Faculty of Resource Science and Technology

Phytochemical Profiling and Potential Application of Sarawak Liberica Coffee Silverskin

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Phytochemical Profiling and Potential Application of Sarawak Liberica Coffee Silverskin

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

……

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ABSTRACT

Liberica coffee is the minority species variety of the *Coffea* family. Internationally, this variety only consisted of 1% of the coffee variety cultivated worldwide. Despite that, this variety is the major species (73%) planted in Malaysia compared to the other varieties. During the roasting of the coffee bean, multiple by-products are produced mainly the coffee silverskin (CS). CS is thin tegument layer outside of the coffee bean, that was generated when treated with high heat intensity. When the coffee production is exponential, this creates an excess of the waste that have multiple potential of application in the industries such as cosmetic, healthcare, and food products. In the recent years, numerous studies had been done on the CS, regarding its beneficial bioactive compounds, as these had shown some promising benefits in multiple industries such as the food production, pharmaceuticals, medicine, and human health. However, most of these studies only focused on the majority variety of the coffee family, thus leaving a knowledge gap on the Liberica CS. As one of the main Liberica cultivators in South-east Asia, the CS variety of the Liberica originate from Sarawak, Malaysia piques the interest in this study. The result showed that, the CS have a high phenolic content in methanol extract and high flavonoid content in ethanol extract, 15.24 ± 0.65 mg GAE/g and 25.14 ± 0.59 mg QE/g, respectively. The DPPH activity was also found to be highest as in the ethanol extract (83.85 ± 1.78%), concurred by the results in the FRAP assay as the highest reduction was also in ethanol extract (11.40 ± 18.57 µmol FSE/g). In terms of the caffeine content, the amount was the highest calculated in the methanol extract with 26.86 ± 5.77 mg/g (1.77 ± 0.38%) of the dry weight of the CS. From the CG-MS employment, the CS was identified to contain 30 bioactive compounds, where four compounds (5-Hydroxymethylfurfural, D-allose, Caffeine, 1,6-Anhydro-β-D-glucofuranose) identified to be the major constituent in the CS, while the rest are in trace

amount. In this study, the CS did not exhibit any antimicrobial effects on both Gram-positive and Gram-negative bacteria. In conclusion, the study shows that Sarawak Liberica CS does contain high beneficial bioactive compounds such as phenolic and flavonoid, while exhibit a significant antioxidant property. The CS may lack in antimicrobial ability, but the high caffeine content of the CS makes it a potential valuable by-product for the industries.

Keywords: Coffee by-product, total phenolic content, total flavonoid content, antioxidant activities, antimicrobial properties, caffeine content

Pemprofilan Fitokimia dan Potensi Kegunaan Kulit Ari Kopi Liberica Sarawak

ABSTRAK

Kopi Liberica adalah spesies minoriti dalam keluarga Coffea. Di peringkat antarabangsa, jenis ini hanya terdiri daripada 1% daripada jenis kopi yang ditanam di seluruh dunia. Walau bagaimanapun, jenis ini merupakan spesies utama (73%) yang ditanam di Malaysia berbanding jenis kopi lain. Semasa proses memanggang biji kopi, pelbagai produk sampingan dihasilkan terutamanya kulit ari (KA). KA adalah lapisan nipis di luar biji kopi, yang tertanggal apabila dipanggang dengan haba yang tinggi. Apabila pemprosesan kopi dilakukan secara besar-besaran, ini akan menghasilkan sisa berlebihan yang mempunyai potensi untuk diaplikasikan dalam pelbagai industri seperti kosmetik, penjagan kesihatan, dan produk makanan. Sejak kebelakangan ini, banyak kajian telah dilakukan ke atas KA, mengenai bahan bioaktifnya yang bermanfaat, dan menunjukkan potensi dalam pelbagai industri seperti penghasilan makanan, ubat-ubatan, perubatan, dan kesihatan manusia. Walau bagaimanapun, kebanyakan kajian ini hanya tertumpu pada kepelbagaian keluarga kopi yang majoriti, sekali gus menghasilkan jurang pengetahuan terhadap KA Liberica. Sebagai salah satu pengeluar Kopi Liberica utama di Asia Tenggara, kepelbagaian KA Liberica Sarawak menjadi fokus dalam kajian ini. Hasil kajian menunjukkan bahawa, KA mempunyai kandungan fenolik yang tinggi dalam ekstrak metanol manakala flavonoid yang tinggi dalam ekstrak etanol, masing-masing 15.24 ± 0.65 mg GAE/g dan 25.14 ± 0.59 mg QE/g. Aktiviti DPPH juga didapati paling tinggi seperti dalam ekstrak etanol (83.85 ± 1.78%), disokong oleh keputusan ujian FRAP kerana pengurangan tertinggi juga dalam ekstrak etanol (11.40 ± 18.57 µmol FSE/g). Dari segi kandungan kafein, yang paling tinggi dikira dalam ekstrak metanol dengan 26.86 ± 5.77 mg/g (1.77 ± 0.38%) daripada berat

bersih KA. Daripada CG-MS, KA telah dikenal pasti mengandungi 30 bahan biokimia, di mana empat sebatian (5-Hydroxymethylfurfural, D-allose, Kafein, 1,6-Anhydro-β-D-glucofuranose) dikenal pasti sebagai juzuk utama dalam KA, manakala selebihnya dalam jumlah yang sedikit. Dalam kajian ini, KA tidak menunjukkan sebarang kesan antimikrob terhadap kedua-dua bakteria Gram-positif dan Gram-negatif. Kesimpulannya, kajian menunjukkan bahawa KA Liberica Sarawak memang mengandungi bahan bioaktif bermanfaat tinggi seperti fenolik dan flavonoid, di samping mempamerkan sifat antioksidan yang ketara. KA mungkin kekurangan keupayaan antimikrob, tetapi kandungan kafein yang tinggi padanya menjadikannya produk sampingan yang berpotensi tinggi sebagai produk berharga dalam pelbagai industri.

Kata kunci: *Produk sampingan kopi, kandungan fenolik, kandungan flavonoid, aktiviti antioksidan, ciri-ciri antimikrobial, kandungan kafein*

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LIST OF ABBREVIATIONS

AAE	Ascorbic acid equivalent
Abs	Absorbance
AST	Antimicrobial Susceptibility Testing
ANOVA	Analysis of variance
ATCC	American Type Culture Collection
±	Approximation
β	Beta
R^2	Correlation coefficient
CLSI	Clinical and Laboratory Standard Institute
CS	Coffee silverskin
CGA	Chlorogenic acid
°C	Celsius
cm	Centimeter
$CHCl_3$	Chloroform
DCM	Dichloromethane
DMSO	Dimethyl sulfoxide
DPPH	2,2-diphenyl-1-piccrylhydrazyl
2,5-DMF	2,5-dimethylfuran
DHT	dihydrotestosterone
eV	Electron volt
E. coli	*Escherichia coli*
FRAP	Ferric Reducing Antioxidant Power

$FeCl_3$	Ferrous chloride
$FeSO_4$	Ferrous sulphate
FSE	Ferrous sulphate equivalent
Fe^{2+}	Ferrous ion
FOS	Fructooligosaccharides
GAE	Gallic acid equivalent
GC	Green chemistry
hr	Hour
HDPE	High-density polyethylene
5-HMF	5-Hydroxymethylfurfural
IC_{50}	Inhibition concentration at 50%
I.D.s	Internal diameters
I	Intermediate
kg	Kilogram
<	Less than
≤	Less or equal
L	Litre
LG	Levoglucosan
MR	Maillard reaction
>	More than
≥	More or equal
µg	Microgram
mg	Milligram
M	Molar
mM	Millimolar

µmol	Micromole
µL	Microlitre
mL	Millilitre
min	Minutes
mm	Millimetre
µm	Micrometre
m/z	Mass-to-charge ratio
MHA	Mueller Hinton Agar
MW	Molecular weight
MF	Molecular formula
MAE	Microwave assisted extraction
MIC	Minimum inhibition concentration
ng	Nanogram
g	Gram
nm	Nanometres
NIST-17	National Institute of Standards and Technology 2017
NB	Nutrient broth
OTA	Ochratoxin A
PLA	Polylactic acid
PBS	Polybutylene succinate
pH	Potential of hydrogen
P	Probability
QE	Quercetin equivalent
RSA	Radical scavenging activities
rpm	Round per minute

R	Resistant
RT	Retention time
RI	Retention index
ROS	Reactive oxygen species
Sdn Bhd	*Sendirian Berhad*
S. aureus	*Staphylococcus aureus*
S	Susceptible
SLE	Solid-Liquid extraction
SWE	Subcritical water extraction
SFE	Supercritical fluid extraction
subsp.	Sub-species
sp.	Species
TPC	Total phenolic content
TFC	Total flavonoid content
TPTZ	2,4,6-Tripyridyl-s-triazine
TE	Trolox equivalent
UV	Ultraviolet
UV-VIS	Ultraviolet visible
UVB	Ultraviolet B
UAE	Ultrasonic assisted extraction
UNIMAS	Universiti Malaysia Sarawak
v/v	Volume-to-volume ratio
w/v	Weight-to-volume ratio
w/w	Weight-to-weight ratio
ZD	Zone diameter

CHAPTER 1

INTRODUCTION

1.1 Study Background

Coffee is one of the most popular beverages worldwide, and its consumption continues to increase every year. In Malaysia alone, the total weight of coffee consumption recently in 2022 was 800,000 of 60 kg bags which bring to total of 48,000 metric tons of coffee (Ahmad, 2022). Quoting from a newspaper report by The Star, the Liberica coffee production in Malaysia was at approximately 325,584 kg in June 2022 (Benjamin, 2022), while the Robusta coffee produced was up to 120,000 kg at the end of 2022 (Statista Research Department). The coffee culture in Malaysia is progressing throughout the year such that the coffee is imported from neighbouring country such as Indonesia, just to satisfy the domestic demands of the consumers. In comparison to Singapore, the third major importer of coffee bean worldwide, Malaysia international trade of shipments was in ratio of 1:6 to Singapore (Volza Grow Global, 2023). Hence, Malaysia is no powerhouse of coffee compared to country such as Brazil, Panama, and Colombia, three main exporters of coffee. Despite that, Malaysia is one of the main Liberica coffee cultivators in the Southeast Asia alongside the Philippines (Anindya, 2021).

As coffee have varieties of species across the globe, Malaysia mainly producing two species which are the Liberica and Robusta variety. Liberica is a rare variety of coffee, and it is grown in small quantity (1%) compared to Arabica and Robusta globally. However, this species accounts for 73% of coffee cultivation in Malaysia, while Robusta coffee comes in second with a 27% coverage, and both are suited for cultivation in Malaysia due to their favourable climate (Ismail et al., 2014). In term of coffee bean production, the Liberica

generated a smaller amount of coffee beans (0.7:10) in kilograms compared to Arabica and Robusta (2:10) in kilograms, by being scarcely available in the market, the Liberica variety serve a higher value in the world coffee market, compared to the other species (Yoong, 2022).

The Sarawak state of Malaysia is well-known for its production of Liberica coffee, a unique minority species of coffee that has adapted to the region's climate and soil conditions. In recent years, researchers have begun to investigate the potential health benefits of Liberica coffee, including its antimicrobial and antioxidant properties. As for the coffee expert and connoisseur, they venture into the exotic taste of the coffee produced from this region, as the taste and aroma of the Liberica coffee were describe as strong, smoky taste, which appreciated by some but not all. Not only that, the Liberica coffee was not a choice of cultivation among the farmers due to the robust and big sized coffee cherries, as this causes inconvenience in post-processing of the coffee. Due to these subjective opinions on the Liberica coffee taste, the species is not the favourite of majority coffee consumer.

In each coffee production, through the dry method of processing, a significant amount of waste would be produced called the coffee silverskin (CS), which accounts for approximately 4% (w/w) of the coffee cherry's weight (Martuscelli et al., 2021). A study by Rodrigues et al. (2015) stated that CS has a high content of cellulose, hemicellulose, and lignin, which makes it a potential source for biofuel production, other than being target of interest in food industries. Despite its potential value as a sustainable biofuel, coffee silverskin is generally considered a waste product and is typically discarded. The further current utilisation and potential future prospect of the CS will be discussed further in this thesis.

1.2 Problem Statement

During the processing of the coffee beans through roasting, approximately 4% (w/w) of the CS would be produced from the coffee cherry. This may seem insignificant on a small production level, however, in the production of 1 tonne of coffee which, approximately 40 kg of CS will be produced, with production of tons of thousands of coffees per year, the CS also produced in excess thus providing us this beneficial by-product to be explore and investigate deeper. In an accumulation of a large amount, the phytotoxic antioxidant such as tannins and polyphenols may emitted pollution towards the soil and water source (Costa et al., 2018).

As far as this study noted, there are no previous reports on Sarawak adapted Liberica coffee silverskin. Previously, a study was done to investigate the antioxidant activity, phenols, flavonoids, antimicrobial, and reducing sugars of the coffee pulp of Sarawak Liberica coffee. The study on Liberica coffee pulp shows promising results as there are high activity of antioxidant observed correlate with the high phenols and flavonoids content (Nillian et al., 2020). This study will contribute to the current knowledge on local Liberica coffee as origin can be a determining factor that contributed to the different in the beneficial compounds level other than roasting, pre and post harvesting process, etcetera.

1.3 Hypotheses

Null hypothesis (H_o): The Liberica coffee silverskin from Sarawak does not possess beneficial bioactive compounds, antioxidant properties, and antimicrobial properties into making it a valuable by-product for various industries.

Alternative hypothesis (H_A): The Liberica coffee silverskin from Sarawak does possess beneficial bioactive compounds, antioxidant properties, and antimicrobial properties making it a valuable by-product for various industries.

1.4 Objectives

This study focal point was to establish a novel look into the potential beneficial properties of Sarawak Liberica coffee silverskin (CS). This was achieved through the following set of objectives:

i. To investigate the effect of ethanol and methanol solvents on the extraction of bioactive compounds in the Sarawak Liberica CS.
ii. To determine the antioxidant activities of Sarawak Liberica CS.
iii. To quantify the caffeine content of the Sarawak Liberica CS.
iv. To determine the antimicrobial properties of Sarawak Liberica CS.

CHAPTER 2

LITERATURE REVIEW

2.1 Coffee Industries in Malaysia

It is important to have some awareness of current issues highlighted in literature. Coffee scenes in Malaysia have seen some increases over the year, particularly after the COVID-19 hits (Ali & Ramanathan, 2021). This can be seen by the recovery of current business in the coffee industries such as the "*kopitiam"*, a Hokkien-Chinese dialect for a coffee shop, that mostly attracted the older communities, and the emerging of trendy coffee specialty shop that targeting the younger generations (Thomas et al., 2021). While both businesses have different targeted audiences, they do have similar purposes which is for the communities to have their beverages while socializing. This was further enhanced as the communities were unable to go out and socialize during the pandemic lockdown, and having the ban lifted gives them a sense of freedom, thus increasing the arising gathering spots for thoughts exchanging and socializing elsewhere than home, while equipped with beverages such as coffee (Thomas et al., 2021).

Not only that, during the pandemic, the trends of coffee drinking also increase as majority of people had to work from home, thus led to significantly more (92.5%) citizen brewing and making their own coffee at home to kickstart their day (Ali & Ramanathan, 2021). A survey was performed by Ali and Ramanathan (2021) such that, Malaysian aged 18-30 consumed mostly coffee (77.6%), followed by tea (76.4%), and malt drinks (60.3%). Not to mention, the culture of the Malaysian, as they prefer to serve coffee beverages during a guest or visitor visits to their home. This can be trace down to the early 19th century as the colonial of the western in the Malaysia which was previously known as "*Tanah Malaya*".

The culture of serving caffeinated drinks especially coffee and tea has influenced the culture of some indigenous Malaysian. Since then, most of the local household have their own coffee at home, either its instant coffee or to a raw green bean as needing roasting before made into a beverage.

2.2 Coffee Industries in Sarawak State

Sarawak is the biggest state in Malaysia (Figure 2.1), being one of the major key industries in the oil-palm plantation, the state also provided other source of income to the country such as oil and gas, and timber. The coffee plantation from Sarawak comes in quite low compared to the other states in Malaysia such as Johor and Sabah. Noted that, Sarawak only produces approximately 20 tons of coffee out of 8,034 tons in Malaysia, 2017 (Goh, 2019), compared to the production in 2021 which was in total of 120,000 tons (Statista Research Department, 2022) of coffee in Malaysia, hence shows some drastic increasing trend.

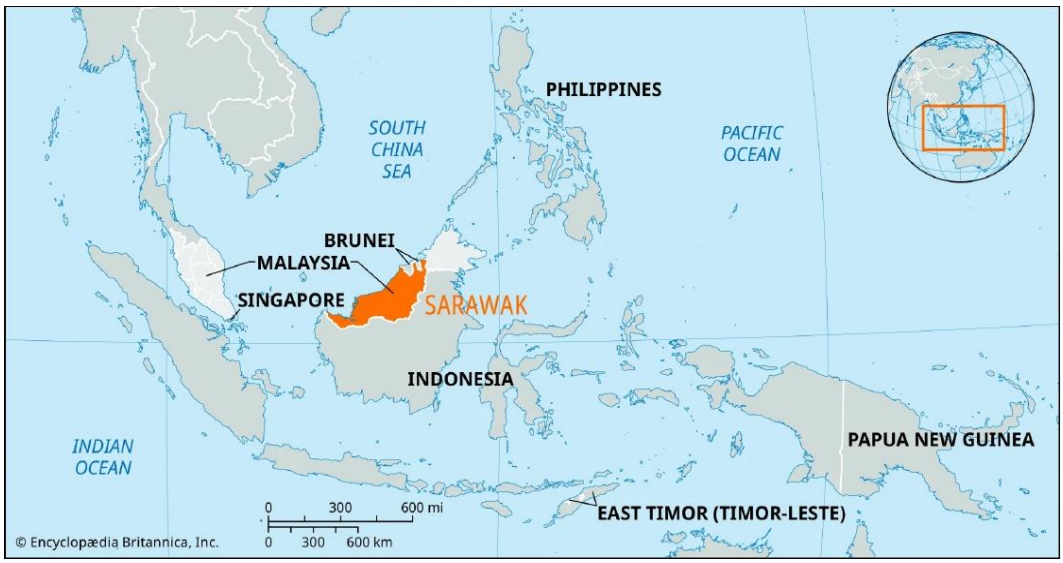

Figure 2.1: Map location of Sarawak state in Malaysia

Note. Figure from "Sarawak" by Britannica, T. Editors of Encyclopaedia, 2024, Encyclopedia Britannica. https://www.britannica.com/place/Sarawak-state-Malaysia.

Nonetheless, with the current support from the government in promoting the planting of the coffee plant, a bright future can be seen with research and development to be made using this as a subject of interest. A local politician, Mr. Lidam Assan from the district of Song in Sarawak said in his interview, a Sarawak coffee development board was proposed to be formed to further monitor the expenditure of the coffee production in Sarawak (Lorna, 2022). This can be related to the ratio of the uncultivated- land in Sarawak to the coffee production in the state is quite low compared to the other states, given that the coffee consumption by the locals is quite high in ratio to our coffee production, such that the government obligated to import coffee beans from oversees to accommodate the demands. the increasing trends and potentials of the state in becoming one of the top cultivators in the country have seen as a potential for an increase expenditure, and unfortunately, waste by-products of the coffee production.

2.3 Liberica Coffee (*Coffea liberica*) Beans

Liberica coffee is a species in the coffee family that is mainly cultivated in the Southeast Asia region which is in the country of Indonesia, Malaysia, and Philippines. The coffee Liberica was first globally introduced in the late of the 19th century around the period of when the pandemic coffee leaf rust (*Hemileia vastatrix*) disease spread originate from the Sri Lanka and had wiped out almost all the Arabica coffee in the world (Yoong, 2022). The coffee market during that time had discover the ability of the Liberica coffee from Liberia, West Africa to be partially resistance to the disease thus witnessing the rising of the coffee species globally accepted through natural selection.

During this period, Arabica and Liberica stand equal as the top coffee species produced in the coffee market globally. According to a review analysed by Davis et al. (2022), Liberica cherries have a bigger size compared to Arabica due to their thick coffee

skin and pulp. Other than that, the characteristics of the coffee itself are having a robust, high yielding profile, and can grow on warm lowland (0-1000 meter elevation) making them well received by the growers in particularly the Middle and Southeastern Asia. Moreover, due to their thick pulp, the cherries are less susceptible to pest such as the coffee borer beetle which targeted the seed for feeding thus damaging the fruit and quality of the beans itself (Yoong, 2022).

The Liberica tree have a robust growth habit such that they can survive in warmer and frequent rainfall, some were observed to more favour this growth condition. The tree can grow as high as 5-11 meter tall, with diameter of 42 cm × 20 cm wide. Compared to Arabica, even the leaves (30 × 25 mm) and fruits (20 × 12 mm) are larger, these were some of the attributes that was appealing to the global market during the time of Arabica downfall (Davis et al., 2022).

However, not long after, the Liberica coffee beans faced it degenerate period in the coffee market. This was or may be due to several factors that caused this such as the unfavourable smell and taste of the coffee which some describe as strong and smoky compared to Arabica. Not only that, but the post-processing process of the coffee is also considered harder due to the fact of the cherries having a thicker pulp, such mishandling of the process is believed to affect the quality and taste of the coffee (Ali & Ramanathan, 2021).

When the taste was indeed a subjective point of opinion such that, some coffee connoisseur may prefer, and some may reject this strong smoky taste. The partial resistance to the coffee leaf rust disease had led the coffee market to switch their attention to another coffee species, the Robusta coffee (*Coffea canephora*). The Robusta species is a superior version compared to the Liberica, as it is extremely resistant towards the leaf's disease, while having all the others fine attributes of Liberica. Towards the end of the 19th century, the

recovery and expansion of Arabica coffee from Brazil to the Southeast Asia coupled with the emergence of rubber estate and plantation particularly in Malaysia had led to further subside of the Liberica coffee production (Davis et al., 2022).

2.4 Coffee Silverskin

On average, the Liberica coffee beans usually have a larger diameter compared to the other coffee species, with two coffee beans found in each coffee cherry. Referring to Figures 2.2 and 2.3, each bean is covered with a thin closely fitting skin called silverskin (CS), outside of which is a looser, yellowish skin called the parchment, followed with the mucilage layer which was formed by the breakdown of the pectin chains by pectolytic enzymes (Klingel et al., 2020), the whole being encased in a pulp which forms the flesh of the cherry.

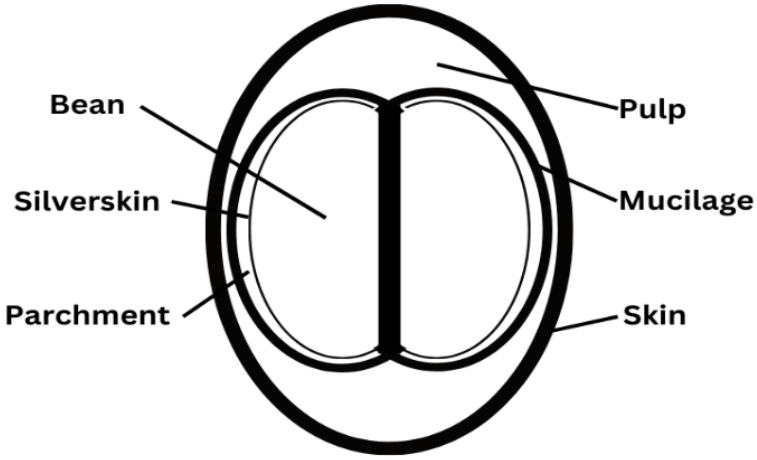

Figure 2.2: Schematic cross-section of a coffee cherry

The CS is a tegument of coffee beans that constitutes a by-product during the roasting procedure and is the most abundant solid by-product generated during the coffee processing. It has no commercial value and is currently discarded either as a solid waste, in some cases, it is minorly used as soil fertilisers, carbohydrate source for biorefineries or as fuel for combustion (Bessada et al., 2018b). Although recently, there are mentions of the CS of the Arabica and Robusta being utilised in food making, cosmetics, and pharmaceutical industries due to its high antioxidant activity (Bessada et al., 2018b; Costa et al., 2018; Rodrigues et al., 2023). However, the majority of these mentions are only on the study level, and not on the commercial scale of ready-made products. This lack of interest in the utilisation of by-product still has negative effects on the environment, as conventionally, most coffee factories and roastery just omitted to discard the waste by-product.

Figure 2.3: Coffee silverskin flakes

In 10 million tons of coffees produced, approximately 400 thousand tons of CS will be produced, as 1 kilogram of the coffee cherries produces 40 grams of CS (Nolasco et al., 2022). The CS constituted only 4% (w/w) of the coffee cherry (Martuscelli et al., 2021), but with the production of tons of thousands per year, the CS is a worthy by-product to investigate. Nevertheless, more than 50% of the coffee cherry component will be discarded as wastes, including the silverskin, as the market usually only focuses on the beans itself (Hejna et al., 2021). The discarding of this waste in an unproper manner will cause an environmental problem such that the CS was investigated to contain high phytotoxic antioxidants such as caffeine, tannins, and polyphenols (Costa et al., 2018). These compounds are beneficial, however, they are toxic if flow into the river or water source, when discarded on landfills as waste, they were reported to cause DNA damage and toxic to the aquatic environment (Fernandes et al., 2017).

2.5 Bioactive Compounds in Coffee Silverskin

The CS are currently known for its beneficial bioactive compounds that are targeted for the current studies recently. These compounds may differ according to the species, pre-processing method, and post-processing methods that were applied to the coffee beans. It is concurred by most research that the CS does have the beneficial compounds needed for the variety of health benefits in a human body including anti-inflammatory, anti-cancer, anti-diabetic, and anti-obesity effects (Bessada et al., 2018b; Iriondo-DeHond et al.,2016; Liang & Kitts, 2014). These health benefits may be due to the potential bioactive compounds in the CS such as chlorogenic acid (Andrade et al., 2022), caffeine (Bessada et al., 2018b), melanoidins, diterpenes (Liang & Kitts, 2014), flavonoids (Kumar & Pandey, 2013), and

fibres (Iriondo-DeHond et al., 2019). There are more compounds found in the CS, but these few are the major class of compounds in the by-product.

2.5.1 Chlorogenic Acid

The chlorogenic acid (CGA) (Figure 2.4), is a group of phenolic compounds that are abundantly found in the CS (Andrade et al., 2022). The formation of a family of esters between trans-cinnamic acids such as, caffeic acid and ferulic acid, together with quinic acid forms the CGA (Liang & Kitts, 2014) (Reaction 2.1). They are known for their antioxidant properties and have been shown to have a range of health benefits, including reducing inflammation, improving glucose metabolism, and protecting against cardiovascular disease.

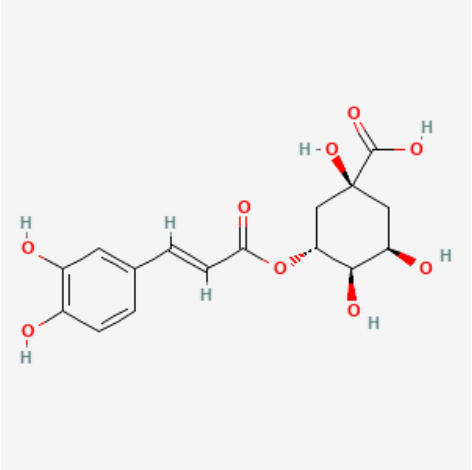

Figure 2.4: Chlorogenic acid chemical structure

In a study by Liang and Kitts, (2014), the CGA has protective effects against oxidative stress on PC12 human cells line which is the cell that store and release the norepinephrine and dopamine in human. The study is also in agreement with (Van Doan et al., 2021), such that CGA can suppress the mucosa inflammation and cell apoptosis thus improves the intestinal development. In an in-vivo study in obese-induced mice, the CGA also exhibits anti-obesity characteristics and improves lipid metabolism of the mice, showing

the benefits of the CGA in improving the glucose metabolism (Iriondo-Dehond et al., 2019). Even though there is a reduction on the CGA content when the coffee beans undergo roasting, the availability of the compounds are there in a reasonable amount in ratio to the molecular weight of the CS (Moon et al., 2009; Mubarak et al., 2019).

$C_9H_8O_4$	+	$C_7H_{16}O_6$	$\longrightarrow$	$C_6H_{18}O_9$
(Caffeic acid)		(Quinic acid)		(Chlorogenic acid)

Reaction 2.1: Esterification of Chlorogenic acid. Catalyst by Chlorogenic acid O-hydroxycinnamolytransferase.

2.5.2 Caffeine

Caffeine (Figure 2.5) is a nervous system stimulant that is found in green coffee beans and is also present in CS. Notable that it is known for its ability to increase alertness and concentration, thus a synonym to a morning drink for most people and has also been shown to have antioxidant and anti-inflammatory effects. Currently, the caffeine compound being a target of interest due to its ability in the cosmetic fields as an active ingredient. The caffeine can exert the lipolytic activity in adipose tissue once penetrating the skin tissue barrier, because of this ability the caffeine is an important ingredient in cosmetic products against gynoid lipodystrophy or commonly known as the cellulite, a red to white coloured stretch marks on the skin (Bessada et al., 2018b).

Other than cellulite, caffeine is also well known for its ability to strengthen the hair, thus helping in fighting against baldness and hair fall. The compound can inhibit 5α-reductase (SRD5A2) activity which converts the testosterone into dihydrotestosterone (DHT) (Reaction 2.2), DHT hormones are bad for the hair follicle as it shortens the anagen phase of a hair cycle (Bessada et al., 2018b). Moreover, the effect of the caffeine in

preventing a UVB-induced carcinogenesis, inhibition of collagenase and elastase, and therapeutic affect due to photoaging by enhancing apoptosis in damaged cells, makes it a valuable component in the derma-cosmetic fields (Rodrigues et al., 2023).

$$\underset{\text{(Testosterone)}}{C_{19}H_{28}O_2} \xrightarrow{\text{SRD5A2}} \underset{\text{(Dihydrotestosterone)}}{C_{19}H_{30}O_2}$$

Reaction 2.2: Reduction of the testosterone to dihydrotestosterone by 5-alpha reductase enzyme.

Figure 2.5: Caffeine chemical structure.

2.5.3 Melanoidins

Melanoidins are a group of brown nitrogenous polymers that are formed as the product of the Maillard reaction (MR). As of now, the chemical structure of the compound is still largely undefined, due to the complexity of the products that are generated in the MR. The MR occurs between the carbonyl group of reducing sugars and the amino group of amino acids or peptides at a high temperature without the presence of enzymatic conditions (Iriondo-DeHond et al., 2021). Findings showed that, the melanoidins content are in proportional to the roasting conditions of the coffee beans as the products of the MR are responsible for the aroma, colour, and taste of different brewed coffee after roasting process

(Liang & Kitts, 2014). They are also present in CS and have been shown to have antioxidant, anti-inflammatory, dietary fibre effect, and prebiotic capacity properties (De La Cruz et al., 2019). Not only on coffee products, but the compound also existed widely in our foods due to the application of heat applied for cooking of these foods such as protein-based melanoidins which is predominant in bakery products. Nevertheless, the presence of melanoidins naturally in food products or even in the CS, synergistically works with phenol compounds to exhibit antioxidant benefits and antibacterial effect.

2.5.4 Flavonoids

Flavonoids are a group of phytochemicals that are abundant in plants or numerous plant-based foods, including CS. Flavonoids occur as methylated, glycosides, and the basic structure form; aglycone, it is usually divided into class of flavonoid and isoflavonoid according to the position of the benzenoid substituent (Kumar & Pandey, 2013). They have been shown to have antioxidant, anti-inflammatory, and anti-cancer effects. Besides that, Baba and Malik in 2015, also reported that flavonoid also exhibits antimicrobial activity, which is due to the inhibition of the nucleic acid biosynthesis and other metabolic processes in the microbe. This is not a surprise as the plant synthesized the flavonoids in response to any microbial infection in the plant. The flavonoids content of a plant often differs according to the species, part of the plant, and even the size of the plant. As an example, in the fermentation of soybeans and their processed products such as soy milk, tempeh, and miso, isoflavone is the predominant source of flavonoid from this type of food for human diet. During the fermentation process, the glycosides hydrolysed to aglycones giving off as high as 30-175 mg isoflavones/L of fresh soy milk (Manach et al., 2004).

2.5.5 Diterpenes

Besides that, diterpenes are a group of compounds that are found in coffee beans and are also present in CS. Specifically, Cafestol and Kahweol (Figure 2.6) are the two main types of coffee-diterpenes compounds that are known to give benefits to human health. In a study completed by Liang and Kitts (2014), Cafestol and Kahweol were able to be recovered from unfiltered coffee beverages. Not only that, diterpenes were detected in a post roasting process of the coffee beans due to the majority of the phenolic compounds were partially destroyed or bound to high molecular weight polymers such as melanoidins and diterpenes which responsible for the strong antioxidant properties of the coffee plant products including the CS (Nzekoue et al., 2020; Giordano et al., 2022). They have been shown to have multiple related interest to human health, such as the unsaponifiable fraction of Cafestol and Kahweol are known to be beneficial against UVB photoprotection, anticarcinogenic, antioxidant, and anti-inflammatory on the skin cells (Rodrigues et al., 2023).

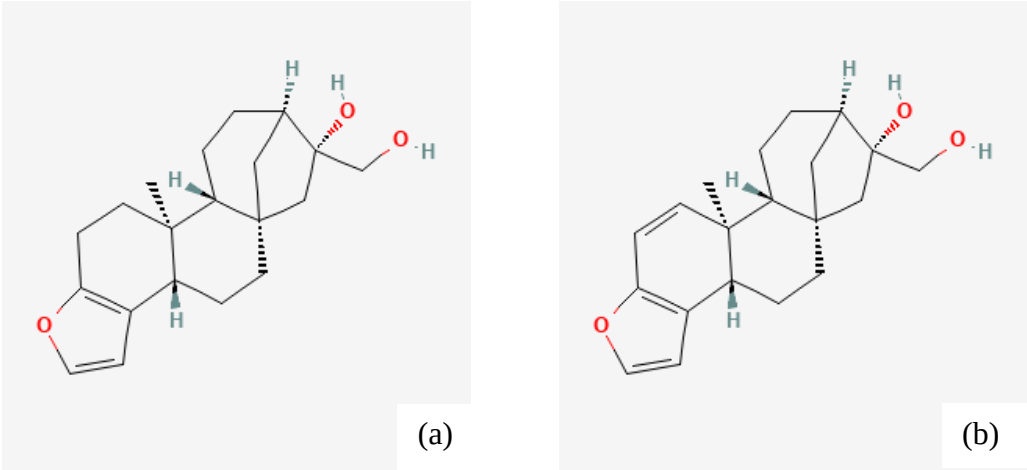

Figure 2.6: (a) Chemical structure of Cafestol **(b)** Chemical structure of Kahweol.

2.5.6 Dietary Fibres

Coffee silverskin is also a rich source of dietary fibre, including both soluble and insoluble fibres. The green coffee beans have been marketed as a dietary supplement due to

the fact it is high in fibre, this would suggest that CS can also be targeted to do the same. Dietary fibre has been shown to have a range of health benefits, including reducing the risk of heart disease, diabetes, and some types of cancer. From a study on antioxidants of coffee beans by Liang and Kitts (2014), a chronic hepatitis C patient was observed to have a reduction in oxidative damage due to the regular coffee consumption with restricted conditions for the study. In the study, the researcher conclude that the antioxidant effect of the dietary fibre was observed from the polyphenol compounds that was bound to the polysaccharide complexes, thus after consumed, was released in the gut, and functioning as an antioxidant agent (Liang & Kitts, 2014). Other than that, CS also has the potential to be beneficial to the intestine and the gut microbiota of humans. The dietary fibre is fermented in the gut by microbiota to produce short-chain fatty acids that contribute to human well-being, thus consumptions of CS effect the conditions of the intestine due to the prebiotic properties of the by-products (Iriondo-DeHond et al., 2019).

2.6 Recent Studies on Coffee and/or Coffee By-Product

In the coming year approaching this study, there are some emerging studies on coffee, and/or coffee by-product. However, most of the work in this field is aimed at investigating the Arabica and Robusta coffee species. For an instant, the study performed by Insanu et al. (2021), indicates a high and significant report for the coffee industries as they were reporting on the high availability of antioxidant activities level in Arabica green beans compared to the roasted beans. Moreover, Arabica and Robusta usually become the substrate of interest for comparison study, this statement can be proven by the study performed by Tan et al. (2016), as they extract and determine the antioxidant activity, phenolic content, and flavonoid content of the CS.

Even though there was comparative study between the coffee species with Liberica coffee such as by Ansori et al. (2021), they only targeted the spent coffee ground as the substrate of interest instead of the CS. In general, prior work is limited to a subset of studies focusing on the Arabica and Robusta coffee species as these two are the major coffee species planted in the world. Despite there being comparative studies with the Liberica coffee, these were focused on other by-product of the coffee production instead of the CS. As from the literature above proven that different species of coffee also affect the different constituent of the coffee plant parts and the by-product.

2.7 Origin Differences of Coffee Silverskin

The current study focused on the Liberica CS originate or indigenous to the Sarawak state. On the prospect of the Liberica coffee, few known research currently has been performed such as the studies by Ismail et al. (2014), Nillian et al. (2020), and Alnsour et al. (2022). Despite that, the study on the Liberica variety only focused on the beans and the pulp of the beans. Even if there were studies on CS, all of them targeted on the Arabica and Robusta CS (Table 2.1).

Despite that, a study by Bessada et al. (2018b) previously had showed that sample of Robusta CS but different region origin, had a significant different ($P < 0.05$) especially on the total phenolic content and antioxidant activity. The Robusta CS extracts from the region of Brazil and Indonesia had a higher TPC and antioxidant activity when compared to the India region in the ratio of Brazil: India (2:1) and Indonesia: India (4:1). This conclusion however, disputed by Alnsour et al. (2022) such that, the findings in the study pointed out on the CGA being the determining factor on the antioxidant level rather than the origin. Noted that the findings do display a slightly higher total phenolic content of coffee beans roasted from Colombia than Kenya, Ethiopia, and Brazil.

The findings by Bessada et al. (2018b) are concurred by Insanu et al. (2021), such that the study highlighted the roasted Liberica coffee bean from Aceh is the highest in terms of TPC and TFC, 22.59 ± 1.6 g/100 g and 4.93 ± 0.36 g/100 g, respectively, in comparison to the beans from Riau and Jambi, Indonesia. The comparison studies in this previous research, had showed a distinguish differences in the antioxidant profiles of coffee beans and silverskin from different geographical origin, thus justify the aim of this study to investigate the Liberica CS from Sarawak region, as this study fill in a gap in the coffee by-products knowledge by ignites more in-depth studies on the CS of the Liberica.

Table 2.1: Caffeine content comparison with current study

Coffee silverskin varieties	Method of extraction	Caffeine content		Source
		mg/g	%	
Liberica	SLE	19.23 ± 6.51-26.86 ± 5.77	1.55 ± 0.47-1.92 ± 0.65	Current study
Arabica mix Robusta	SLE	17.45 ± 0.69	N. R	Martuscelli et al., 2021
Arabica	UAE	N. R	1.00-3.59	Nzekoue et al., 2020
Arabica	UAE	41.88 ± 2.36	N. R	Zengin et al., 2020
Arabica	UAE	32.7 ± 1.0	N. R	Wen et al., 2019
Arabica	SLE	N. R	3.5 ± 0.2	De La Cruz et al., 2019
Arabica	SLE	12.43 ± 0.21	N. R	Xuan et al., 2019
Robusta	SLE	6.76 ± 0.05 – 12.15 ± 0.11	N. R	Bessada et al., 2018b
Arabica mix Robusta	SLE	0.91 ± 0.09	0.8-1.0	Toschi et al., 2014
Arabica	SLE	10.0 ± 1.1	N. R	Bresciani et al., 2014

Note. N. R – not reported.

Plants and plant materials such as fruits, leaves, cherries, bark, and even by-products produce secondary metabolite such as polyphenols, terpenes, and methylxanthines. These are some of the secondary metabolites that are categorised under the three main classifications which are phenolic, terpenoid, and nitrogen/sulphur-containing compounds, respectively (Pott et al., 2019). These metabolites in fruits carry out most of the fruit functional attributes in a plant, this includes the attractiveness to pollination of the fruit and its resistance towards the pathogens and plant diseases. Nonetheless, the secondary metabolites are the compounds which are responsible for the unique aroma of the fruits including the coffee beans and its beneficial effects towards the health of human when consumed (Pott et al., 2019). The variability in the origin of the coffee variety including the coffee by-product also affects the secondary metabolite contents, hence directly influences the value of the coffee variety.

Moreover, the origin of the coffee beans also related to the human activities, such that post-harvest handling of the green coffee beans, and the roasting of the green beans itself which would affect the metabolite content in the beans. Haile and Kang, in 2019 mentioned that the post-harvest processing or handling of the green beans is contributing to approximately 60% of the bean's quality. During the roasting process for instant, the duration of the roasting will affect the degree of roasting which coherently affects the Maillard reaction and contributes to the different in the aromas and the taste of the beans (Iriondo-DeHond et al., 2021). The aroma and the brownish colour of the beans are due to the melanoidins compounds which proven the effect of the post-harvest activities. In conclusion, different location may treat their beans differently thus providing different results in the metabolite content.

2.8 Current Utilisation of Coffee Silverskin from Arabica and Robusta Coffee.

According to Bessada et al. (2018a), consumers generally prefer natural antioxidants products compared to synthetic ones. This can be observed from the increasing demands for this environmentally sustainable resource cosmetic products. Recently, it has been reported that CS is a rich source of antioxidants constituents due to the presence of phenolic, flavonoids, and alkaloids compounds that worked synergistically (Alnsour et al., 2022), not to mention the availability of the bioactive compounds such as fibre, minerals, and caffeine (Bessada et al., 2018a). In a study by Iriondo-DeHond et al. (2016), the application of the CS extract on human skin cells could prevent the effects causes by oxidative stress damage, as there are compounds present in the CS that can protect the cells from UV-induced photodamage. Overcoming the damage done by the UV ray is one of the most effective ways of decreasing the effects of photoaging.

Moreover, Machado et al. (2020) reported on the CS having potential to be utilised in food products and food addictive as it also shows promising source of relevant amino acids in helping the improvement of cognitive and physical attribution. The sum of all amino acids in an analysis performed by them reached approximately 9% of the CS content. As the CS is the by-product of the coffee roasting, the sample was subjected to a high temperature roasting, this process causes the Maillard reaction in the coffee bean (Reaction 2.3). From the reaction, high content of melanoidins was formed as one of the main constituents in the CS alongside caffeine and fibre. Melanoidins compounds were reported to have the potential to be used as a functional food ingredient, thus exploiting the usage of the CS in food industries, as it has been classified as antioxidant dietary fibre previously (De La Cruz et al., 2019). There was also a study by Gocmen et al. (2019), whereas the addition of the CS into cookies for baking had not affected the outcome of the cookies compared to the control,

except for the distinctively bitter taste, which however are quite subjective to the sensory testers. Overall, the marks given to the cookies in general acceptance does not significantly differ from the control cookies, except that the CS added cookies are proven to be higher in antioxidant capacity and beneficial phenolics, thus increasing the beneficial nutrition for the food.

Not only that, but there are also reports on study as the CS is developed into a lignocellulosic waste filler for high-density polyethylene (HDPE) composite (Hejna et al., 2021). Based on this study, the waste filler has an increase in thermo-oxidate resistance compared to the conventional waste filler. Other than that, the CS also developed in polylactic acid (PLA)/ polybutylene succinate (PBS) composites. The addition of the CS into the PLA and PBS composites would increase the elastic modulus of the materials while retain the thermal stability and hydrophobicity characteristics (Ghazvini et al., 2022). Other than that, CS serves as a feedstock for antifungal chemicals in wood preservation due to its antifungal properties. Even though the reported CS addition may not be performing as a commercial wood preservative, the ecotoxicity of the silverskin was significantly lower and better for the environment (Barbero-López et al., 2020). In addition, the CS was also studied to produce an alternative carbohydrate source in butanol biorefineries. This was done via in-vitro acetone-butanol-ethanol (ABE) fermentation of the CS, to act as a feedstock for the *Clostridium* genus, through this fermentation, the bacteria will produce biobutanol biomass (Procentese et al., 2018; Hijosa-Valsero et al., 2018).

Recent studies over the years shows trend such as utilizing the waste by-product in cosmetics, functional foods, and industrial application in the sense of managing it properly instead of just discarding or combustion (Gocmen et al., 2019; Zengin et al., 2020; Hejna et al., 2021; Ghazvini et al., 2022). By comparing to earlier study on the utilisation of the CS,

the approach was more on novel discovery of the CS potentials and beneficial bioactive compounds description (Narita & Inouye, 2012; Bresciani et al., 2014). In short, multiple studies have shown and supports that the CS of the coffee waste by-product is beneficial source of substrate to be investigated further as this compound may help create a new stream of revenue while helps managing the output of the CS waste into a proper value-added product.

2.9 Potential Prospect of Coffee Silverskin from Arabica and Robusta Coffee.

As current studies on CS show promising in the utilisation of the waste, there are also some potential prospects for it as well. Studies have shown that CS is a rich source of antioxidants, including polyphenols, chlorogenic acids, and melanoidins, which have been found to exhibit anti-inflammatory, anticancer, antidiabetic and antibacterial properties (Bessada et al., 2018a; Klingel et al., 2020; Angeloni et al., 2020). CS can be future exploits in the pharmaceutical field as study has shown that the CS originated from roasting of Robusta coffee species exhibit antimicrobial properties when tested against *Staphylococcus aureus* bacteria (Cendekia et al., 2020). From the study, the zone of inhibition of the sample extract tested shows similarity to the positive control of the study, thus deducing the ability of the CS extract to inhibit microbial growth.

This however contradicts with the study made by Nillian et al. (2020), as they had executed a Kirby-Bauer disk diffusion methods on the coffee pulp of the Liberica coffee sp., but no antibacterial activity of the coffee pulp extract against a Gram-positive and a Gram-negative bacteria observed as there are no zone of inhibition appears on the test media. As there are no known studies done on the CS of the Liberica variety, this study will be the primary data on antimicrobial that can be found regarding the CS of Liberica variety.

In relation to that, there is also proof of studies regarding the utilisation of the CS in treatment of Type-2 diabetes complication. Diabetes mellitus is a metabolic disorder such that the body does not create and perform insulin secretion properly or insulin deficiency and usually characterised as hyperglycaemia (Iriondo-DeHond et al., 2019). The CS which are known to have beneficial proteases from the oxidation of polyphenols and phenol oxidases can act as inhibitor of key enzymes that are involved in lipid and carbohydrates metabolism. This by chance can reduces blood sugar levels, and act as a supplement to the conventional drugs during the treatments (Nolasco et al., 2022). This finding concurred with the study performed by Zengin et al. (2020) such that Type-2 diabetes can be inhibited by caffeic and CGA contained in the CS. To further support, a study conducted using model rats showed significant result as the rats were administered with CS extract daily before injection with a diabetes inductor and were able to reduce the pancreatic oxidative stress thus preventing the rats from developing diabetes (Iriondo-DeHond et al., 2019).

Recent investigations have demonstrated that the CS also has the potential to be used in terms of environmental view of interest. The CS can be used as biochar in fertilisers, as the CS also contains nitrogen and phosphorus besides other beneficial compounds, which are crucial for the growth of a plant. This was displayed by the study performed by Machado et al. (2020), such that the total nitrogen content of the CS was measured via the total real protein content, and thus showed approximately 12% of the fresh weight represent the real protein content. This also supported by Martuscelli et al. (2021), stated that the CS have high contents in mineral such as nitrogen and phosphorus with up to 15.4% of the total CS. With CS utilised as the biochar, it can fill the spaces between the raw materials and reduce heat losses during composting, not to mention the increase of microorganisms' activity and

proliferation due to the increase in aeration, nutrient retention, water, and toxin adsorption (Picca et al., 2022).

These benefits for the CS to be channel back to the environment as soil supplement or fertiliser supplement, may need to be reassess as some reports does mention the present of toxic bio compound such as Ochratoxin A (OTA). however, this primarily influenced by the preparation and storage of the CS before subjected to testing or utilisation but also by roasting (Narita & Inouye, 2012; Toschi et al., 2014). There is also an agreement that the amount of the toxins may only be at a minimum amount set by the European Commission, a maximum weekly intake of 120 ng/kg body weight was derived for OTA, and maximum content for ground and roasted coffee is 5 μg/kg, while soluble coffee is 10 μg/kg (European Food Safety Authority, 2010). Although there is no threshold amount set by the European Commission on the OTA in CS, the amount of OTA detected in the by-products of the coffee was in the range of 2.7 – 4.3 μg/kg (Klingel et al., 2020), thus providing an acceptable suggestion for the CS to be added as bio-fertiliser, as this amount of OTA does not affect the soil toxicity. But still, preliminary study on the toxicity should be tested before jumping to any deduction.

2.10 Plant Extraction Method

The method of plant extraction plays a crucial role in obtaining the targeted or non-targeted beneficial compounds. In this study, the method of conventional extraction was performed to initiate the analysis on the Liberica CS. The conventional method, maceration is known to be the simplest method for plant extraction such that, the design of the experiment only required basic vessel or container for operation. That said, there are no specific set of skills required by the researchers to employ this method. Not only that, as this

method needs only the basic set-up for the design, the cost for this method is also generally lower compared to UAE method. Besides, when implementing the SLE, the method is more suitable for high temperature sensitive compounds that may undergo thermal degradation such the polyphenol compounds, which responsible for the antioxidant properties of the plant extracts (Rasul, 2018; Antony & Farid, 2022).

Another conventional way that was used to extract the plant sample is the Soxhlet apparatus method (Figure 2.7). This method was created by Franz Ritter von Soxhlet, in the year of 1879. The method was first introduced for fats separation in milk. However, due to its automated mechanism, the method remains popular and important in the chemistry and biochemistry industries. This method usually can be used to extract plant material on a large amount at one time, as the mechanism can hold more volume of solvent compared to the maceration. Moreover, the extraction method is considered as a self-independent mechanism, such that less monitoring needs to be done. The solvent is enclosed in a continuous system which will repeatedly interact with the solute or sample in the extraction chamber. Thus, the sample will be fixed in the extraction cellulose thimble, leaving only the solvent plus extract to return to the round-bottom flask. Hence, this method does not need a filtration step after the extraction and can proceed to the drying of the extract directly when done (Rasul, 2018).

This method was employed with the maceration process due to some limitation of the maceration method, such as time consuming and less dried sample can be extract at one time. Despite that, the Soxhlet method also had its downside such as the method involved a long and high exposure of the sample to temperature, thus increasing the probability of thermal degradation on heat labile compound (Rasul, 2018). Nonetheless, both methods were utilised to their availability for this study.

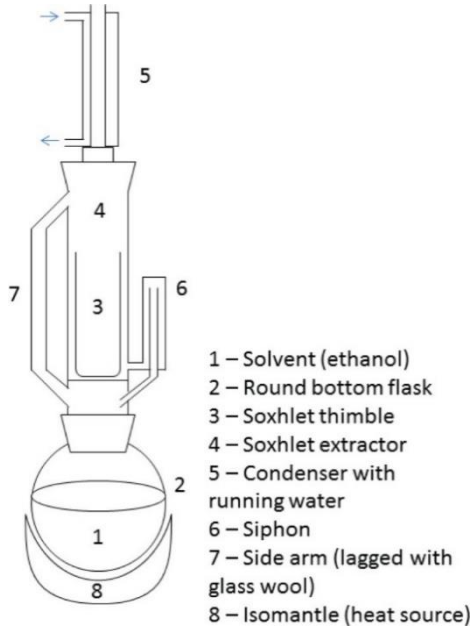

Figure 2.7: Soxhlet apparatus set-up

Note. From "Using Soxhlet Ethanol Extraction to Produce and Test Plant Material (Essential Oils) for Their Antimicrobial Properties", by J. Redfern, M. Kinninmonth, D. Burdass, and J. Verran, 2014, *Journal of Microbiology and Biology Education, 15*(1), p. 45-46. Copyright 2014 by the American Society for Microbiology. Reprinted with permission.

2.11 Chapter summary

This chapter discuss the Liberica coffee scene in Malaysia particularly in Sarawak region. Next, this chapter touched briefly on the Liberica coffee background and history of its emergence to the South-east Asia region. Moreover, the beneficial constituent of the coffee beans was reviewed within focal point shift towards the compounds in the coffee silverskin of the Liberica coffee variety. Several known beneficial bioactive compounds also put in review from previous studies, as there are several stand out compounds such as the caffeine, chlorogenic acid, diterpenes, melanoidins, flavonoids, and dietary fibres. Lastly, we also review on the current and future potential of the utilisation of the Liberica CS in term of becoming a value-added product.

CHAPTER 3

METHODOLOGY

3.1 Phytochemical Properties of Sarawak Liberica Coffee Silverskin

3.1.1 Sample Preparation

In collaboration with a local coffee company, the Sarawak Liberica CS sample was obtained from Reka Jaya Plantation Sdn Bhd, an industrial partner in Kuching, Sarawak. Liberica CS was collected directly from the roaster and grounded using a basic blender to break down the CS further into smaller sizes (1-10 mm) in diameter, to maximise extraction. Then, 1 g of CS was macerated with 20 mL of solvents methanol, ethanol, and distilled water inside a 50 mL conical flask, respectively. The mixture was then placed in the incubator shaker for 30 min at 60–65 °C. After completed the extraction run, the extracts were centrifuged (Benchmark Scientific, LC-8) for 15 min at 350 rpm, and the supernatant was filtered using the Whatman No. 1 paper filters (0.45µm). The extracts were then concentrated using the rotary evaporator (IKA RV8) for a few minutes depending on the solvent used. After that, the dried weight of the CS extracts was weighted and recorded. The extracts were then stored at 3 - 5 °C inside an amber vial, for further analysis. The extracts re-diluted in its constituent solvents (ethanol, methanol, distilled water) to make 1 mg/mL (w/v) concentration for further analysis (Ballesteros et al., 2014).

3.1.2 Total Phenolic Content

The Folin-Ciocalteau technique (Utami et al., 2013) was used to estimate the total phenolic content (TPC). In a brief, 0.75 mL of the Folin-Ciocalteau reagent was combined with 0.1 mL of the extracts respectively (1 mg/mL) and allowed to react for 5 min. Then, an aliquot of 0.75 mL of Na_2CO_3 6% (w/v) solution was added into the mixture and was left for

90 minutes in the dark and at room temperature. The absorbance of the extracts at 725 nm was measured using a UV-VIS spectrophotometer (Shimadzu UV-1900i, Shimadzu Corp, Japan). The procedure was performed in triplicate to obtain a mean reading for significant analysis. The calibration curve for gallic acid (Merck, Germany) obtained was $R^2 = 0.998$ within a linearity range of 0.2 to 1.0 mg/mL. The linear equation obtained was $y = 0.5002x - 0.0211$ (Figure 4.1). The results were expressed as milligram of gallic acid equivalents per gram of CS (mg GAE/g).

3.1.3 Total Flavonoid Content

According to Rodrigues et al. (2016) , total flavonoid content (TFC) can be measured using the colourimetric assay. In a brief, 0.075 mL of 5% $NaNO_3$ solution (w/v) was mixed with 0.125 mL of the CS extracts as described in 3.1.1 (1 mg/mL), the mixtures were left for 6 min in dark room temperature. The mixtures were then added with 0.15 mL of 10% $AlCl_3$ (w/v). The final volume of 2.5 mL was then reached by adding 0.75 mL of NaOH (1M) and 1.4 mL of distilled water into the mixtures. After five minutes of incubation in dark room temperature, the absorbance of the mixtures was then measured at 510 nm via the UV-VIS spectrophotometer (Shimadzu UV-1900i, Shimadzu Corp, Japan). A quercetin (Merck, Germany) calibration curve was employed with linearity range of 0.2 to 1.0 mg/mL to obtain a linear equation of $y = 0.0785x + 0.00002$ and correlation coefficient (R^2) of 0.99 (Figure 4.2). The results were reported as milligram of quercetin equivalents per gram of dried CS weight (mg QE/g).

3.1.4 2,2-diphenyl-1-piccrylhydrazyl (DPPH) Assay

DPPH's radical-scavenging activity (RSA) were used to measure the extracts' antioxidant activities. A 1.0 mL of DPPH solution was added into a range concentration of the sample (0.2 - 1.0 mg/mL). After 35 min in the dark, the absorption was read at 515 nm

(Rodrigues et al., 2016). A calibration curve of ascorbic acid with a linearity range of 2 – 10 µg/mL and R^2 = 0.99 was prepared, while the inhibitory concentration needed to decrease the initial DPPH by 50% (IC_{50}) was obtained from the linear equation obtained. The results were expressed as microgram of ascorbic acid equivalent per gram of sample (mg AAE/g). The RSA was calculated in percentage according to the following equation:

$$RSA\% = \frac{Abs_{Control} - Abs_{Sample}}{Asb_{Control}} \times 100 \qquad \text{Equation 3.1}$$

Where $Abs_{Control}$ is the control's absorbance reading, while Abs_{Sample} is the sample's absorbance reading at 515 nm, respectively.

As for the DPPH value, the calculation of the ascorbic acid equivalent per gram of the sample (mg AAE/g) was done as following equation:

$$DPPH\ value\ (\text{mg AAE/g}) = \left(\frac{IC_{50}(control)}{IC_{50}(sample)}\right) \times \left(\frac{C}{W}\right) \qquad \text{Equation 3.2}$$

Where IC_{50} can be obtained from the linear equation generated by the RSA against concentration graph, C is the initial concentration (mg/mL) of the control (ascorbic acid), and W is the dry weight (g) of the CS extract.

3.1.5 Ferric Reducing Antioxidant Power (FRAP) Assay

For the FRAP assay, according to (Benzie & Strain, 1996) 3 mL of the FRAP reagent (10:1:1 of 300 mM sodium acetate buffer at pH 3.6, 10 mM TPTZ, and 20 mM $FeCl_3$) were combined with 0.1 mL of a range of sample concentrations (0.2 - 1.0 mg/mL), the mixture was then incubated at 37 °C for 20 min. The increase in absorbance at 592 nm was read using a spectrophotometer. It was calibrated using a $FeSO_4$ with linearity range of 1.32 – 6.57 µmol/mL and R^2 = 0.99. The results were expressed in terms of micromole ferrous

sulphate equivalents per gram of sample (µmol FSE/g). The FRAP value was calculated according to the equation:

$$FRAP\ value\ (\mu mol\ FSE/\ g) = C \times V \times \frac{t}{m} \qquad \text{Equation 3.3}$$

Where C is the $FeSO_4$ concentration (µmol/mL) of the corresponding standard curve of the CS sample, V is the CS sample volume (mL), *t* is the dilution factor, and *m* is the weight of the CS dry matter (g).

3.2 Bioactive Compounds Composition

3.2.1 Sample Preparation

The CS was obtained from Reka Jaya Plantation Sdn Bhd, after undergoing roasting process. The CS was first grounded using a pestle and mortar to increase the surface area of the sample and maximise compound extracted when in contact with the solvents. The CS then extracted using the Soxhlet apparatus with organic solvent ethanol and methanol based on study by Nillian et al. (2020). For the Soxhlet extraction, 10 g of the grounded CS was inserted into a thimble (33 mm × 118 mm, Whatman, GE Healthcare Life Sciences), then 200 mL of the solvents, respectively was poured into the round bottom flask and placed on the heating mantle. The temperature on the mantle was set to range 78-80 °C. The extraction was allowed to run until the solvent appeared colourless in the apparatus or for maximum of 8 hr. After completing the run, the collected extract in the solvent was concentrated using the rotary evaporator (IKA RV8). Next, the dried extracts were weighted and transferred to an amber vial for storage and keep in temperature 4 °C, before proceeding to analysis. The dried sample was resuspended in GCMS vial with dichloromethane (DCM) to make concentration 15 mg/mL for analysis.

3.2.2 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.s, 0.25 mm film thickness, BPX-5 fused-silica capillary column containing 5% phenyl polysilphenylene-siloxane (Trajan Scientific and Medical, Australia) was employed. With a scan mass range of 28–400 m/z and an electron impact ionization energy of 70 eV, the interface temperature was fixed at 250 °C. The oven was preheated to 40 °C for 1 min, then raised to 220 °C at a rate of 5 °C per minute, total run time was 37 min. 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 splitting ratio of 20:1 was applied. The chemical constituents of the CS extract were identified by comparing their mass spectra with those of National Institute of Standards and Technology 2017 (NIST-17) mass spectral library incorporated in the data system to identify its name, molecular weight, and structure.

3.3 Caffeine Content

3.3.1 Determination of Caffeine Content via GC-MS

The standard curve was made by calibrating standards over a known range concentration of 25 - 100 µg/mL of caffeine (1,3,7-Trimethylxanthine) solution. By plotting the peak area to the caffeine concentration on the chromatogram, the calibration curve's slope and *y*-intercept were calculated (Adams et al., 2010). An acceptable linearity was obtained with correlation coefficient (R^2) of 0.97. The linearity equation for the calibration curve was y = 31903x – 253622, where y value is the intercept, while x value is the slope of the curve. The caffeine content was reported in mg/g of dry basis, and percentage of caffeine

content per extract concentration. The caffeine content percentage was calculated as below equation:

$$Caffeine\ \% = \frac{C_e}{M} \times 100 \qquad \text{Equation 3.4}$$

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

3.3.2 Determination of Caffeine Content via UV-VIS Spectrophotometer

3.3.2.1 Sample Preparation

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

3.3.2.2 Caffeine Extraction

The CS extract was then transferred into the separatory funnel for caffeine separation and extraction (Vuletić et al., 2021). Two gram of sodium bicarbonate was added into the extract in the funnel and 1:1 (v/v) ratio of extract to chloroform ($CHCl_3$) was used as the separating agent of the caffeine compound. The mixed solution was shaken gently and inverted 2-3 times, to allow maximum contact of the chloroform with the extract and obtain a maximum amount of caffeine from the extract. After letting the mixture to rest for a few minutes, the bottom layer of the separation was removed precisely to avoid the top layer. An

aliquot of 100 µL taken from the separated bottom layer and added with 10 mL of chloroform before reading with UV-VIS Spectrophotometer at 274 nm wavelength (Shimadzu UV-1900i, Shimadzu Corp, Japan).

3.3.2.3 Calibration Curve

A standard curve was plotted with caffeine standard (1,3,7-Trimethylxanthine). In brief, 10 mg of caffeine was diluted in 100 mL of chloroform to make stock concentration of 100 ppm. The stock solution was then diluted to 5-point in manner of 2-fold dilution to make concentration of 3.125 - 50 ppm. The absorbance of the range was measured using the UV-VIS Spectrophotometer at 274 nm (Shimadzu UV-1900i, Shimadzu Corp, Japan), while the calibration curve was plotted on the concentrations of the caffeine (Merck, Germany) standard against the absorbance value and linearity equation obtained was $y = 0.0002x + 0.0081$, with correlation coefficient (R^2) value of 0.93. The caffeine content is reported as mg/g of caffeine per dry basis of CS, and percentage of caffeine content based on the extract concentration. The percentage caffeine content was calculated as Equation 3.4.

3.4 Antimicrobial Susceptibility Testing

3.4.1 Sample Preparation

The CS extraction was performed according to the method by Vuletić et al. (2021) as in 3.3.2.1. The dried extracts were then resuspended in dimethyl sulfoxide (DMSO, 2.5%) for further AST analysis.

3.4.2 Inoculum Preparation

To test the antimicrobial activity, two test bacteria which are Gram-positive (*S. aureus* ATCC 25923) and Gram-negative (*E. coli* ATCC 25922) were obtained from the microbiology laboratory culture collection, Faculty of Resource Science and Technology (FRST), UNIMAS. For each experiment, both bacteria were subculture in Nutrient broth

(NB) (Difco Laboratories, Detroit, MI) to assess culture viability. The cultures were left for overnight at 37 ℃. The turbidity of the suspension was adjusted to 0.5 McFarland standard concentration and use within 15 min.

3.4.3 Kirby-Bauer Disk Diffusion

The antimicrobial activities were tested according to Clinical and Laboratory Standards Institute (CLSI), M2-A9 document (Clinical and Laboratory Standards Institute, 2020). Briefly, 3-point dilutions of CS extracts were performed with DMSO. CS concentrations ranging from 5 - 20 mg/mL were tested, by impregnated onto an empty Whatman 6 mm testing disk (GE Healthcare Life Sciences). Inoculum from previous step (3.4.2) was swabbed 3 times onto a Mueller Hinton Agar (MHA) and left to dry for few minutes. Then, the test sample together with the positive control (Gentamicin 10 µg, Oxoid, United Kingdom) and negative control (DMSO, 2.5%) were placed onto the MHA with spaces approximately >24 mm from middle to middle of each consecutive disks. After 18 hr of incubation at 35 ± 2 °C, bacterial growth was visually compared for each concentration and zone of inhibition was measured using a ruler. All determinations were performed in triplicate for each assay to obtain a mean average result. For result was reported as either susceptible (S), Intermediate (I), or Resistant (R) based on the interpretation chart by CLSI.

3.5 Data Analysis

All data was collected and analysed using the Excel for Microsoft Office 365, version 2021. In this study, the methods were performed and collected in triplicate to obtain the mean average and distribution of the data deviation. The one-way ANOVA test was performed on the data, to test the significant of the results, follows with Tukey's Honest Significant Difference (HSD) post-hoc test.

CHAPTER 4

RESULTS

4.1 Total Phenolic and Flavonoid Content

Based on Table 4.1, the methanol extract showed an exceptionally high amount of phenolic (15.24 ± 0.65 mg GAE/g). This is then followed by ethanol (11.2 ± 0.12 mg GAE/g), and water (9.48 ± 0.32 mg GAE/g) extracts.

Table 4.1: Total phenolic content and total flavonoid content of Sarawak Liberica coffee silverskin extracts

Solvent	TPC (mg GAE/g)	TFC (mg QE/g)
Water	9.48 ± 0.32**	6.45 ± 2.79**
Methanol	15.24 ± 0.65*	21.05 ± 4.28*
Ethanol	11.2 ± 0.12*	25.14 ± 0.59*

Note. All data is expressed as mean ± standard deviation. '**' represent significantly different at 0.05. While '*' represent not significantly different at 0.05.

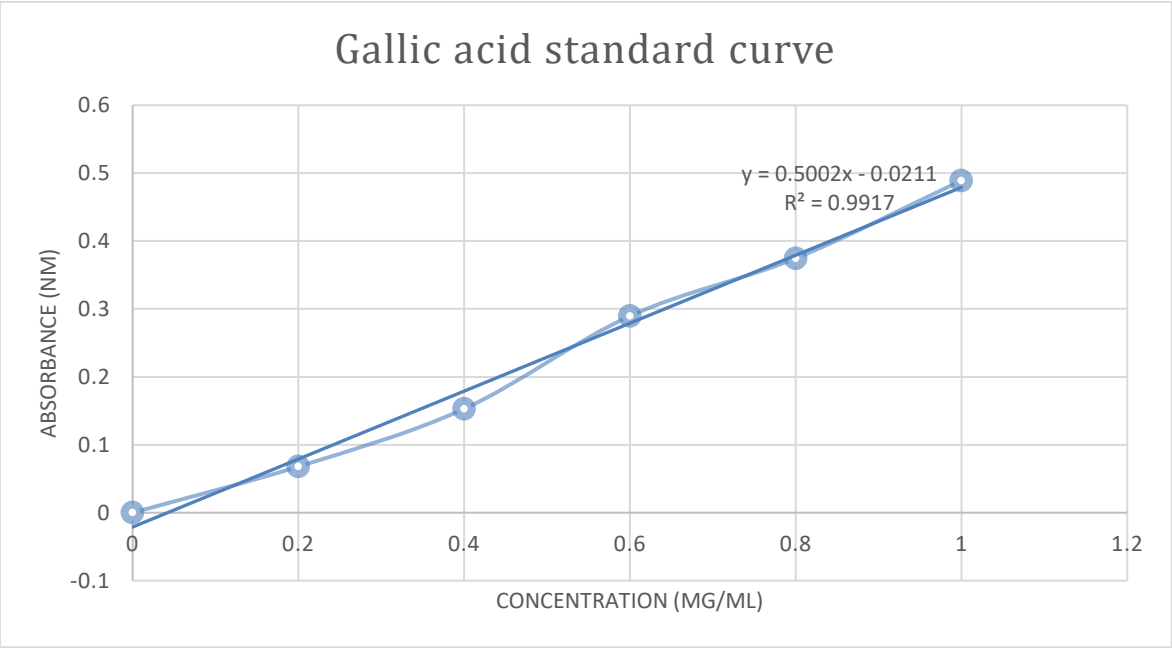

Figure 4.1: Gallic acid standard curve.

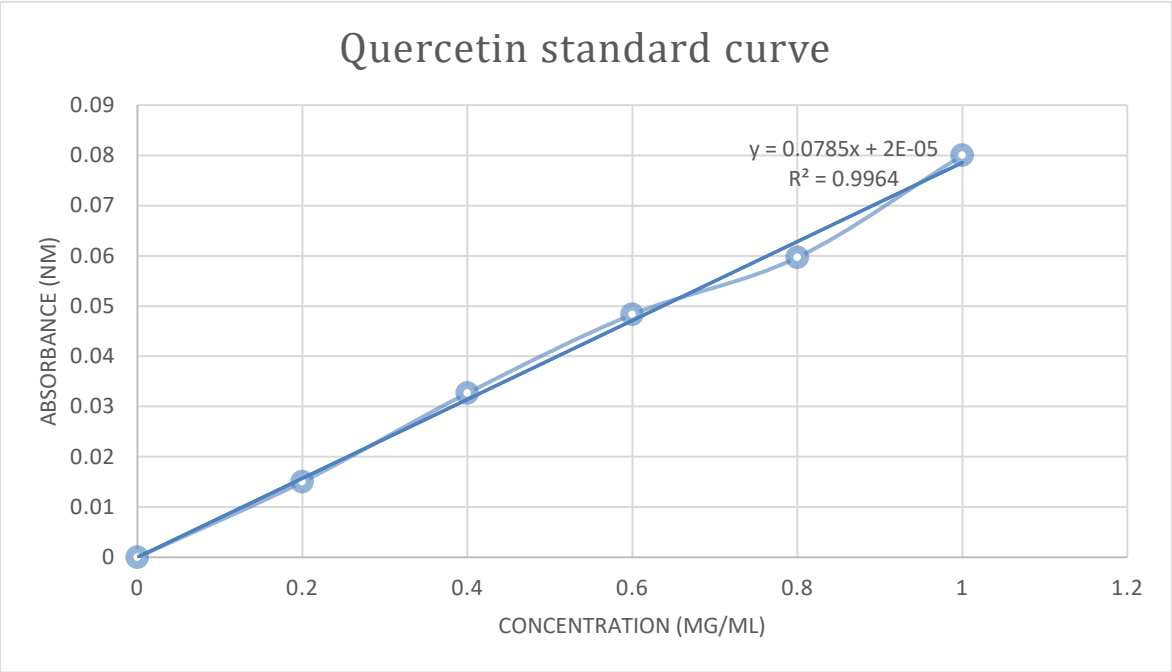

Figure 4.2: Quercetin standard curve.

4.2 Antioxidant Activities

The RSA values were calculated using Equation 3.1, while the value of the ascorbic acid equivalents was calculated as Equation 3.2. The CS extract was allowed to counteract the DPPH free radical by donating hydrogen at concentrations ranging from 0.2 - 1.0 mg/ml. Based on Table 4.2, the IC_{50} was calculated from the regression line of RSA (%) against concentration (mg/mL) of each sample. The IC_{50} of the CS extract in ethanol solvent is the lowest with 0.40 ± 0.004 mg/mL. This is followed by methanol and lastly water extract with 0.59 ± 0.01 mg/mL, and 0.81 ± 0.03 mg/mL, respectively. The DPPH scavenging activity in this study was correlated directly proportional with the antioxidant of the sample.

Table 4.2: Antioxidant activity of Sarawak Liberica coffee silverskin extracts

Sample	RSA (%)	IC_{50} (mg/mL)	DPPH value (mg AAE/g)	FRAP value (µmol FSE/g)
Water	62.84 ± 2.98**	0.81 ± 0.03**	0.78 ± 0.03**	2.50 ± 3.98**
Methanol	78.77 ± 0.99*	0.59 ± 0.01*	1.81 ± 0.03*	11.14 ± 17.83*
Ethanol	83.85 ± 1.78*	0.40 ± 0.004*	2.60 ± 0.02*	11.40 ± 18.57*

Note. All data is expressed in mean ± standard deviation. '**' represents a significant difference at 0.05. While '*' represents not significantly difference at 0.05.

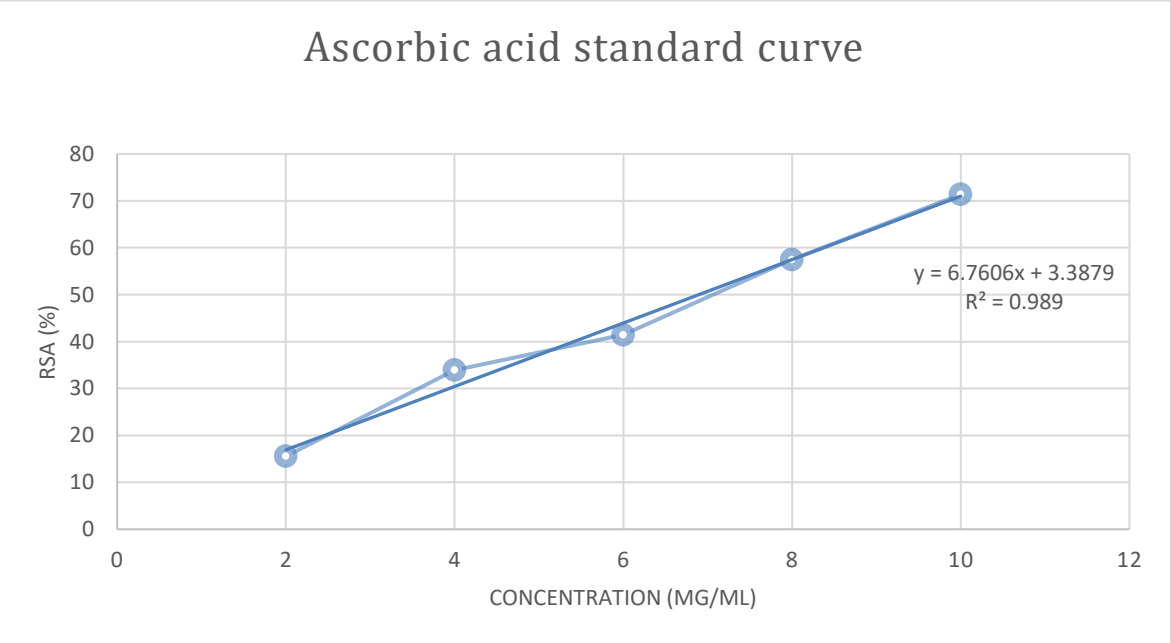

Figure 4.3: Ascorbic acid standard curve.

The trend in RSA was plotted in Figure 4.4. From the graph plotted, the scavenging activities of ethanol extract was higher from concentration 0.2 - 0.8 mg/mL, compared to the methanol and water extract. The scavenging percentage is significantly different ($P < 0.05$) between the alcohol and the aqueous extracts. This shows that the antioxidant ability of the extracts (methanol and ethanol) was much more rapid in comparison to the water extract. Based on the RSA graph, the plateau region of the ethanol extract appeared approximately at concentration 0.8 mg/mL, comparing to the methanol and water extracts. The earlier onset of the plateau region indicates the maximum capacity of the extract to scavenge free radicals, thus further increase in the concentration does not significantly increase the RSA. Earlier

onset of the plateau region represents a higher RSA capacity of the extract against DPPH radicals (Nabavi et al., 2010).

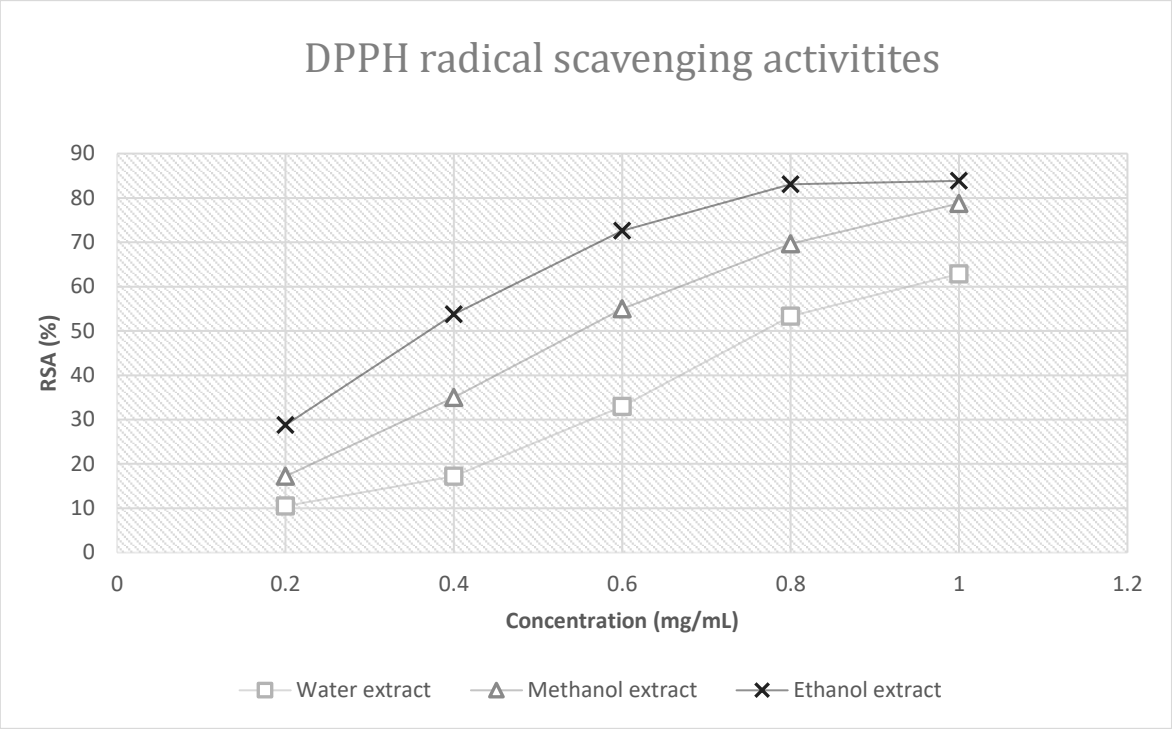

Figure 4.4: Radical scavenging activities of Liberica coffee silverskin extracts

Note. Range of concentration tested 2 - 10 µg/mL. Ascorbic acid is the standard used as control.

Alluding from Table 4.2, it showed that the FRAP value of the CS sample is the highest when extracted with ethanol solvent with value 11.40 ± 18.57 µmol FSE/g. This then followed by methanolic extract sample which were not that significant of a difference (P> 0.05) comparing to the ethanolic extract with value of 11.14 ± 17.83 µmol FSE/g, but still considered lower than the FRAP value by methanol. Lastly water extract with value 2.50 ± 3.98 µmol FSE/g. The FRAP value was calculated as in Equation 3.3. The P-value is lower than 0.05, hence, the FRAP value is significantly different between extracts such that, the difference in type of solvent is notable in affecting the capability of the samples to reduce ferric into ferrous (Fe^{2+}) ions.

Different standard used to calibrate will give different value of FRAP, such as shown in Benzie and Strain (1996) as they investigated different possible standard that be used a standard compound for references and different standard showed different value of FRAP. This also supported by research completed by Ansori et al. (2021), as there was 24.41 ± 0.49 mg TE/g of FRAP value estimated from Liberica coffee ground spent. In current study, the result of the Liberica CS shown only as high as 11.40 ± 18.57 µmol FSE/g of FRAP value when compared to standard $FeSO_4$ instead of using Trolox. The results also show distinguish difference of result as different parts were tested from the Liberica coffee plant. However, both current results and previous study show agreement in such that there is reduction of ferric. Thus, supporting the ability of the Liberica CS as a potential antioxidant agent.

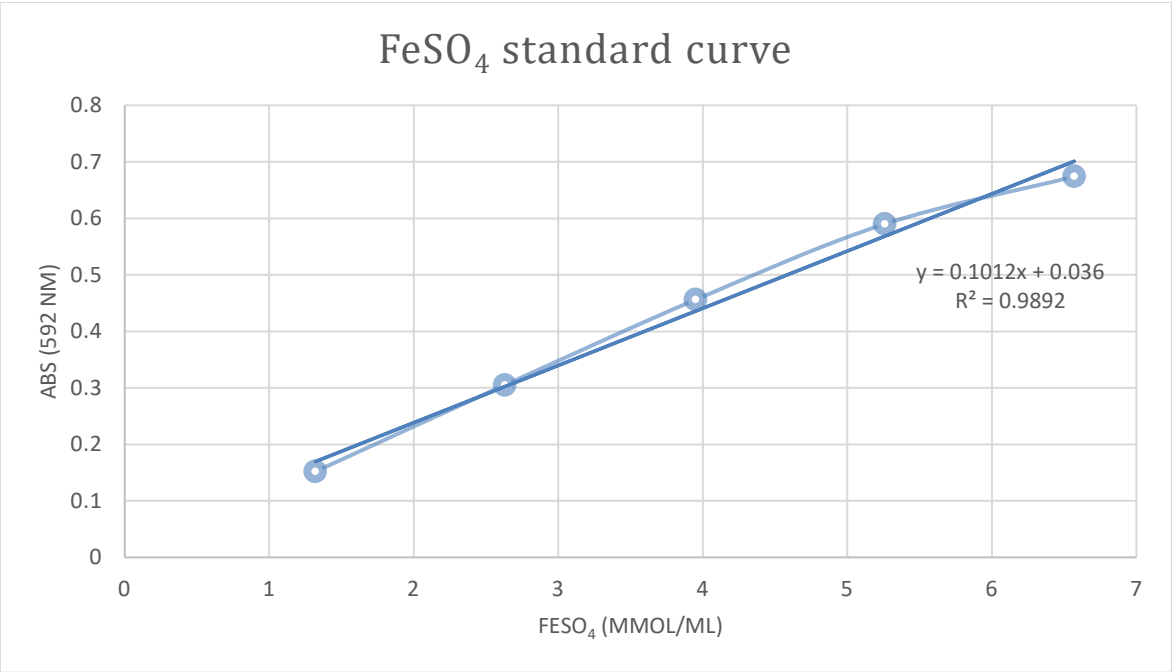

Figure 4.5: Ferrous sulphate standard curve.

4.3 Bioactive Compounds Composition

The present study was undertaken to investigate the bioactive compounds present in the ethanolic extract and methanolic extract of the CS by using gas chromatography coupled with mass spectroscopy. 30 chemicals compounds were identified based on comparison with the NIST-17 GC-MS library. The active compounds with their area (%) are presented in Table 4.3 respectively for ethanol and methanol extract. While four major compounds from the screening are listed in Table 4.4, with their molecular formula (MF), molecular weight (MW), and peak area. In the current study, four identified compounds are the highest in the CS extracts.

Table 4.3: 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-dimethylethyl)-	n.d	0.81

Note. Table 4.3 listed the organic compounds identified via GC-MS using the protocol in 3.2.2. n.d represent not detected.

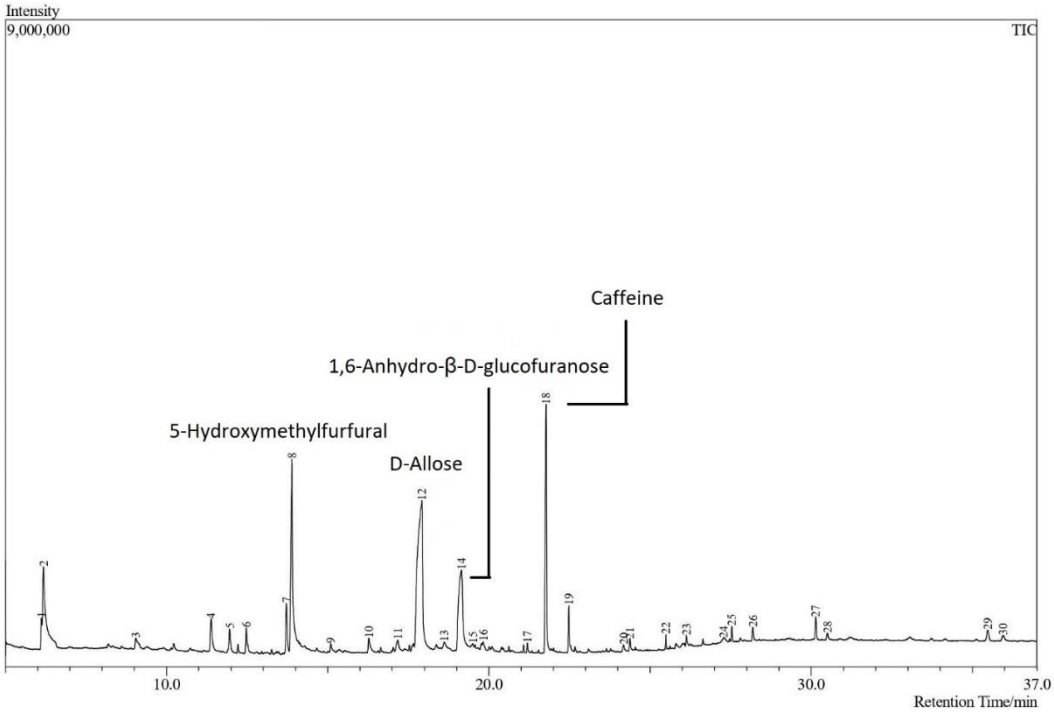

Figure 4.6(a): Chromatogram of ethanol extract of Sarawak Liberica coffee silverskin.

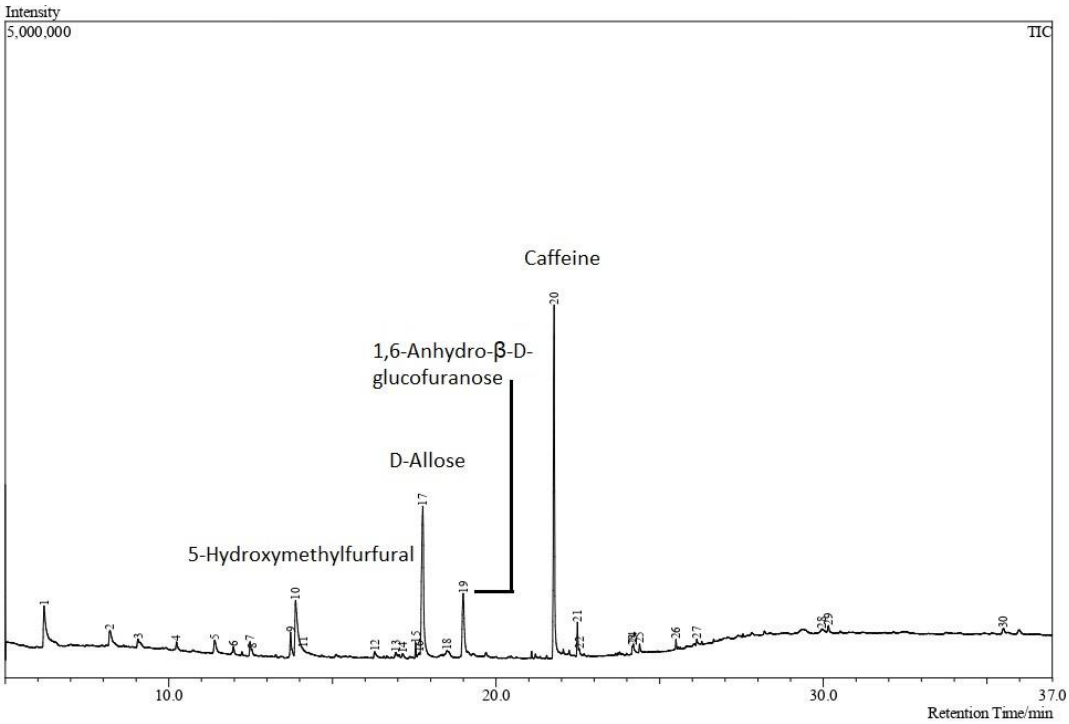

Figure 4.6(b): Chromatogram of methanol extract of Sarawak Liberica coffee silverskin.

Note. The Figure 4.6 (a,b) shows the total ion chromatogram of ethanol and methanol extract, respectively based on the conditions mentioned in 3.3.2.

Table 4.4: 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

4.4 Determination of Caffeine Content

Coffee beverages are known to have high amounts of caffeine; this is one of the main sources for people worldwide to get their daily dose of the stimulant. In this study, the major compound screened from the CS via the GC-MS is caffeine besides the other 3 compound (Table 4.4). The caffeine content in the CS of the ethanol, methanol, and water extracts were ascertained to the concentration of 23.3 ± 7.12 mg/g, 26.86 ± 5.77 mg/g, and 19.23 ± 6.51 mg/g respectively on the sample dry weight basis. The percentage of caffeine contents are 1.55 ± 0.47%, 1.77 ± 0.38%, and 1.92 ± 0.65% respectively (Refer to Table 4.5) when calculated as in Equation 3.4. The results accept the null hypothesis, such that there are no significant differences ($P > 0.05$) among the mean caffeine content of the CS extracts. The comparison of current study with previous caffeine studies will be further discussed in Chapter 5.6.

Table 4.5: 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 was performed in triplicate and expressed in mean ± standard deviation. Absent of significant detonation "*" indicates $P > 0.05$.

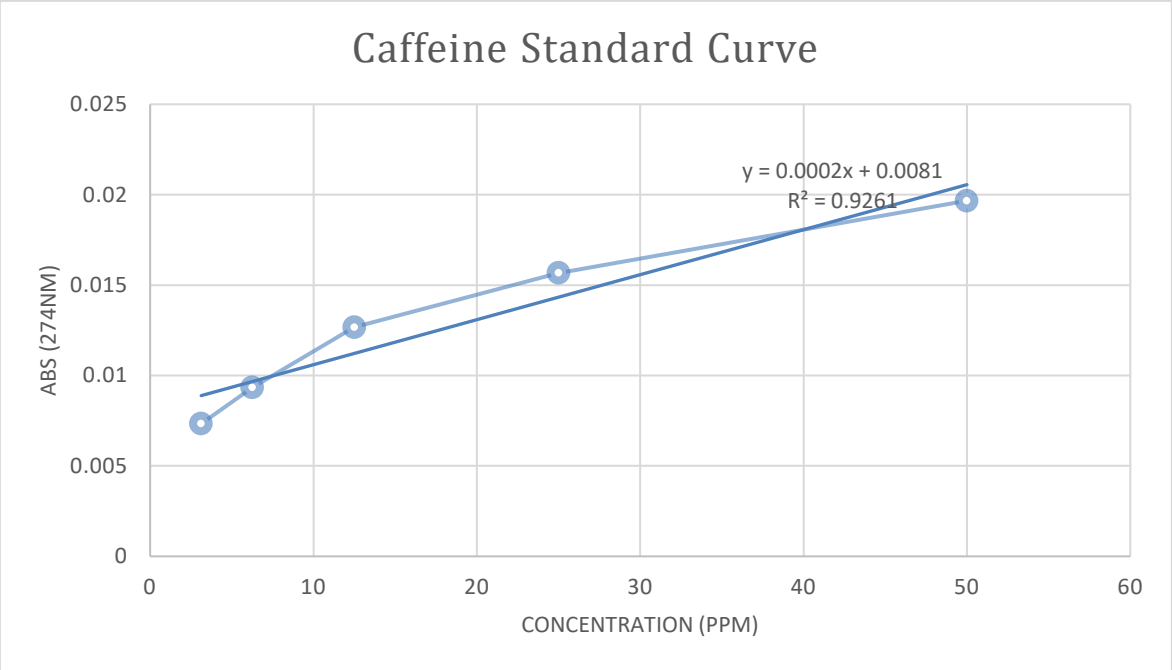

Figure 4.7: Caffeine standard curve via UV-VIS Spectrophotometer.

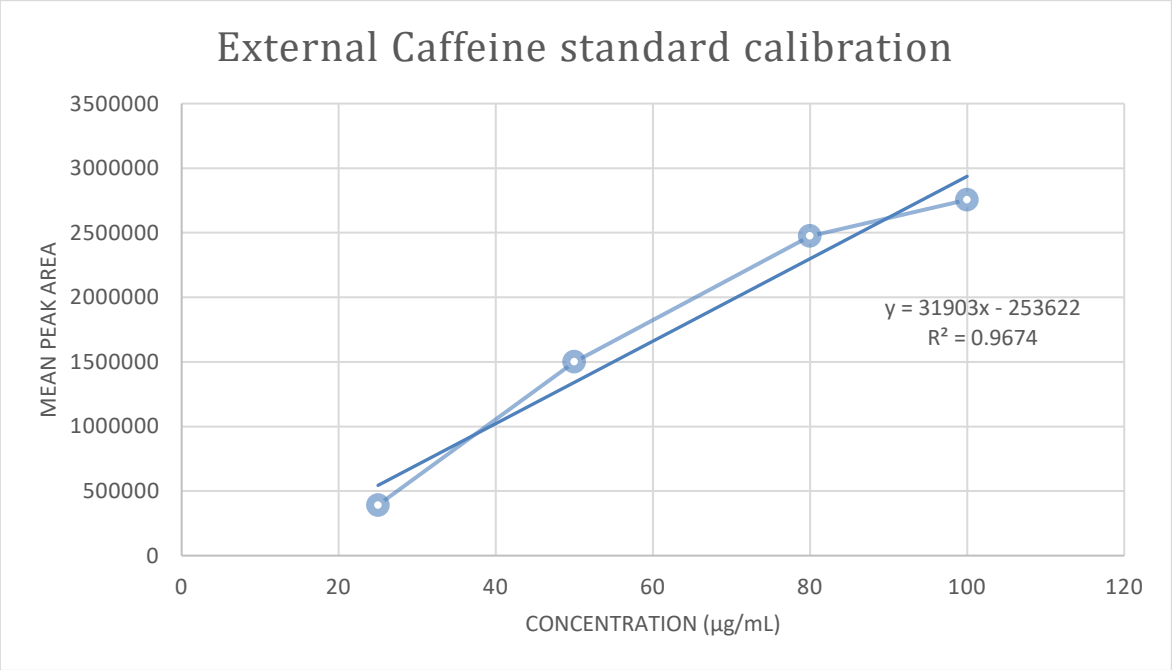

Figure 4.8: Caffeine standard curve via GC-MS.

4.5 Antimicrobial Susceptibility Testing of Coffee Silverskin

Alluding from Table 4.6, the CS extracts in water, ethanol, and methanol solvent does not show any susceptibility against *E. coli* and *S. aureus*. The current testing was done according to the CLSI standard protocol, and the positive control used was Gentamicin (10 µg), while the negative control was DMSO (2.5%) without the addition of any extract or antibiotic. According to the breakpoint standard set by the CLSI, the interpretation of the AST is divided into three categories which are Resistant (R), Intermediate (I), and Susceptible (s). in this case of assay with the test bacteria *E. coli* and *S. aureus*, the breakpoints are ≤12 mm, 13-14 mm, and ≥15 mm, respectively for each category.

Table 4.6: Antimicrobial susceptibility testing of Sarawak Liberica coffee silverskin

Disk/ extract	**Disks concentration**	***Escherichia coli***		***Staphylococcus aureus***	
		ZD (mm)	**Interpretation [a]**	**ZD (mm)**	**Interpretation [a]**
Gentamicin (positive control)	10 µg	14	I	15	S
DMSO (negative control)	2.5%	0	R	0	R
Water extract	5 mg/mL	0	R	0	R
	10 mg/mL	0	R	0	R
	20 mg/mL	0	R	0	R
Ethanol extract	5 mg/mL	0	R	0	R
	10 mg/mL	0	R	0	R
	20 mg/mL	0	R	0	R
Methanol extract	5 mg/mL	0	R	0	R
	10 mg/mL	0	R	0	R
	20 mg/mL	0	R	0	R

Note. Table 4.6 shows the zone diameter (ZD) of CS extracts tested against a Gram-positive bacterium and a Gram-negative bacterium. Where "[a]" was evaluated based on the inhibition zone diameter breakpoint standard by Clinical and Laboratory Standards Institute, 2020.

The *E. coli* and the *S. aureus* were both observed to be resistant to all the extract which are the water, methanol, and ethanol extract of the CS. The result was confirmed with the observation being compared to the negative control of the test (DMSO, 2.5%), which was the solvent used to dilute the extract for the assay. On the other hand, both test bacteria were shown intermediate and susceptible to Gentamicin (10 µg) respectively. The result was validated as was compared based on the chart by CLSI. The current trend of the assay thus showcasing that the CS extracts did not exhibit any antimicrobial susceptibility abilities, even when tested with concentration of the extract as high as 20 mg/mL.

CHAPTER 5

DISCUSSION

5.1 Phenolic and Flavonoid Content

The result obtained from this study complies with the result from the study done by Nzekoue et al. (2020) on CS extraction to quantify the bioactive compounds, the solvent ratio of ethanol to water (70:30) was employed and TPC obtained was as high as 40.4 to 73.4 mg GAE/g. Even though this study achieved lower phenolic compounds compared to the previous study, this study still proven the presence of a significant number of phenolic compounds in the Liberica CS. The result from this study is also in agreement with the study performed by Rodrigues et al. (2016). Whereas a high TPC was observed using the organic ethanol solvent as well compared to the water. As high 35.25 ± 0.25 mg GAE/g of TPC was observed compared to water with only 1.08 ± 0.23 mg GAE/g. The difference in the TPC among the solvent was expected as the CS had showed a high amount of CGA in study concluded by Siva et al. (2016). However, TPC from this study was also relatively lower when compared to its own coffee pulp in a study completed previously (Nillian et al., 2020). The coffee pulp was extracted with ethanol solvent, and they managed to obtain as high as 24.24 mg GAE/g. This was expected as coffee pulp are known to have higher content of bioactive compounds such as caffeine, melanoidins, and CGA (Geremu et al., 2016; Nillian et al., 2020), thus highlighting a higher result of TPC.

As referring to Table 4.1, the flavonoid content of the Liberica CS was significantly ($P < 0.05$) highest observed by extracting with organic ethanol and methanol solvent, 25.14 ± 0.59 mg QE/g and 21.05 ± 4.28 mg QE/g respectively, compared to extraction with aqueous distilled water (6.45 ± 2.79 mg QE/g) subsequently. The flavonoid content is

deemed higher in ethanol compared to methanol and water extract. This is mostly due to the solubility characteristics of the compound's group (Kumar & Pandey, 2013; Costa et al., 2018). This certainly one of the main factors that impacted the absorption of the flavonoid by the human body. However, the result obtained from this study were consistent to the studies before (Ballesteros et al., 2014; Rodrigues et al., 2016; Costa et al., 2018) such that the total flavonoid content estimated was highest when extracted by ethanol solvent even though in different coffee species.

5.2 Antioxidant properties

Referring to Table 4.2, the data showed a higher percentage of free-radical scavenging activity of DPPH by the CS sample in ethanol extract which then follows by methanol and water extract, 83.85 ± 1.78%, 78.77 ± 0.99%, and 62.84 ± 2.98%, respectively. These results obtained agree with the past studies (Ballesteros et al., 2014; Costa et al., 2018) especially on the ability of the organic solvent to extract antioxidant compounds from natural sources. Other than that, the RSA obtained in this study also in an agreement to the study done by Nzekoue et al. (2020), such that the IC_{50} of the DPPH scavenging was the highest in methanol extract with 101.7 ± 5.5 µg/ml and the lowest was in water extract 362.1 ± 65.7 µg/ml**.**

This is also predicted as the DPPH are known to have hydrophobic properties thus are less soluble in aqueous solution compared to organic solution (Costa et al., 2018). As the CS extracts are reported to have high number of bioactive compounds especially due to the roasting process regardless the species, the synergistic manner of the main bioactive compounds may be responsible for this scavenging activity of the DPPH free radicals. However, as it was suggested, there is no evidence to show the correlation between the DPPH value with the TPC of the CS extracts. Thus, this suggests that the antioxidant of the

CS may also came from the activities of the melanoidins and diterpenes and not entirely depending on the CGA and caffeine (Nzekoue et al., 2020).

5.3 Selection of Solvent

When doing extraction, a few factors come in crucial impact on the extracts such as the target compound to be extracted. The factors such as extraction method, extraction conditions applied, and type of solvent utilised. In this study, the type of solvent used are ethanol, methanol, and distilled water to extract the compounds in the CS sample. The current study was performed on solid-liquid extraction of the CS sample. Due to the novelty of this study on the Sarawak Liberica CS, selection of the extraction solvents concentration was tested on ground zero (100%) without the performance of solvent dilution with any other solvents neither organic or water, or dilution based on the concentration. These solvents were selected due to the previous studies referred on utilises ethanol and methanol as it is most effective on the extraction of plant bioactive compounds (Al-Hadrami et al., 2016). The selection of solvents also decided based on the Green Chemistry (GC) concept such as the solvent must be considered of reduction or elimination in products, by-products, or reagents which are hazardous to human health and environments (Joshi & Adhikari, 2019).

However, there are other non-toxic and preferred solvents in extracting the phenolic and flavonoid compounds such as acetone and ethyl acetate (Joshi & Adhikari, 2019). Despite that, alcohol solvents still performing better in phenolic and flavonoid extraction such as in the study by Dirar et al. (2019), ethanolic extracts showed higher phenolic and flavonoid content extracted compared to acetone and dichloromethane (DCM). The usage of DCM is very much undesirable as this solvent is very low density, irritable, and more toxic compared to the water and alcohol solvents. Despite that, the utilisation of DCM in the extraction of caffeine compound plays a different outcome as caffeine dissolves better in

DCM alongside chloroform compared to water, ethanol, and methanol (Shalmashi & Golmohammad, 2010).

Based on Table 4.3, the ethanol extracts do exhibit higher carbohydrates of a total 47.71% of the total compounds identified. Comparing to the methanol extracts which displayed a higher percentage on the alkaloid extracted (29.77%). The difference between the bioactive compounds detected from the GC-MS was suggested by the nature of the compound's solubility (Dirar et la., 2019). In this study, the CS extracts dissolve in ethanol exhibited better affinity for the carbohydrates compound (47.71%) significantly higher than in the methanol extract (5.26%). Carbohydrates encompasses a diverse group of compounds such as sugars, polysaccharides, etcetera, this suggests that ethanol might be more effective in extracting certain carbohydrates or carbohydrates-rich compounds from the CS compared to methanol (Choi et al., 2021).

In the caffeine content study, the extracts were then further isolated with chloroform and dichloromethane (DCM) because caffeine is more soluble in these solvents compared to water (Chaugule et al., 2019). In the investigation of studies done on the caffeine content, the caffeine is known to be partially polar due to the resonance stability structure. This means that caffeine is both soluble in water and polar organic solvents such that in an event of proton transfer can change the polarity of caffeine (Bahrami et al., 2013; Edwards et al., 2015). This nature of the caffeine compound makes it significantly insoluble in non-polar solvents such as pentane, hexane, and diethyl ether. Figure 5.1 below illustrates the solubility of the solvents in extracting caffeine compound (Shalmashi & Golmohammad, 2010).

On the other hand, in the antimicrobial assay of the CS extract, the extracts were further suspended in DMSO before the assay because the solvent able to dissolve both organic and inorganic compounds well for the antimicrobial procedure (Bubonja-Šonje et al., 2020). As plant compounds usually extracted with a more polar solvents such as water, methanol, and ethanol, due to the fact they can managed to extract various bioactive compound such as flavonoids, alkaloid, and terpenoids (Al-Hadhrami et al., 2016). However, the usage of solvent such as ethanol, may exhibit false positive on the antimicrobial testing, as the solvent is known to have bactericidal effect on *Staphylococcus* genera even at low concentration >2.5% (Chatterjee et al., 2006; Bubonja-Šonje et al., 2020). To overcome the possibility of a false positive on the antimicrobial properties, the employment of the solvent (DMSO) as the negative control was taken. This is to consider of any possible interference of the solvent on the bacterial growth.

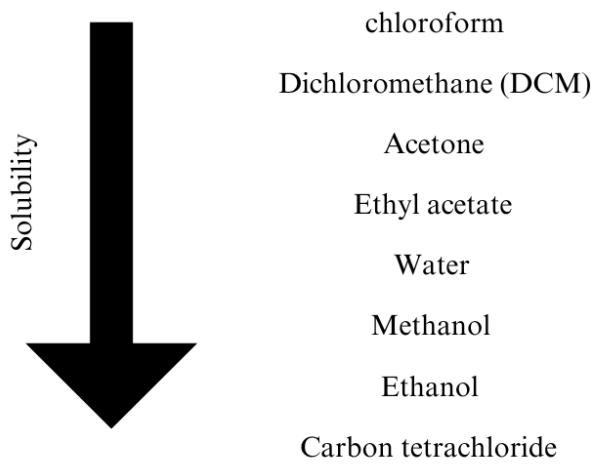

Figure 5.1: Solubility of different type of solvents in caffeine extraction

5.4 Extraction Parameter of Coffee Silverskin

As for the time and temperature of extraction on the CS, there are optimum conditions established for the extraction of the bioactive compounds from the CS sample, even though they were performed on other coffee varieties instead of Liberica (Narita & Inouye, 2012; Costa et al., 2014; Tangguh & Kusumocahyo, 2017; Ginting et al., 2022). However, for this study, the effect of the time and temperature on the extraction of bioactive compounds such as the phenolic, flavonoid, and alkaloid was not the focus, as this study was a novel study on the Sarawak Liberica CS, the focal point is more shift towards proving the presence of these compounds. Thus, this study only refers to previous study on the time and temperature for the extraction of CS with minor modification (Nillian et al., 2020; Vuletić et al., 2021).

Despite that, the time and temperature selected for this study may not be the optimum conditions for the extraction of the Liberica CS. This is due to the optimization from the previous studies may differ to the current CS used in this study, as there are few factors such as the coffee variety, coffee parts used and geographical factor that may affect the conditions variable. Despite that, this opens a way for further research on the different concentrations of the solvent can be employed, while employing different parameters of extraction such as time and temperature to obtain highest bioactive compounds can be extracted. In a nutshell, this study achieved its objective of phytochemical profiling of the Sarawak Liberica CS, even though the conditions of extraction employed were based on previous parameter explored by previous studies.

5.5 Extraction Method of Coffee Silverskin

In terms of extraction method, the current study utilises the conventional maceration method as to allow reproducibility by other researchers. This method is inexpensive, quick,

and does not require the usage of complex and expensive machineries. In terms of the result, the TPC value (15.24 ± 0.65 mg GAE/g) does not differ significantly while extracted with methanol solvent compared to a study before (8.94 ± 0.01 mg GAE/g) (Table 2.1) which utilises an ultrasonic machine for extraction of the plant crude (Wen et al., 2019), even lower if pointed out. This also concurred by research of Tangguh and Kusumocahyo (2017), such that the conventional extraction achieved a high antioxidant activity of 68.8% compared to ultrasonic-assisted extraction (UAE) method.

During thermal treatment on the CS sample during extraction, the temperature applied mostly at room temperature, or at low temperature (<100 °C) for large scale industries extractions, while left soaked for time span of hours to days. Hence, the downside of the maceration process is time consuming during extraction. Despite that, in this study, the maceration process was coupled with heat (<100 °C) as adapted from previous study (Vuletić et al., 2021) and continuous stirring at 300 rpm. This allows for the acceleration of the extraction process instead of running for days, as agitation was introduced into the extraction process, thus increasing the volume of contact between the CS sample with the extraction solvent. The results achieved in this current study after 10 minutes of extraction are indeed in par with the previous studies utilizing the SLE as in comparison shown in Table 2.1 in Chapter 2.

The UAE on the other hand, is an example of the modern extraction method alongside methods such as microwave-assisted extraction (MAE), subcritical water extraction (SWE), and supercritical fluid extraction (SFE). The modern methods were developed to mainly reduce the volume of solvent used during extraction, reduce the extraction time, increase extraction kinetics while improving the extraction efficiency, to automate a long extraction process, and reduce additional steps such as sample filtration,

leads to the favours of these method utilisation (Sasidharan et al., 2011). While the UAE is commonly used to target the extraction of temperature sensitive compounds and sustaining their nature that may degrade at high temperature, such as polyphenols, carotenoids and phenolics (Bitwell et al., 2023).

From the previous studies (Table 2.1), the UAE observed to be a better alternative to the SLE for the extraction of caffeine compound from the CS extracts. As from previously discussed, the UAE have certain better aspects compared to the SLE such as time consumption, solvent usage, and extraction yield. Despite that, the SLE was selected as the method of extraction in this study as the previous research (Bresciani et al., 2014; Toschi et al., 2014; De la Cruz et al., 2019; Martuscelli et al., 2021) which involved in the caffeine content determination study, opt for this method due to the ease-use of the method mechanism overall. In terms of extraction yield, it is acknowledged most studies shown that the UAE extracted more yield than the SLE, but this still contradicted by the study done by Mota et al. (2021), such that the Soxhlet and the UAE showed similar extraction yield, however, the Soxhlet displayed a more favourable phytochemical attribute to be used as feedstock.

5.6 Major Compounds in Coffee Silverskin

The current study shows that the CS extract predominantly in 4 different compounds which are 5-Hydroxymethylfurfural (5-HMF), D-allose, 1,6-Anhydro-β-D-glucofuranose, and caffeine (Table 4.4). From the total ion chromatogram in Figure 4.6 (a) and (b), the compounds shows that methanol extract profiled a more caffeine compounds than ethanol extract. This is due to the polarity of the methanol, which is higher compared to ethanol, thus attracting more polar compounds such as caffeine (alkaloid) (Table 4.3).

5.6.1 5-Hydroxymethylfurfural

The compound 5-HMF (Figure 5.2) is one of the highly utilised compounds in the food industries, especially as a quality marker (Martins et al., 2022). The compound most commonly found in food products due to the thermal treatment that were applied on them such as the roasting of the coffee bean, this thermal treatment performed to increase the food safety, other than to increase the shelf life of the food product, it's also to change the properties of the food product such as improve or removing of the smell, to indite changes to the colour, to give a more fulfilling taste to the food, and to improve the flavour such as done on the coffee beans (Iriondo-DeHond et al., 2021).

Overall, the thermal treatment applied on a food product such as the coffee bean, is to improve the consumer acceptability of the product, whether it is the physical look, or the taste entirely. 5-HMF produced during the Maillard reaction or caramelization of the food product, which both involves the treatment of heat on the product. For vegetables, and fruits, the content of the 5-HMF is higher compared to other food products due to the high natural sugar and amino acids in the food (Mathew et al., 2018). When paired with long and low moisture during storage, this level increases (Martins et al., 2022).

The 5-HMF utilisation is varies in the industries such as the compound itself acted as the middle compound to produces more beneficial compound such as the 2,5-dimethylfuran (2,5-DMF) through a simple chemical reaction such as the oxidation and reduction reaction of the side group and furan ring of the 5-HMF structure. Beneficial compound such as the 2,5-DMF is becoming a target of interest due to the potential of it becoming an alternative to the gasoline and diesel as biofuel. The process is possible via catalytic hydrogenolysis of the 5-HMF, the 2,5-DMF is known to have high octane number,

high boiling point while having low miscibility with water, high energy density, and low volatile, which makes it a promising biofuel candidate (Wang et al., 2018).

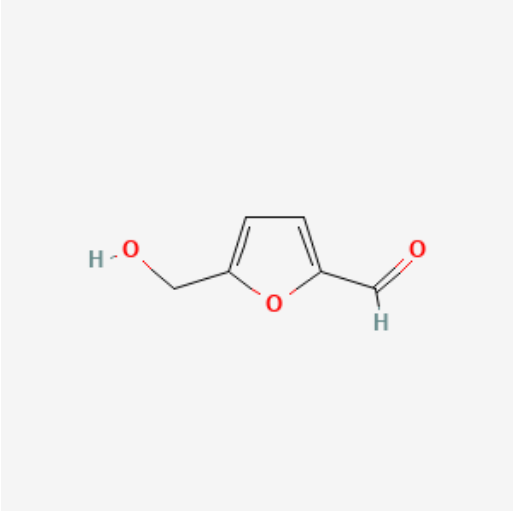

Figure 5.2: Chemical structure of 5-Hydroxymethylfurfural.

In the current study, the CS displayed a relatively large peak area of 5-HMF of 14.87% and 10.96%, ethanol extract and methanol extract, respectively; at retention time of 13.8 min (Table 4.4). this may be correlated to the intensity of the heat used during the roasting of the Liberica coffee beans and the high acidity of the environment, which causing a significant level of 5-HMF (Wang et al., 2018). However, the level of 5-HMF suspected to be a bell shape curve instead of linear of increasing pattern, such as a study performed by Diviš et al. (2019) showed that, the highest level of the compound was found at medium roasting temperature and time for the Arabica and Robusta coffee beans, instead of the highest temperature and time tested.

According to Zhao et al. (2013), the inhibitory of the 5-HMF on human cancer cell proliferation (melanoma A375 cell) suggests that the compound have the potential to be developed into a natural antioxidant with particularly applications in cancer chemotherapy. Besides antioxidant activities, the 5-HMF also associated with antimicrobial properties. The

compound was tested to be suppressing the production of virulence phenotypes and biofilm formation in *Pseudomonas aeruginosa* pathogens which commonly caused the nosocomial infections (Rajkumari et al., 2019).

5.6.2 D-allose

On the other hand, the compound D-allose (Figure 5.3) is considered as a rare functioning reducing sugars in nature, as the compound is very scarce and has high production costs if needed to be produced synthetically and not from nature based (Choi et al., 2021). Even the studies of rare sugars monosaccharides were limited by scarce methods for major production in the industries. The derivative of the compound D-allulose, is grouped as the third-generation sweetener, that only consists of 80% of sweetness relative to the first-generation, table sugar. The compound was studied to be anti-cancer, antioxidant, anti-hypertensive, and anti-inflammatory (Choi et al., 2021).

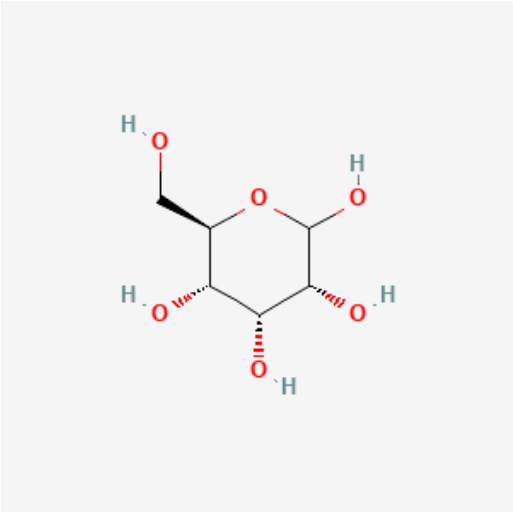

Figure 5.3: Chemical structure of D-allose.

However, by having low calories and non-toxic, the compound D-allose has multiple possible physiological functions to be applied to such as, food additive, clinical therapy, and medical field. In addition, as a reducing sugar, the compound involves in the Maillard

reaction during thermal treatment of food product to improve the flavour, taste, and visual of the food. By the addition of this compound in food, the long-term effect is to assist in losing weight, thus helping targeted patients with diabetes and hypertension complication. Currently, the compound D-allose is used in surgery and organ transplant as immunosuppressive agent to decrease the tissue damage during operation, thus increasing the probability of success when doing so (US patent no. 5620960A, 1997) (Chen et al., 2018).

In plants, D-allose is known as a critical compound involves in a plant immunity defences mechanism such that, the compound act as a regulator in the reactivity of the reactive oxygen species (ROS) accumulation and expression (Zhang et al., 2020). While the D-allose was studied to have a growth inhibitory effect in plant, but the study also concurred with the latter such that, D-allose proven to be a candidate for disease resistant agent in agriculture (Kano et al., 2010).

In the roasting of coffee, where thermal treatment was induced on the coffee beans, the degradation of carbohydrates produces monosaccharides such as D-allose, for such in a study by Kim et al. (2021) on the Arabica coffee beans, they detected that the reduction of glucose content by approximately 80% in beans and increase the formation of furans. This also induced the accumulation of D-allose from isomerization of D-glucose to D-fructose, to D-allulose, and finally D-allose in that order (Chen et al., 2018). Even though there are no studies on the monosaccharides of the CS currently, the relation of the coffee beans to the CS are considered proportional as the phytochemical of the two are considered similar (Gottstein et al., 2021).

5.6.3 1,6-Anhydro- β-D-glucofuranose

The CS is known to have high insoluble polysaccharides such as the hemicellulose and cellulose contained in the composition. In the CS, cellulose content (60%) is more abundant compared to the hemicellulose, which helps in the production of enzymes such as amylase and fructooligosaccharides (FOS) by many types of bacteria and moulds (Martuscelli et al., 2021; Nolasco et al., 2022). As the CS undergoes thermal treatment during the roasting of the beans, the high temperature burning at low or no oxygen present, naturally will induce the pyrolysis of the cellulose in the CS. This will produce the compound Levoglucosan (LG) (1,6-Anhydro-β-D-glucopyranose) (Figure 5.4b) which is known to have high demand in the chemical industries to produce surfactants, propellants, plastics, resins, and in the pharmaceuticals industry (Junior et al., 2020).

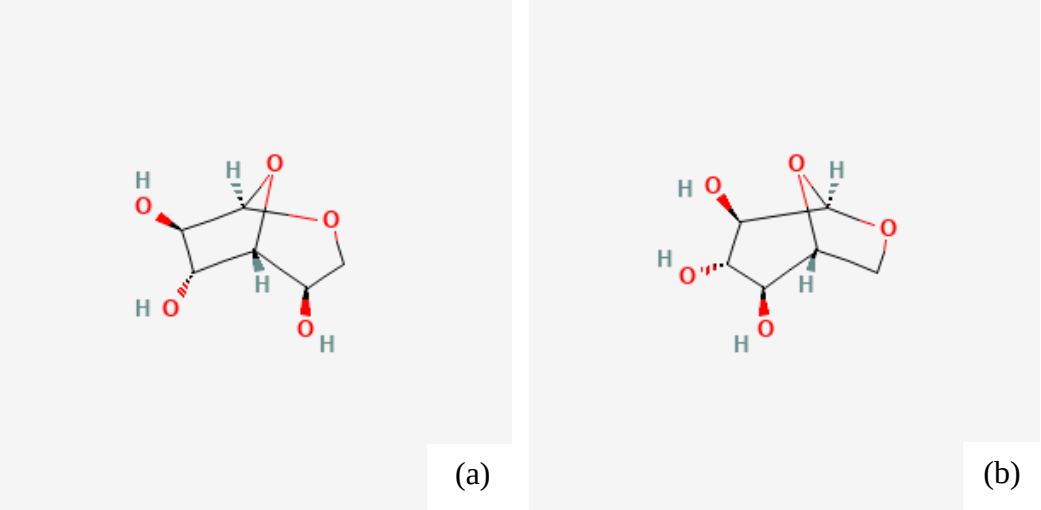

Figure 5.4: (a) Chemical structure of 1,6-Anhydro-β-D-glucofuranose.
(b) Chemical structure of Levoglucosan.

The isomer of this beneficial compound is the 1,6-Anhydro-β-D-glucofuranose (Figure 5.4a), which is found to be one of the major compounds detected in this study (Table 4.4). LG is a 6-membered ring, while the 1,6-Anhydro-β-D-glucofuranose is a 5-membered

ring cyclic ring structure, in terms of molecular structure, both having similar $C_6H_{10}O_5$ arrangements, but by having different aldehydes attachment on the hydroxyl group resulting in two different cyclic form. The D-glucofuranose is a rare monosaccharide comparing to the D-glucopyranose due to the equilibrium factor that effect the compound to produce a more stable 6-membered ring cyclic compound. During the hemiacetal formation of the D-glucose compound, majority of the isomers produced are D-glucopyranose (99%), while the D-glucofuranose only consists of the minority (1%) (Ashenhurst, 2023).

5.7 Caffeine Content Studies Comparison

This current study shown that the caffeine content of Sarawak Liberica coffee silverskin has higher content compared with the findings by Martuscelli et al. (2021) such that 17.45 ± 0.69 mg/g of caffeine was obtained from the study recently. The study however was performed on the CS of Arabica mixed with Robusta coffee varieties, thus proven that the Liberica CS have a higher caffeine content comparing to the major varieties of the coffee family. Moreover, based on Table 5.1, it is shown that the caffeine content of the Liberica CS is deemed higher compared to the Robusta variety which was concluded to be 6.76 ± 0.05 – 12.15 ± 0.11 mg/g (Bessada et al., 2018b). For the Arabica variety also displayed a lower caffeine content compared to the current study, such that only 10.0 ± 1.1 mg/g of caffeine detected from the study by Bresciani et al. (2014) and 12.43 ± 0.12 mg/g reported by Xuan et al. (2019).

Despite that, the current study is deemed lower compared to the study by Zengin et al. (2020), such that, the caffeine content was 41.88 ± 2.36 mg/g. Not only that, the study conducted in 2019 also reported a higher caffeine content of 32.7 ± 1.0 mg/g (Wen et al., 2019). Moreover, the study reported by Nzekoue et al. (2020) also shows high caffeine

content in the Arabica variety of 1.00 - 3.59% which compared to the current study as high as 1.55 ± 0.47 - 1.92 ± 0.65% of the Liberica CS extracts.

This however was concluded that the findings were higher due to the extraction method and the solvent of choice applied to the CS. Not only that, the post-harvest processing of the CS may also be a factor in influencing the overall bioactive compounds constituent (Bessada et al., 2018b). In addition, the temperature and time for roasting affects the bioactive constituent in the CS due to the formation and degradation of the precursors for the Maillard reaction (Martuscelli et al., 2021; Giordano et al., 2022; Nolasco et al., 2022).

5.8 Antimicrobial Properties of Coffee Silverskin

The test bacteria *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) were observed to be resistant towards the CS extracts (Table 4.6). In this study, the Kirby-Bauer assay or the disk diffusion method was employed according to the standard outlined by Clinical and Laboratory Standard Institute, 2020. According to the interpretation chart by CLSI, the bacteria show resistant (R) towards the CS extracts tested on MHA. The R category according to CLSI is that the bacterial growth would not be inhibited by the usual dosage or concentration of the agent, thus not an appropriate choice for treating infection against the bacterium. The opposite of this which shows by the positive control (Gentamicin 10 µg), the susceptible (S) category, is an appropriate pick for the treating the infection caused by *S. aureus* but not towards *E. coli* which shows only intermediate (I) category.

Not to be mistaken with R, the I category still can be used in treatment against the bacterium, but the response rates may be lower. This is might due to the reason that the *E. coli* is a Gram-negative bacterium that have outer layer membrane, which leads to lower or slower permeability of the antibiotic into the cell (Jiménez-Zamora et al., 2015). The use of

the bacterial strain in this study *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) is the strain commonly found in and on the human body such as the intestinal and the skin microbiota. Both strains are recommended by CLSI as reference strain commonly used in quality controlling of laboratory procedures such as the antimicrobial susceptibility testing (AST). The *S. aureus* (ATCC 25923) is suitable as standard control as the strain is sensitive to variety of antibiotics, including methicillin (Treangen et al., 2014).

The test subject or the CS extract concentration used in this study was in the range of 5, 10, and 20 mg/mL. the minimal inhibitory concentration (MIC) of the extracts required maybe needs to be higher than the concentrations tested. Currently, there are scarce sources of references on the AST of the CS regardless of the coffee variety. However, few of these studies recently agrees with the current study such as the study performed by Jiménez-Zamora et al. (2015), where the Arabica CS was tested against the standard culture of *S. aureus* (subsp. a6538P) and *E. coli* (ATCC 11775), showed no significant antimicrobial effect. Despite that, in the study, the interference of the microbial displayed a significant different result ($P < 0.05$) when the CS was mixed with coffee melanoidins, thus exhibiting the antimicrobial effect merely due to the melanoidins instead of the CS alone. Other than that, the current study also concurred with previous study that executed an AST on *S. aureus* (ATCC 6538), *E. coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 9027). The assay resulted in no inhibition of any of the bacteria strains with stock CS concentration of 4.096 mg/mL (Nzekoue et al., 2020).

Even though this study reported on the compound with proven antimicrobial effect such as the caffeine compound (Gaul & Donegan, 2015), the compound absolute amount may not be sufficient for the MIC required for bacterial growth inhibition. For example, the MIC of caffeine against *E. coli* and *S. aureus* was previously reported as 10 mg/mL (Al-

Janabi, 2011; Rathi et al., 2022), by comparison in this study, the caffeine content is estimated to be 19.23 ± 6.51 - 26.86 ± 5.77 mg/g (Table 4.5) which also equivalent to 0.10 ± 0.03 - 0.27 ± 0.06 mg/mL. Hence for this study to achieve the MIC of the caffeine, an extra 97.3% of the caffeine concentration would be needed, which hamper the possibility for this study, as a laboratory scale study. However, this does not cease the possibility of the antimicrobial properties of the CS extract, although the current study can conclude that there is no visible effect on the microbial growth at the stock concentration of 20 mg/mL.

5.9 Chapter summary

This chapter discussed the effect of selecting solvent, extraction method, and extraction parameter on the result obtained from this study. Moreover, this study also discusses regarding the crucial effect of the CS origin and differences in the geographical setting coupled with variant climate will affect the metabolite content of the coffee bean and CS. Next, we also discussed the major compounds identified from the CS constituent. The availability of these compounds in a considerably high amount in the CS of the Liberica variety open a future prospect in the utilisation of the CS in multiple industries such as the sustainable biofuel production, the chemical industries, and the pharmaceutical industry coupled with employment in the medical scenes. The caffeine content was investigated in this study, which leads to the comparison of the current study with the previous caffeine study on the CS. Lastly, the antimicrobial result was also compared with previous study using the CS and identified the possible reason for the negative result.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Overall, this study accepts the null hypothesis such that Sarawak Liberica coffee silverskin do possess beneficial bioactive compounds, antioxidant properties, and antimicrobial properties making it a valuable by-product for various industries. The results of this study could have significant implications for the utilisation of Liberica CS as a functional food ingredient, cosmetic active ingredient, and provide insight into the potential health benefits of consuming Liberica coffee, while pique an interest into the nutraceutical approach for the CS. Furthermore, this study could contribute to the development of sustainable coffee production practices by providing an innovative solution for the utilisation of coffee waste. Finally, this study can benefit the local indigenous communities of Sarawak, as to increase the local coffee production through effective and sustainable coffee cultivation, while introducing a new revenues stream for them by exploiting the value-added waste by-product of the coffee production.

Moreover, the data in this study such as the TPC (9.48 ± 0.32 - 15.24 ± 0.65 mg GAE/g), TFC (6.45 ± 2.79 - 25.14 ± 0.59 mg QE/g), and DPPH's radical scavenging (62.84 ± 2.98 - 83.85 ± 1.78 %) can be employed in the coffee beverages industry such as to monitor the period of coffee roasting through the melanoidins content of the CS. In addition, crops with high nutritional value tend to be more resistant to environmental stressors, pests, and or even diseases. Thus, understanding of the phenolic and flavonoid content will indirectly abet the sustainable and survival of the coffee species. Although there was no antimicrobial (R) effect visible from the CS tested in this study, the present of the promising compound such

as caffeine (19.23 ± 6.51 - 26.86 ± 5.77 mg/g) would provide an insight on the future prospect application of the CS in health products.

6.2 Recommendations

This study was conducted based on the novel look into the Liberica CS as a beneficial by-product. As there is no established optimum extraction on the Liberica CS, a multifactorial experimental design is recommended for future studies on the by-product. Moreover, further studies should be done on more specific compounds such as CGA and melanoidins extraction and investigation. This is due to in this study, both beneficial compounds did not appear during the GC-MS application. Perhaps in a more targeted set of methodology, these compounds can be detected even in a trace mount. This is due to these compounds are known to have beneficial effects as discussed in Chapter 2. The compounds screened from this study also only visualized the peak of the compound in response to the amount of the compound itself but did not further quantify into concentrations of the amount presence. Further studies are recommended on quantifying these compounds, as they show some promising utilisation, thus adding value to the CS.

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APPENDICES

Appendix A: Journal Publications

1. **Buyong, N. L.** & Nillian, E. (2023). Physiochemical Properties of Sarawak's adapted Liberica Coffee Silverskin Utilizing Varying Solvents. *Food Science & Nutrition.* http://dx.doi.org/10.1002/fsn3.3541.
2. Nillian, E., **Buyong, N. L**., Lesen, D., Bebey, G., & Zulkarhain, A. (2021). Detection of Beneficial Lactic Acid Bacteria (LAB) and Yeast in Sarawak Fermented Food. Akademia Baru Publishing (M) Sdn Bhd.
3. Nillian, E., **Buyong, N. L**., Lesen, D., Basiron, M., Ahad, N., W.N.Q., W. J., Jamaludin, M., Fadilah, M., Azra, T., & R., T. (2023, January 15). A survey study on the assessment of food handler's compliance to personal hygiene practices regulation in selected Malaysia food outlets. *Food Research*, *7*(1), 64–75. https://doi.org/10.26656/fr.2017.7(1).620
4. Elexson, N., Ja'afar, A. Z., Joel, W., **Buyong, N. L**., Lesen, D., & Tze, T. Y. (2023, June 21). Random amplified polymorphic DNA (RAPD) and enterobacterial repetitive intergenic consensus (ERIC) PCR of Vibrio cholerae from a foodborne outbreak in Limbang, Sarawak. *International Food Research Journal*, *30*(3), 591–600. https://doi.org/10.47836/ifrj.30.3.04
5. 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

Appendix C: Kirby-Bauer disk diffusion

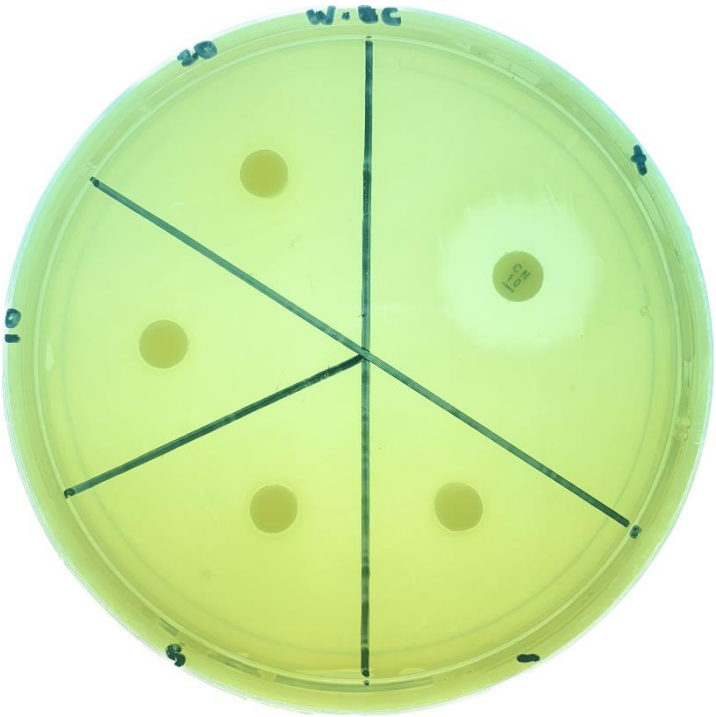

Figure 2(a): Water extract against *E. coli*

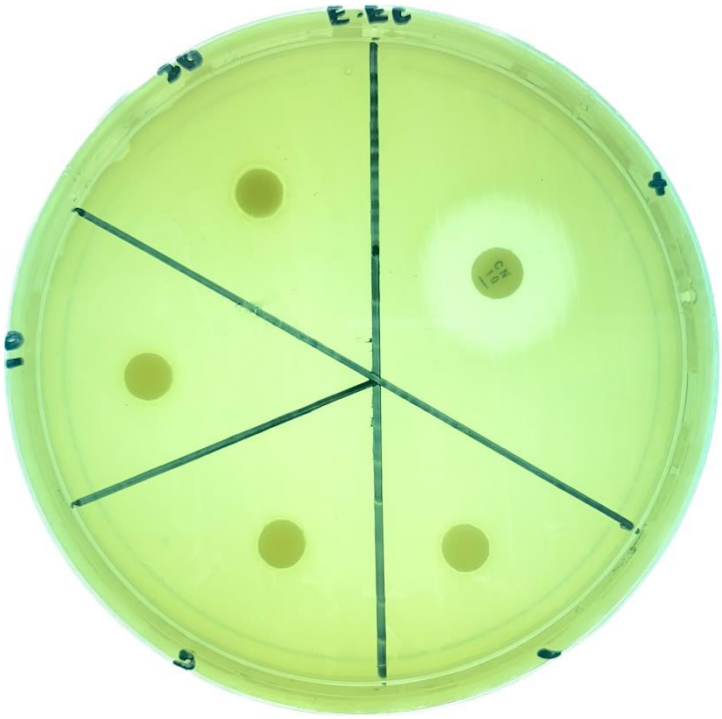

Figure 2(b): Ethanol extract against *E. coli*

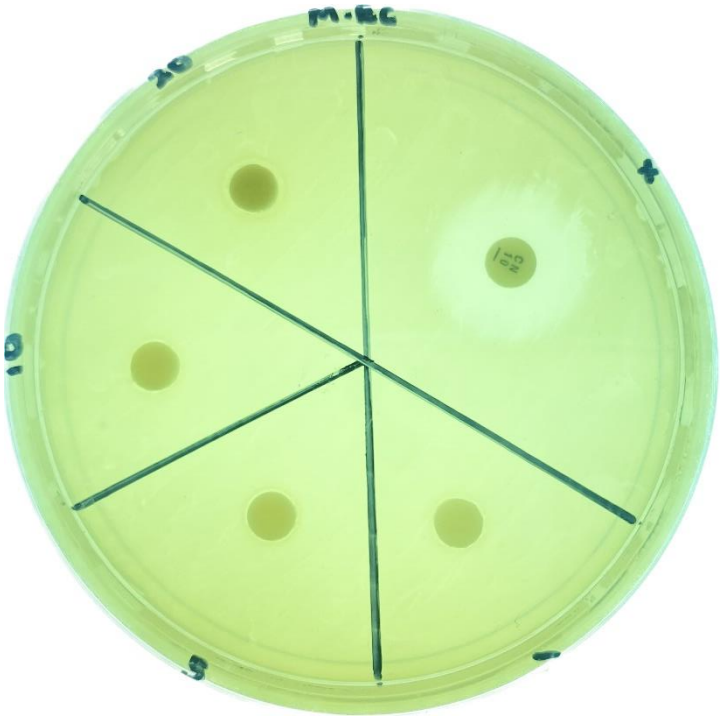

Figure 2(c): Methanol extract against *E. coli*

Note. Figure 2(a, b, c) shows the disk diffusion of CS extracts against *E. coli* on MHA. The concentration of the extracts used were 5, 10, 20 mg/mL. The positive control was Gentamicin (10 µg). The negative control was DMSO.

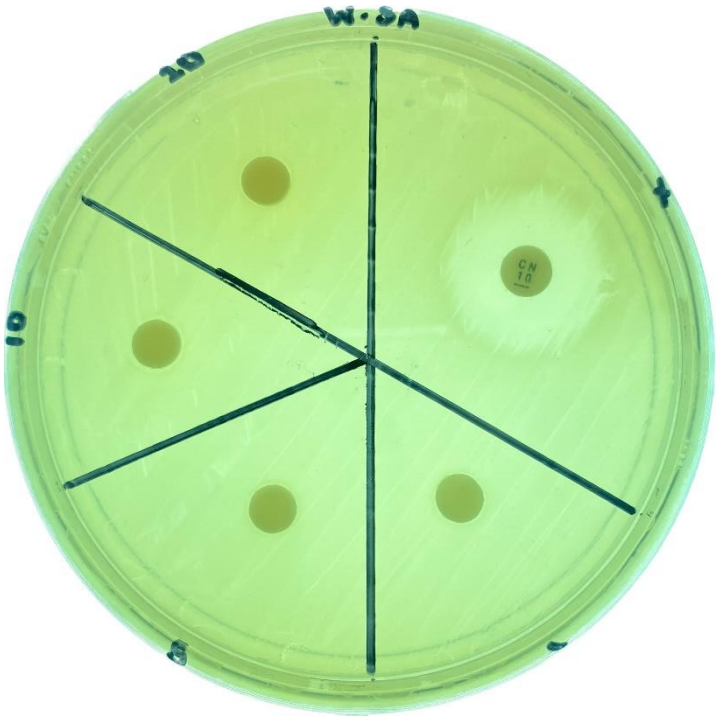

Figure 3(a): Water extract against *S. aureus*

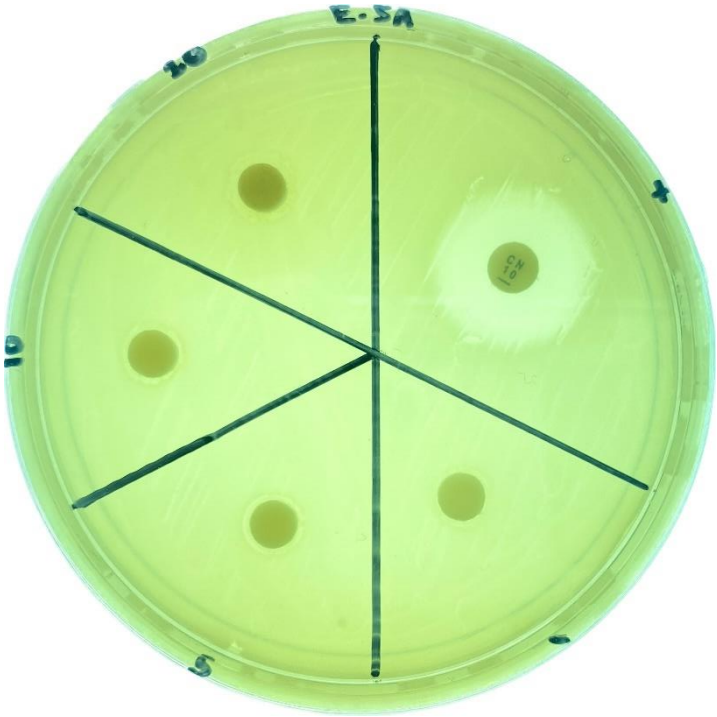

Figure 3(b): Ethanol extract against *S. aureus*

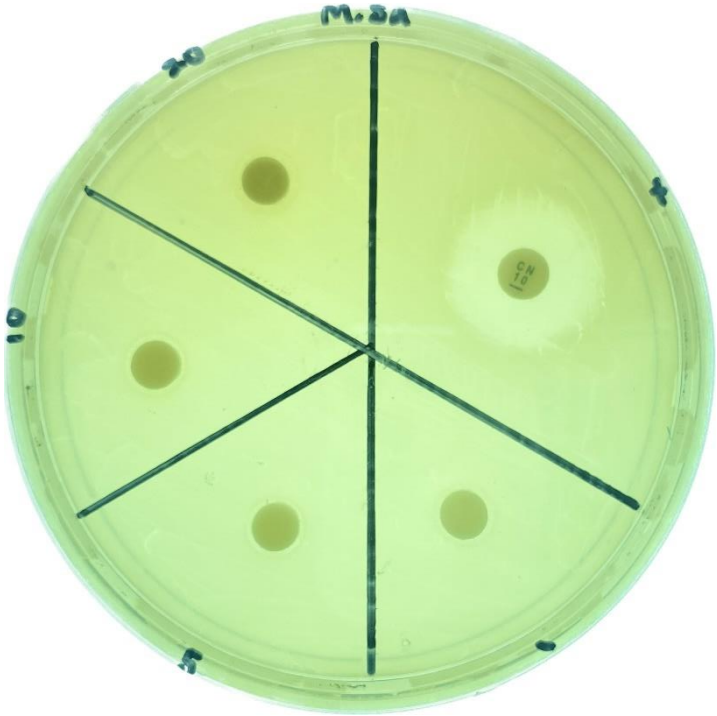

Figure 3(c): Methanol extract against *S. aureus*

Note. Figure 3(a, b, c) shows the CS extract against *S. aureus* on MHA. The concentration of the extracts used were 5, 10, 20 mg/mL. The positive control was Gentamicin (10 µg). The negative control was DMSO.

CHAPTER 7