



Faculty of Resource Science and Technology

**Feasibility Study on Effect of Microwave-Assisted Extraction Method on
Physicochemical Characteristic of Sarawak *Coffea liberica* sp. Pulp
Waste**

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Feasibility Study on Effect of Microwave-Assisted Extraction Method on
Physicochemical Characteristic of Sarawak *Coffea liberica* sp. Pulp Waste

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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

Coffee is one of the most widely consumed beverages around the globe. Its unique aroma, flavor, and stimulating effects have made it an indispensable part of daily life for many individuals. The coffee industry is notably dominated by two species, *Coffea arabica* and *Coffea canephora* (Robusta), however, a third species, *Coffea liberica*, although less common, holds significant potential. The present study aims to advance the usage of Sarawak *Coffea liberica* sp. for the development of functional products and aids in reducing environmental pollution by utilisation of coffee pulp waste. This study investigates the optimisation of microwave-assisted extraction (MAE) using response surface methodology (RSM) to obtain maximum yield of total phenolic, flavonoid, and carbohydrate content from Sarawak *C. liberica* sp. pulp. Optimal MAE conditions were found to be a microwave power of 700 W, irradiation time of 180 s, and a solvent-to-feed ratio of 87:1. Besides, the effectiveness of microwave-assisted extraction (MAE) was compared with Soxhlet extraction (SE) and conventional maceration extraction (CME) in this study. Comparison with CME confirmed the efficiency of MAE as it revealed the advantages of higher extraction yield within a shorter time of extraction. No significant difference was observed ($p < 0.05$) between MAE and SE on the extraction efficiency of TPC, TFC, and TCC. The antioxidant properties of the coffee pulp waste extract were evaluated, with a DPPH scavenging activity assay revealing a dose-dependent antioxidant activity. Maximum inhibition was observed at a concentration of 2.5 mg/mL, indicating the presence of bioactive compounds. However, no antibacterial activity was detected against *Escherichia coli* and *Staphylococcus aureus* under the experimental conditions. Characterisation of the extract via Fourier transform-infrared (FTIR) analysis identified the presence of various functional groups associated with carbohydrates, lipids, organic acids,

and phenolic compounds. High Performance Liquid Chromatography (HPLC) quantified the caffeine concentration, revealing a concentration of 2.62 ± 0.31 ppm in the liquid extract and $0.05 \pm 0.01\%$ (w/w) on a dry basis. This study contributes to understanding the potential of Sarawak *C. liberica* sp. pulp as a source of bioactive compounds, while also demonstrating the advantages of MAE over traditional extraction methods. Despite the absence of antibacterial activity, the extract exhibited potential antioxidant properties, emphasizing the potential health benefits and applications of coffee by-products.

Keywords: Sarawak *C. liberica* sp. pulp, microwave-assisted extraction (MAE), DPPH, fourier transform-infrared (FTIR), high performance liquid chromatography (HPLC)

Kajian Kebolehlaksanaan tentang Pencirian Fizikokimia Pulpa Kopi liberica sp.

Sarawak

ABSTRAK

Kopi adalah salah sejenis minuman yang paling banyak diminum di seluruh dunia. Aroma unik, rasa, dan kesan rangsangannya telah menjadikan ia tidak dapat dipisahkan dalam kopi iaitu kehidupan harian bagi kebanyakan individu. Industri kopi didominasi oleh dua spesies, Coffea arabica dan Coffea canephora (Robusta), namun, spesies ketiga, Coffea liberica, meskipun kurang diketahui, memiliki potensi yang signifikan. Kajian ini bertujuan untuk meningkatkan penggunaan Kopi Liberica sp. Sarawak dari aspek fungsinya dan membantu mengurangkan pencemaran dengan memanfaatkan limbah pulpa kopi. Kajian ini fokus kepada pengoptimuman ekstraksi menggunakan bantuan mikro gelombang (MAE) dengan metodologi permukaan respons (RSM) bagi mendapatkan hasil maksimum total fenolik, flavonoid, dan kandungan karbohidrat dari Pulpa Kopi Liberica sp. Sarawak. Pemerhatian mendapati MAE pada kuasa mikro gelombang 700 W, masa pancaran 180 s, dan nisbah pelarut-ke-bahan makanan 87:1 adalah keadaan optimum untuk rawatan pulpa kopi tersebut. Selain itu, keberkesanan ekstraksi bantu mikro gelombang (MAE) dibandingkan dengan ekstraksi Soxhlet (SE) dan ekstraksi macerasi konvensional (CME) dalam kajian ini. Perbandingan dengan CME mengesahkan keberkesanan MAE kerana ia menunjukkan kelebihan hasil ekstraksi yang lebih tinggi dalam masa ekstraksi yang lebih pendek. Tidak ada perbezaan signifikan yang diperhatikan ($p < 0.05$) antara MAE dan SE pada kecekapan ekstraksi TPC, TFC, dan TCC. Sifat antioksidan ekstrak pulpa kopi telah dinilai menggunakan ujian aktiviti penangkapan DPPH dan ia menunjukkan aktiviti antioksidan bergantung pada dos. Penghambatan maksimum diamati pada kepekatan 2.5 mg/mL, menunjukkan keberadaan sebatian bioaktif. Namun, tidak ada aktiviti antibakteria

yang dikesan terhadap Escherichia coli dan Staphylococcus aureus dalam kondisi eksperimen. Pencirian ekstrak melalui analisis Fourier transform-infrared (FTIR) mengenal pasti kehadiran pelbagai kumpulan berfungsi berkaitan dengan karbohidrat, lemak, asid organik, dan sebatian fenolik. Kromatografi Cairan Prestasi Tinggi (HPLC) menunjukkan kepekatan kafein ialah 2.62 ± 0.31 ppm dalam ekstrak cecair dan $0.05 \pm 0.01\%$ (w/w) berdasarkan berat kering. Kajian ini menyumbang kepada pemahaman tentang potensi Pulpa Kopi Liberica sp. Sarawak sebagai sumber sebatian bioaktif, di samping menunjukkan kelebihan MAE berbanding kaedah ekstraksi tradisional. Meskipun tidak ada aktiviti antibakteria, ekstrak menunjukkan potensi sifat antioksidan, potensi untuk manfaat kesihatan dan potensi aplikasi produk sampingan daripada kopi.

Kata kunci: liberica sp. Sarawak, bantu-mikro gelombang (MAE), DPPH, fourier transform-infrared (FTIR), Kromatografi Cecair Prestasi Tinggi (HPLC)

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
CCD	Central Composite Design
CME	Conventional Maceration Extraction
DDPH	2,2-diphenyl-1-picrylhydrazyl
DMSO	Dimethyl sulfoxide
<i>E. coli</i>	<i>Escherichia coli</i>
FTIR	Fourier Transform Infrared
HPLC	High Performance Liquid Chromatography
MAE	Microwave-assisted Extraction
R ²	Regression Coefficient
RSM	Response Surface Methodology
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
SE	Soxhlet Extraction
TCC	Total Carbohydrate Content
TFC	Total Flavonoid Content
TPC	Total Phenolic Content
UV-VIS	Ultraviolet visible

CHAPTER 1

INTRODUCTION

1.1 Background Study

Coffee is one of the most popular beverages in the world, with billions of cups consumed every day. According to the International Coffee Organization's report, global coffee consumption for the coffee years 2021 and 2022 was estimated to be around 170.3 million 60 kg bags (Ralph, 2023). This would be roughly equivalent to 9.998 million metric tons (ICO, 2021). Arabica (80%) and Robusta (20%) are the two most widely grown and traded varieties of coffee, whereas Liberica accounts for less than 1% of global production (Ismail et al., 2022). In Malaysia, however, Arabica is rarely grown in highland areas, with Liberica (73%) and Robusta (27%) being the two most popular coffee kinds. According to Azmil (1991), Robusta and Liberica coffee may be successfully grown in Malaysia because of its ideal growing temperature range of 18°C to 28°C (maximum at 34°C). Nevertheless, Arabica can only be cultivated in altitude regions, such as the Cameron Highlands in Pahang, where temperatures must be below 23°C.

Coffee manufacturing generates a variety of by-products, including wasted coffee grounds, coffee wastewater, coffee pulp, coffee husks, coffee silver skin, and coffee parchment (Reichembach & Petkowicz, 2020). Coffee pulp makes up most of the solid waste produced during the wet processing of coffee. Studies on coffee pulp have confirmed coffee pulp to be abundant in health-promoting nutrients such as fibre, proteins, carbohydrates, minerals (especially potassium), tannins, polyphenols, and caffeine (Murthy & Madhava Naidu, 2012). In addition, Sarawak *C. liberica* sp. pulp has been found to have high levels of phenolic and flavonoid compounds, reducing sugar analysis, and a higher

level of radical scavenging activity (Nillian et al., 2020), indicating its potential as a natural source for obtaining bioactive compounds of interest and further develop into functional food. For example, antioxidant-rich food products such as energy bars, cereal bars, or snack bars can be developed from the extracted phenolic and flavonoid compounds. Besides, it can also be used as an ingredient in prebiotic supplements, such as capsules or powders, due to its high level of reducing sugars, which promote the growth of beneficial bacteria in the gut. However, the coffee pulp harms the ecosystem by contaminating lakes and rivers close to coffee processing facilities. Suppose marketable by-products are extracted from the coffee pulp in large-scale commercial secondary processing installations using a technology that benefits the environment. This issue might become an opportunity (Manasa et al., 2021). Therefore, it may be better to exploit the coffee pulp to extract bioactive compounds.

Extraction is the initial and most crucial stage to isolating bioactive ingredients from natural sources. The knowledge of effective bioactive compound extraction from *Liberica* needs to be improved. It has been discovered that traditional extraction methods, including maceration, infusion, and simple extraction, take a long time, cost a lot to operate, and provide minimal output (Fang et al., 2011). However, Ince et al. (2014) found that microwave-assisted extraction (MAE) may be used to substitute traditional extraction methods and increase bioactive compounds' yield while also speeding up the extraction process and using less solvent. One of the cutting-edge extraction methods, MAE, has been widely used to separate, evaluate, and quantify bioactive components. In MAE, solvents are heated when they encounter solid or liquid samples (or heated samples, such as fresh tissues), facilitating the partition of chemicals relevant to the sample into the solvent. According to published research, several variables, for example, the type of solvent, power,

microwave irradiation time, sample-to-solvent ratio, and matrix type, affect MAE yields (Mandal et al., 2007). Because coffee pulp may be used to obtain bioactive compounds, it will be helpful to determine the experimental conditions for extracting bioactive compounds with the highest yield using MAE.

Advanced analytical techniques, such as high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), and Fourier-transform infrared spectroscopy (FTIR), have revolutionised the science industry by providing powerful tools to characterise and quantify bioactive compounds in complex matrices. These techniques have facilitated the discovery and identification of novel compounds with potential applications in various fields, such as food, pharmaceuticals, and cosmetics (Rostagno et al., 2013). Implementing these advanced analytical methods has not only led to a deeper understanding of the composition and properties of natural products. However, it has also contributed to developing quality control standards, ensuring the safety and efficacy of the products derived from these compounds. Furthermore, these techniques have fostered interdisciplinary research, bridging the gaps between chemistry, biology, and engineering, and driving innovation across the science industry. These techniques have changed how scientists approach the study of bioactive compounds and their potential benefits by supplying precise and trustworthy data, ultimately improving human health and well-being (Scalbert et al., 2005).

1.2 Problem Statement

Coffee pulp, a by-product generated during the wet processing of coffee, has traditionally been considered waste due to its potential negative impact on the environment and its limited suitability for animal feed. When used as animal feed, the presence of

tannins and their monomers in the pulp has been associated with anti-nutritive or toxic effects. Additionally, the coffee pulp has been known to contribute to pollution in rivers and lakes near coffee processing facilities. However, this challenge could be transformed into an opportunity by extracting potentially valuable bioactive compounds from the coffee pulp using field-friendly, large-scale commercial methods for secondary processing (Manasa et al., 2021). Identifying and recovering bioactive compounds from coffee pulp could lead to developing new products, simultaneously addressing environmental concerns and creating additional revenue streams.

Liberica coffee, which originated in Liberia, West Africa, is a lesser-known coffee variety that accounts for less than 2% of global coffee production (Longhi, 2019). Research on the physicochemical properties and structural characterisation of biological compounds from Sarawak *C. liberica* sp. pulp is still in its infancy. Limited information is available regarding the mechanisms underlying their biological activities. Investigating these properties could provide valuable insights into the potential applications of Liberica coffee pulp in various industries, such as food, beverage, pharmaceuticals, and cosmetics.

Developing effective extraction techniques for bioactive compounds from Sarawak *C. liberica* sp. pulp is crucial, as different extraction parameters can significantly influence the functional characteristics of the extracted compounds. These parameters may include time, solvent volume, solvent type, pH, power, and concentration. A study by Nillian et al. (2020) investigated the aqueous extraction of bioactive compounds from Sarawak *C. liberica* sp. pulp, providing initial insights into the potential value of this coffee variety. However, research on microwave-assisted extraction of bioactive compounds from Sarawak *C. liberica* sp. pulp and the effects of microwave variables on extraction efficiency has not been reported.

Exploring microwave-assisted extraction methods for bioactive compounds from Sarawak *C. liberica* sp. pulp could lead to more efficient and sustainable extraction techniques. This approach could enable the recovery of high-quality bioactive compounds while reducing energy consumption and extraction time (Mandal et al., 2007), ultimately contributing to developing value-added products and promoting a circular economy in the coffee sector (Mushtaq et al., 2020). Future research should focus on optimising extraction parameters, characterising the bioactive compounds obtained, and evaluating their potential applications in various industries. This would enhance our understanding of Sarawak *C. liberica* sp. pulp and support the development of innovative and sustainable solutions for utilising coffee by-products (Ballesteros et al., 2018).

1.3 Objectives of the Study

1. To optimise microwave-assisted extraction (MAE) conditions to obtain maximum yield of total phenolic content, total flavonoid content, and total carbohydrate content from Sarawak *C. liberica* sp. pulp.
2. To compare the effectiveness of microwave-assisted extraction (MAE) with conventional maceration extraction (CME) and Soxhlet extraction (SE).
3. To characterise the antioxidant, antibacterial, and selected chemical properties of Sarawak *C. liberica* sp. pulp extract.

1.4 Hypotheses of the Study

1. Microwave-assisted extraction (MAE) conditions can be optimised using response surface methodology (RSM) to obtain maximum yield of total phenolic content,

total flavonoid content, and total carbohydrate content from Sarawak *C. liberica* sp. pulp.

2. MAE is more efficient than conventional maceration extraction (CME) and Soxhlet extraction in terms of extraction yield and time efficiency.
3. Extracts from Sarawak *C. liberica* sp. pulp exhibit dose-dependent antioxidant activity as determined by the DPPH scavenging activity assay.
4. The extracts from Sarawak *C. liberica* sp. pulp will exhibit antibacterial activity against bacterial strains, such as *E. coli* and *S. aureus*.
5. The FTIR spectrum and HPLC analysis of the Sarawak *C. liberica* sp. pulp extract will reveal the presence of bioactive compounds and quantify the caffeine concentration, respectively.

CHAPTER 2

LITERATURE REVIEW

2.1 Sarawak *Coffea liberica* sp.

2.1.1 Origin and Taxonomy of Sarawak *Liberica* sp. Coffee

Coffea liberica sp. is an exotic and lesser-known coffee species that has gained attention in recent years in the coffee industry. Several studies have examined the historical context of Liberica coffee, including the Sarawak *C. liberica* sp. variety. According to Topik (2009), Liberica coffee originates from West Africa, specifically Liberia, and was introduced to Southeast Asia during the colonial period to diversify the coffee industry and reduce the impact of diseases afflicting Arabica and Robusta coffee varieties. The introduction and subsequent cultivation of Liberica coffee, including the Sarawak *C. liberica* sp. variety, shaped the local coffee industry and the livelihoods of smallholder farmers in the region. This historical context illuminates the cultural and economic significance of Sarawak *C. liberica* sp. and its current function in the coffee industry.

The cultivation and refining methods of Liberica coffee cast light on the traditional practises of smallholder farmers and the difficulties they face in maintaining the crop's quality and sustainability. In the context of Sarawak *C. liberica* sp., it is essential to comprehend these cultivation and refining techniques in order to preserve the product's quality and marketability. Studies such as Jaramillo et al. (2013) have highlighted the need for enhanced agronomic practises, modern processing techniques, and adequate infrastructure to improve the quality of Liberica coffee, which can be applied to the Sarawak *C. liberica* sp. variety. Furthermore, studies on the socioeconomic factors influencing the adoption of sustainable practices in coffee cultivation (Bacon et al., 2017)

can direct the creation of policies and support systems for Sarawak *C. liberica* sp. producers.

Several studies have examined the environmental impact of coffee cultivation, including that of Liberica coffee. Researchers such as Nojonen et al. (2013) have studied the environmental impact of coffee cultivation on factors including greenhouse gas emissions, water consumption, and biodiversity. Understanding the environmental implications of Sarawak *C. liberica* sp. cultivation is crucial for promoting sustainable practises and minimising coffee production's negative effects on the environment. This information can guide the development of sustainable agricultural practises, such as agroforestry systems, integrated pest management, and soil conservation techniques, as well as the implementation of conservation efforts for the preservation of the unique genetic resources of Liberica coffee. In addition, research on the socio-environmental factors influencing coffee producers' adoption of sustainable practises (Rueda et al., 2017) can support the development of targeted interventions and policy measures for promoting environmentally friendly cultivation of this unique coffee variety.