17 GC-MS library. The active compounds and their area percentages are presented in Table 4. Figure 4 shows the chromatogram of the compounds present in the methanol extract of Liberica coffee leaves. Three major compounds from the screening are listed in Table 5, along with their molecular formula (MF), molecular weight (MW), and peak area.

These compounds, including 1-pentadecene, 1-nonadecene, and phytol, contribute to the unique chemical profile and potential bioactivities of Liberica coffee leaves. 1-Pentadecene, which constitutes 9.55% of the Liberica coffee leaves, is an unsaturated hydrocarbon known for its antibacterial activity based on the study by Kumar (2011). The second most abundant chemical in the Liberica coffee leaves is 1-nonadecene, which makes up about 9.23%. 1-Nonadecene is noted for its aroma and potential bioactive properties, including anticancer and antifungal activities (Arora & Meena, 2017). Phytol (5.18%) is an acyclic diterpene alcohol that has various pharmacological effects on the nervous system, such as anxiolytic and antidepressant effects

Table 4: Bioactive compounds composition of Sarawak Liberica coffee leaves

Classification	Compound Name	Area (%)
Alkane	Dodecane	0.91
	Tetradecane	4.13
	1-pentyl-2-propyl-cyclopentane	4.16
	1-methyl-1-(1-methylethyl)-2-nonyl-cyclopropane	3.27
	Heptadecane	4.91
	7-methyl-heptadecane	1.07
	Hexacosane	2.45
	9-Methylheneicosane	0.79
	2,6,11,15-tetramethyl-hexadecane	0.61
	Heneicosane	0.97
Alkene	1-Tetradecene	3.52
	1-Pentadecene	9.55
	3-methylene-tridecane	4.60
	(E)-5-octadecene	2.7
	1-Octadecene	0.56
	Neophytadiene	1.46
	1-Nonadecene	14.20
	Hexadecanoic acid, methyl ester	3.81
	2-methyl-1-pentadecene	1.44
	(E)-5-Eicosene	3.92
	1-Hexacosene	1.76
	1-Heptadecene	1.12
Alcohol	2-butyl-1-octanol	4.5
	2-methyl-1-decanol	1.84
	Phytol	5.18
Carboxylic Acids and Esters	Dichloroacetic acid, 4-hexadecyl ester	0.78
	12-methyl-tridecanoic acid, methyl ester	0.48
	trifluoro-acetic acid, undecyl ester	0.69
	(Z,Z)-9,12-Octadecadienoic acid, methyl ester	1.32
	(E)-9-Octadecenoic acid, methyl ester	4.36
	Methyl stearate	1.78
Ketone	2-Pentadecanone, 6,10,14-trimethyl-	1.36
Oxathiane	6-dodecyl-1,2-oxathiane,2,2-dioxide	0.72
Borate Ester	Trihexadecyl borate	2.25
Phenol	2,4-Di-tert-butylphenol	1.2
Siloxane	dodecamethylcyclohexasiloxane	0.94

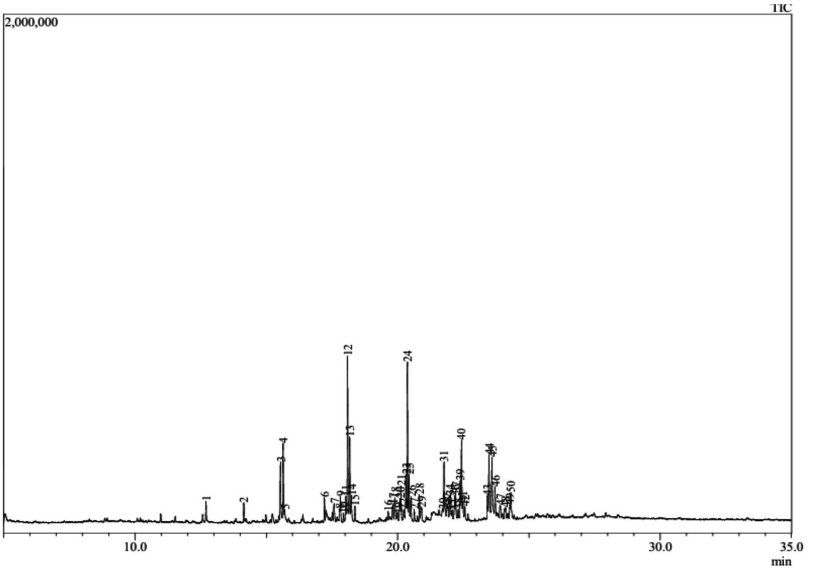

Figure 4: Chromatogram of methanol extract of Sarawak Liberica coffee leaves

Table 5: Major compounds in the extracts of Sarawak Liberica coffee leaves

No.	Name	MF	MW	Peak Area (%)
1	1-Nonadecene	$C_{19}H_{38}$	266	14.20
2	1-Pentadecene	$C_{15}H_{28}$	208	9.55
3	Phytol	$C_{20}H_{30}O$	286	5.18

(Costa *et al.*, 2014). Liberica coffee leaves exhibit a wide range of chemical constituents that contribute to their unique chemical profiles and potential for bioactivity, including antibacterial, anticancer, antifungal, anti-inflammatory, and antioxidant properties. The detailed GC-MS analysis results can provide a foundation for their potential use across a broad range of industries and in developing products such as cosmetics, flavourings, and even health products.

Total Phenolic and Flavanoid Content

Table 6 reveals that the TPC and TFC values of Sarawak Liberica coffee leaves are 356.79 ± 18.41 mg GAE/g and 1514.63 ± 16.74 mg quercetin/g, respectively. The TPC value of Sarawak Liberica coffee leaves is relatively high, suggesting a significant presence of phenolic compounds in these leaves. However, there have not been many studies analysing the TPC of Liberica leaves. According to a study by

Table 6: The TPC and TFC value of methanol extract of Sarawak LIberica coffee leaves

	TPC (mg GAE/g)	TFC (mg quecertin/g)
Methanol extract of Sarawak Liberica coffee leaves	356.79 ± 18.41	1514.63 ± 16.74

Note: All data were collected in triplicate and expressed as mean ± standard deviation. Absent significant detonation "*" indicates $p > 0.05$.

Fibrianto *et al.* (2020), the TPC value of Liberica coffee leaves ranged from 14.6 ± 0.31 to 42.0 ± 0.79 mg GAE/100 mL using the decoction method, indicating consistency in the presence of phenolic compounds across different studies. The relatively high TFC in the Sarawak Liberica coffee leaves may be related to the freshness and maturity of the sample leaves collected. The maturity of the leaves and the period in which they are collected will also affect the phenolic content, as the phenolic content of the leaves decreases as they mature (Alba *et al.*, 2024).

There have been a few studies on the TFC readings of other parts of the coffee tree, such as the pulp. According to Ismail *et al.* (2020), the TFC reading for Liberica coffee pulp was 39.39 mg QE/g, suggesting that the sample had only a small amount of flavonoid content. The TFC reading for Liberica coffee leaves obtained in this study is remarkably high compared to the coffee pulp of the Liberica coffee trees. One study conducted by Maxiselly *et al.* (2022) stated that the TFC value for Robusta coffee leaves was 13.27 ± 1.18 mg catechin equivalent/100 g dry weight, with catechin being used as the standard curve. The high TFC value in coffee leaves may be due to several factors, such as the choice of solvent and the conditions of extraction. The solvent chosen for this study was methanol, using the MAE method. According to a study conducted by Buyong *et al.* (2023), it was found that ethanol was the most efficient solvent for obtaining flavonoids from Liberica coffee silverskin (CS). Additionally, the flavonoid content can also be affected by the extraction parameters employed, such as temperature, duration, and solvent concentration. For instance, studies by Buyong *et al.* (2023) may have optimised the flavonoid extraction process by using a 30-minute extraction at 60°C to 65°C.

Antioxidant Activity Analysis

In this study, 54.37 ± 0.10% of the antioxidant activity in Liberica coffee leaves was calculated. This finding implies that the antioxidant capacity is moderate, indicating the presence of bioactive substances capable of efficiently neutralising free radicals. The results for Liberica coffee leaves can be compared with a study by Kurang and Kamengon (2021), which found that the antioxidant activity for Arabica coffee leaves ranged from 17.126% to 80.459%. *Coffea arabica* leaf extracts demonstrated high antioxidant potential, as evidenced by their strong radical scavenging activity, with IC_{50} values ranging from 7.47 ± 0.12 to 122.76 ± 1.38 µg/mL in the DPPH assay, indicating their effectiveness in neutralising free radicals and supporting their role as a rich source of natural antioxidants. One of the active ingredients, 5-caffeoylquinic acid, showed antioxidant activities that inhibit inflammation in mice (Segheto *et al.*, 2018).

The antioxidant activity of Liberica coffee leaves is within the range observed for Arabica coffee leaves. The antioxidant activity of Liberica was found to be 54.37%, which is considered high. A study conducted by Baay *et al.* (2024) showed high concentrations of alkaloids, flavonoids, and saponins in the ethanolic extract of Liberica coffee leaves, while indicating a moderate antioxidant potential with a free radical scavenging activity, as evidenced by an LC_{50} value of 33.48 µg/mL. Liberica coffee leaves exhibit significantly higher antioxidant activity due to their richer phenolic compound profile, genetic differences that lead to a higher synthesis of antioxidants, and potentially more favourable environmental conditions for growing Liberica coffee. These factors combined contribute to the enhanced antioxidant capacity observed in Liberica coffee leaves.

The efficiency of extraction methods significantly influences the antioxidant potential of coffee extracts. Optimum brewing conditions for Liberica coffee leaves were established at 95.52°C for 6.03 minutes, resulting in a total phenolic content of 562.72 ± 20.21 mg GAE/g, a tannin content of 434.86 ± 34.05 µg TAE/g, and an IC_{50} antioxidant activity of 12.68 ± 1.89 ppm, demonstrating high antioxidant activity (Hariyadi & Dewi, 2020). A comparative study on maceration and ultrasonic extraction methods for *Arabica*, *Robusta*, and *Liberica*

green coffee beans demonstrated that ultrasonic extraction yielded higher antioxidant activity, with Liberica exhibiting the most potent radical scavenging potential and the lowest IC_{50} value of 45.37 ppm (Firdaus *et al.*, 2025). Ultrasonic extraction can be an alternative method for maximising the bioactivity of coffee leaf extracts and underscores the promising value of Liberica byproducts for future applications.

Conclusions

In conclusion, the GC-MS analysis revealed the presence of 41 chemical compounds, with four major ones: 5-Hydroxymethylfurfural (5-HMF), D-allose, 1,6-Anhydro-β-D-glucofuranose, and caffeine in the CS extract using both methanol and ethanol as solvents. The percentage of caffeine content in the CS extracts showed no significant difference ($p < 0.05$) across the different extraction solvents, with ethanol (1.55 ± 0.47%), methanol (1.77 ± 0.38%), and water (1.92 ± 0.65%). The GC-MS analysis also identified 36 chemical compounds in the methanol extract of Sarawak Liberica coffee leaves, with the three major compounds being 1-pentadecene, 1-nonadecene, and phytol. The total phenolic content (TPC) and total flavonoid content (TFC) of the Sarawak Liberica coffee leaves were 356.79 ± 18.41 mg GAE/g and 1514.63 ± 16.74 mg quercetin/g, respectively, with an antioxidant activity of 54.37%. The presence of these compounds in relatively high amounts in the CS and leaves of the Liberica variety suggests potential applications for coffee by-products in various industries, including sustainable biofuel production, the chemical industry, food production, and pharmaceuticals.

Acknowledgements

The research was funded by International Matching Grant INT/F07/UB/86419/2024 (UNIMAS). This research was a collaborative work between Faculty of Research Science and Technology of UNIMAS and the Faculty of Agricultural Technology, Universitas Brawijaya in Indonesia.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

References

Abduh, M. Y., Nofitasari, D., Rahmawati, A., Eryanti, A. Y., & Rosmiati, M. (2023). Effects of brewing conditions on total phenolic content, antioxidant activity, and sensory properties of cascara. *Food Chemistry Advances*, *2*, 100183. https://doi.org/10.1016/j.focha.2023.100183

Alba, T. M., Tessaro, E., & Sobottka, A. M. (2022). Seasonal effect on phenolic content and antioxidant activity of young, mature, and senescent leaves from *Anredera cordifolia* (Ten.) Steenis (Basellaceae). *Brazilian Journal of Biology*, *84*, e254174. https://doi.org/10.1590/1519-6984.254174

Alnsour, L., Issa, R., Awwad, S., Albals, D., & Al-Momani, I. (2022). Quantification of total phenols and antioxidants in coffee samples of different origins and evaluation of the effect of degree of roasting on their levels. *Molecules*, *27*(5), 1591. https://doi.org/10.3390/molecules27051591

Arora, S., & Meena, S. (2017). GC-MS profiling of *Ceropegia bulbosa* Roxb. var. *bulbosa*, an endangered plant from Thar Desert, Rajasthan. *Pharma Innovation*, *6*(11), 568-573.

Ashenhurst, J. (2023, February 17). Pyranoses and furanoses: Ring-chain tautomerism in sugars. *Master Organic Chemistry*. https://www.masterorganicchemistry.com/2017/07/13/pyranoses-and-furanoses-ring-chain-tautomerism-in-sugars/

Baay, D. L. J. M., Borromeo, R. V., & Paglinawan, F. C. (2024). *In vitro* phytochemical screening, antioxidant activity, and cytotoxicity of *Coffea liberica* ethanolic leaf extract. *International Journal of Botany Studies*, *9*(8), 31-35.

Bessada, S. M. F., Alves, R. C., Costa, A. S. G., Nunes, M. A., & Oliveira, M. B. P. P. (2018). *Coffea canephora* silverskin from different geographical origins: A comparative study. *Science of the Total Environment*, *645*, 1021-1028. https://doi.org/10.1016/j.scitotenv.2018.07.201

Bessada, S. M. F., Alves, R. C., & Oliveira, M. B. P. P. (2018). Coffee silverskin: A review on potential cosmetic applications. *Cosmetics 2018*, *5*(1), 5. https://doi.org/10.3390/cosmetics5010005

Bresciani, L., Calani, L., Bruni, R., Brighenti, F., & Del Rio, D. (2014). Phenolic composition, caffeine content and antioxidant capacity of coffee silverskin. *Food Research International*, *61*, 196-201. https://doi.org/10.1016/j.foodres.2013.10.047

Buyong, N. L., & Nillian, E. (2023). Physiochemical properties of Sarawak's adapted *Liberica* coffee silverskin utilising varying solvents. *Food Science & Nutrition*, *11*, 6052-6059. https://doi.org/10.1002/fsn3.3541

Chen, X. (2018). A review on coffee leaves: Phytochemicals, bioactivities, and applications. *Critical Reviews in Food Science and Nutrition*, *59*(6), 1008-1025. https://doi.org/10.1080/10408398.2018.1546667

Chen, Z., Chen, J., Zhang, W., Zhang, T., Guang, C., & Mu, W. (2018). Recent research on the physiological functions, applications, and biotechnological production of D-allose. *Applied Microbiology and Biotechnology*, *102*(10), 4269-4278. https://doi.org/10.1007/s00253-018-8916-6

Choi, M. N., Shin, K. C., Kim, D. W., Kim, B. J., Park, C. S., Yeom, S. J., & Kim, Y. S. (2021). Production of D-allose from D-allulose using commercial immobilised glucose isomerase. *Frontiers in Bioengineering and Biotechnology*, *9*. https://doi.org/10.3389/fbioe.2021.681253

Costa, J. P., da Silva Oliveira, J., Mario Rezende Junior, L., & de Freitas, R. M. (2014). Phytol: A natural diterpenoid with pharmacological applications on the central nervous system—A review. *Recent Patents on Biotechnology*, *8*(3), 194-205. https://doi.org/10.2174/1872208308031506051627 45

Defri, I., Palupi, N. S., & Yuliana, N. D. (2021). Physicochemical and sensory characteristics of Kawa Daun from West Sumatra at different smoking times. *Advances in Biological Sciences Research*, *13*. https://doi.org/10.2991/absr.k.210609.020

Dirar, A. I., Alsaadi, D. H. M., Wada, M., Mohamed, M. A., Watanabe, T., & Devkota, H. P. (2019). Effects of extraction solvents on total phenolic and flavonoid contents and biological activities of extracts from Sudanese medicinal plants. *South African Journal of Botany*, *120*, 261-267. https://doi.org/10.1016/j.sajb.2018.07.003

Faudone, G., Arifi, S., & Merk, D. (2021). The medicinal chemistry of caffeine. *Journal of Medicinal Chemistry, 64*(11), 7156-7178. https://doi.org/10.1021/acs.jmedchem.1c00261

Fibrianto, K., Daryanto, K. A., Sholihah, N., Wahibah, L. Y., Hasyati, N., Al-Baarri, A. N., & Hariyadi, D. M. (2020). Sensory profiling of Robusta and Liberica coffee leaves functional tea by modifying brewing temperature. *IOP Conference Series: Earth and Environmental Science*, *475*(1), 012028. https://doi.org/10.1088/1755-1315/475/1/012028

Firdaus, S., Permadi, A., & Sulistiawati, E. (2025). Comparative study of ultrasonic and maceration extraction in enhancing antioxidant and SPF properties of green coffee bean serum. *Current Research in Biosciences and Biotechnology*, *6*(2). https://doi.org/10.5614/crbb.2025.6.2/GAMCE9IN

Gottstein, V., Bernhardt, M., Dilger, E., Keller, J., Breitling-Utzmann, C. M., Schwarz, S., Kuballa, T., Lachenmeier, D. W., & Bunzel, M. (2021). Coffee silver skin: Chemical characterisation with special consideration of dietary fibre and heat-induced contaminants. *Foods*, *10*(8). https://doi.org/10.3390/foods10081705

Hariyadi, D., & Dewi, R. M. (2020). Optimisation of brewing time and temperature for caffeine and tannin levels in Dampit coffee leaf tea of Robusta (*Coffea canephora*) and Liberica (*Coffea liberica*). *Potravinarstvo Slovak Journal of Food Sciences*, *14*, 197-206. https://doi.org/10.5219/1212

Hejna, A., Barczewski, M., Kosmela, P., Mysiukiewicz, O., & Kuzmin, A. (2021). Coffee silverskin as a multifunctional waste filler for high-density polyethylene green composites. *Journal of Composites Science*, *5*(2). https://doi.org/10.3390/jcs5020044

Iriondo-Dehond, A., Rios, M. B., Herrera, T., Rodriguez-Bertos, A., Nuñez, F., Andres, M. I. S., Sanchez-Fortun, S., & Del Castillo, M. D. (2019). Coffee silverskin extract: Nutritional value, safety, and effect on key biological functions. *Nutrients*, *11*(11). https://doi.org/10.3390/nu11112693

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. http://www.pertanika.upm.edu.my/

Ismail, N. S., Boli, M. E., Buyong, N. L., Sing, N. N., Awang Adeni, D. S., & Awang Hussini, A. A. S. (2020). The feasibility study of the physicochemical properties of Sarawak Liberica sp. coffee pulp. *Pertanika Journal of Tropical Agricultural Science*, *43*(4). https://doi.org/10.47836/pjtas.43.4.05

Itodo, A. U., Abdulrahman, F. W., Hassan, L. G., Maigandi, S. A., & Itodo, H. U. (2010). Application of GC/MS quantitation in the adsorption of herbicides by sorbents from two abundant nut shells. *Researcher*, *2*(2), 64-73.

Junior, I. I., Marcelo, A. D. N., De Souza, R. O. M. A., Dufour, A., & Wojcieszak, R. (2020). Levoglucosan: A promising platform molecule? *Green Chemistry*, *22*(18), 5859-5880. https://doi.org/10.1039/d0gc01490g

Kano, A., Gomi, K., Yamasaki-Kokudo, Y., Satoh, M., Fukumoto, T., Ohtani, K., Tajima, S., Izumori, K., Tanaka, K., Ishida, Y., Tada, Y., Nishizawa, Y., & Akimitsu, K. (2010). A rare sugar, D-allose, confers resistance to rice bacterial blight with upregulation of defence-related genes in *Oryza sativa*. *Phytopathology, 100*(1), 85-90. https://doi.org/10.1094/PHYTO-100-1-0085

Kim, Y. J., Choi, J., Lee, G., & Lee, K. G. (2021). Analysis of furan and monosaccharides in various coffee beans. *Journal of Food Science and Technology*, *58*(3), 862-869. https://doi.org/10.1007/s13197-020-04600-5

Kumar, V. (2011). Antibacterial activity of crude extracts of *Spirulina platensis* and its structural elucidation of bioactive compounds. *Journal of Medicinal Plants Research*, *5*(32). https://doi.org/10.5897/jmpr11.1175

Kurang, R. Y., & Kamengon, R. Y. (2021). Phytochemical test and antioxidant activity of methanol extract in Arabica coffee leaves by using DPPH method (1,1-Diphenyl-2-Picrylhydrazyl). *Walisongo Journal of Chemistry*, *4*(2), 113-118. https://doi.org/10.21580/wjc.v4i2.8032

Lim, T. K. (2013). Coffea liberica. In Lim T. K. (Ed.), *Edible medicinal and non-medicinal plants* (pp. 1–10). Springer, Dordrecht.

Martins, F. C. O. L., Alcantara, G. M. R. N., Silva, A. F. S., Melchert, W. R., & Rocha, F. R. P. (2022). The role of 5-hydroxymethylfurfural in food and recent advances in analytical methods. *Food Chemistry*, *395*, 133539. https://doi.org/10.1016/J.FOODCHEM.2022.133539

Martuscelli, M., Esposito, L., Di Mattia, C. D., Ricci, A., & Mastrocola, D. (2021). Characterisation of coffee silver skin as potential food-safe ingredient. *Foods*, *10*(6). https://doi.org/10.3390/foods10061367

Mathew, A. K., Abraham, A., Mallapureddy, K. K., & Sukumaran, R. K. (2018). Chapter 9 - Lignocellulosic biorefinery wastes, or resources? In Bhaskar, T., Pandey, A., Mohan, S. V., Duu-jong, L., & Khanal, S. K. (Eds.), *Waste biorefinery: Potential and perspectives* (pp. 267-297). Elsevier. https://doi.org/10.1016/B978-0-444-63992-9.00009-4

Maxiselly, Y., Anusornwanit, P., Rugkong, A., & Chiarawipa, R. (2022). Phytochemical analysis of leaves and cherries of coffee and sensory evaluation of tea products of Robusta coffee in Songkhla, Thailand. *AGRIVITA Journal of Agricultural Science*, *44*(3). https://doi.org/10.17503/agrivita.v41i0.3072

Nolasco, A., Squillante, J., Velotto, S., D'Auria, G., Ferranti, P., Mamone, G., Errico, M. E., Avolio, R., Castaldo, R., Cirillo, T., & Esposito, F. (2022). Valorisation of coffee industry wastes: Comprehensive physicochemical characterisation of coffee silverskin and multipurpose recycling applications. *Journal of Cleaner Production*, *370*, 133520. https://doi.org/10.1016/j.jclepro.2022.133520

Nzekoue, F. K., Angeloni, S., Navarini, L., Angeloni, C., Freschi, M., Hrelia, S., Vitali, L. A., Sagratini, G., Vittori, S., & Caprioli, G. (2020). Coffee silverskin extracts: Quantification of 30 bioactive compounds by a new HPLC-MS/MS method and evaluation of their antioxidant and antibacterial activities. *Food Research International*, *133*. https://doi.org/10.1016/j.foodres.2020.109128

Samling, B. A., Assim, Z., Tong, W. Y., Leong, C. R., Ab Rashid, S., Nik Mohamed Kamal, N. N. S., Muhamad, M., & Tan, W. N. (2021). *Cynometra cauliflora* L.: An indigenous tropical fruit tree in Malaysia bearing essential oils and their biological activities. *Arabian Journal of Chemistry*, *14*(9). https://doi.org/10.1016/j.arabjc.2021.103302

Segheto, L., Santos, B. C. S., Werneck, A. F. L., Vilela, F. M. P., de Sousa, O. V., & Rodarte, M. P. (2018). Antioxidant extracts of coffee leaves and its active ingredient 5-caffeoylquinic acid reduce chemically-induced inflammation in mice. *Industrial Crops and Products*, *126*, 48-57. https://doi.org/10.1016/j.indcrop.2018.09.027

Tesfaye, G., Alemu, D., Kuma, B., & Shiferaw, A. (2024). Determination of the caffeine contents of Guji Zone coffee beans in Southern Ethiopia using ultra violet-visible (UV-Vis) spectrometer analysis. *International Journal of Nutrition and Food Sciences*, *12*(2), 1-5. https://doi.org/10.11648/ijnfs.20241301.11

Vuletić, N., Bardić, L., & Odžak, R. (2021). Spectrophotometric determinination of caffeine content in the selection of teas, soft and energy drinks available on the Croatian market. *Food Research*, *5*(2), 325-330. https://doi.org/10.26656/fr.2017.5(2).482

Wang, Y., Brown, C. A., & Chen, R. (2018). Industrial production, application, microbial biosynthesis, and degradation of furanic compounds, hydroxymethylfurfural (HMF). *AIMS Microbiology*, *4*(2), 261-273. https://doi.org/10.3934/MICROBIOL.2018.2.261

Wanyika, H. N., Gatebe, E. G., Gitu, L. M., Ngumba, E. K., & Maritim, C. W. (2010). Determination of caffeine content of tea and instant coffee brands found in the Kenyan market. *African Journal of Food Science*, *4*(6), 353-358.

Wen, L., Zhang, Z., Rai, D., Sun, D. W., & Tiwari, B. K. (2019). Ultrasound-assisted extraction (UAE) of bioactive compounds from coffee silverskin: Impact on phenolic content, antioxidant activity, and

morphological characteristics. *Journal of Food Process Engineering*, *42*(6). https://doi.org/10.1111/jfpe.13191

Wong, J. C. J., & Nillian, E. (2023). Microwave-assisted extraction of bioactive compounds from Sarawak *Liberica* sp. coffee pulp: Statistical optimisation and comparison with conventional methods. *Food Science and Nutrition*, *11*(9), 5364-5378. https://doi.org/10.1002/fsn3.3494

Xuan, S. H., Lee, K. S., Jeong, H. J., Park, Y. M., Ha, J. H., & Park, S. N. (2019). Cosmeceutical activities of ethanol extract and its ethyl acetate fraction from coffee silverskin. *Biomaterials Research*, *23*(1). https://doi.org/10.1186/s40824-018-0151-9

Zengin, G., Sinan, K. I., Mahomoodally, M. F., Angeloni, S., Mustafa, A. M., Vittori, S., Maggi, F., & Caprioli, G. (2020). Chemical composition, antioxidant, and enzyme inhibitory properties of different extracts obtained from spent coffee grounds and coffee silverskin. *Foods*, *9*(6). https://doi.org/10.3390/FOODS9060713

Zhang, H., Jiang, M., & Song, F. (2020). D-allose is a critical regulator of inducible plant immunity in tomato. *Physiological and Molecular Plant Pathology*, *111*. https://doi.org/10.1016/j.pmpp.2020.101507

Zielińska, E., Baraniak, B., & Karaś, M. (2017). Antioxidant and anti-inflammatory activities of hydrolysates and peptide fractions obtained by enzymatic hydrolysis of selected heat-treated edible insects. *Nutrients*, *9*(9), 970.