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RESEARCH ARTICLE

Preliminary Evaluation of Supercritical Fluid Extraction Conditions for Phenolic and Flavonoid Compounds Recovery from *Swietenia macrophylla* Seeds

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Abstract *Swietenia macrophylla* is a tropical hardwood tree recognized for its bioactive compounds, including phenolics and flavonoids, which offer potential antioxidants, anti-inflammatory, and other therapeutic benefits. However, the extraction process has not been thoroughly investigated, particularly due to their oily nature. This study investigates the influence of supercritical fluid extraction (SFE) parameters on the total phenolic content (TPC) and total flavonoid content (TFC) of *S. macrophylla* seeds, incorporating ethanol as the polar modifier. The effects of CO_2 flow rate, temperature, and pressure on bioactive compound recovery were analysed. The finding indicates that a CO_2 flow rate of 2 mL/min, a temperature of 60°C, and a pressure of 20 MPa yielded the highest TPC (74.00 mg GAE/g) and TFC (42.34 mg QE/g). The *S. macrophylla* seeds extract exhibited a scavenging activity of 21.062±1.45 mg TE/g using the FRAP assay. Gas chromatography-mass spectrometry (GC-MS) identified eight major bioactive compounds, with linoleic (62.88±0.71%) and oleic acids (15.24±0.90%) as the predominant fatty acids. The presence of eugenol, a polar compound, highlights the effectiveness of ethanol as a modifier in enhancing SFE, enabling the extraction of both polar and non-polar compounds from S. macrophylla seed oil. The results demonstrate the enhanced efficiency of SFE with ethanol as a modifier compared to SFE alone. These findings provide valuable insights into optimizing SFE parameters for the efficient recovery of phenolic and flavonoid compounds from S. macrophylla seeds. Future research utilizing a Design of Experiments (DOE) approach will further deepen our understanding of the extraction process and its potential applications.

Keywords: *Swietenia macrophylla,* total phenolic content, total flavonoid content, supercritical fluid extraction.

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Introduction

Swietenia macrophylla King, commonly known as ‘sky fruit’ or ‘Tunjuk Langit’ in Malaysia, derives its name from the upward orientation of its fruits. *S. macrophylla* trees are easily found in Malaysia and is found in over 40 countries, including Brazil, Bolivia, Mexico, Guatemala, and other parts of Central America [1]. *S. macrophylla* is classified within the Meliaceae family, which encompasses several economically and ecologically significant species. The tree is valued not only for its timber but also for its medicinal properties, making it a focal point in ecological and pharmacological research [2-3]. *S. macrophylla* has been extensively used in traditional medicine, especially in Asia and other regions where it is widely distributed [4]. The seeds of *S. macrophylla* are recognized for their therapeutic applications, including the treatment of hypertension and diabetes, as well as their antimicrobial properties [5]. In Malaysia, the seeds are used traditionally as they believe *S. macrophylla* seeds can

alleviate high blood pressure and diabetes, as well as pain reliever [6]. The pharmacological effects attributed to the seeds are primarily due to their rich composition of bioactive compounds.

Recent studies on *S. macrophylla* have highlighted its rich phytochemical profile, particularly focusing on phenolic and fatty acid compounds. The seeds of this plant are remarked for their diverse array of bioactive constituents, including limonoids such as swietenine, which have demonstrated significant pharmacological effects, especially in the management of diabetes. Swietenine has demonstrated a strong antidiabetic effect, enhanced the insulin sensitivity and helped to regulate blood sugar levels [4]. The presence of this limonoid, in conjunction with phenolic compound, play a significant role in the antioxidant activity of the seed oil, which has demonstrated protective effects against oxidative stress and inflammation [7]. Supercritical fluid extraction (SFE), particularly utilizing supercritical carbon dioxide (SC-CO_2), has emerged as a highly effective technique for extracting fatty acid compound. This method operates under mild temperature and pressure conditions, which aids in preserve the quality of sensitive compounds while optimizing their recovery [8], [9]. The ability of SC-CO_2 to act as a non-toxic and non-flammable solvent further enhances its appeal as an environmentally friendly alternative to traditional organic solvents [8,10]. Moreover, SFE can be customized to extract a diverse array of phytochemicals by manipulating their important parameters such as temperature, pressure, and the incorporation of polar modifiers [11]. The addition of polar co-solvents can increase the solubility and extraction efficiency of polar compounds, thereby increase the yield of phenolic and fatty acid extracts [12]. A study by Kaur [13] indicated that the utilization of ethanol and water mixtures as co-solvents in SC-CO_2 extraction substantially enhanced the TPC, TFC, and antioxidant activity of the resulting extracts. Co-solvents, such as ethanol, modulate solvent potency and improve the solubilization of polar compounds when used with SC-CO_2 [13].

Despite the increasing interest in medicinal properties of *S. macrophylla* seeds, research has yet to investigate the combination of SFE with a polar modifier, such as ethanol, to enhance the extraction of its valuable bioactive compounds. The non-polar nature of SC-CO_2 extraction has traditionally limited its application to extracting non-polar compounds, highlighting a gap in optimizing conditions for extracting polar bioactive components such as TPC and TFC. This limitation emphasizes the necessity for preliminary studies to examine extraction parameters before delving deeper into optimizing conditions for these valuable compounds. Consequently, this study aims to fill these gaps by optimizing SC-CO_2 parameters with ethanol as a polar co-solvent to enhance the recovery of TPC and TFC from *S. macrophylla* seeds.

Materials and Methods

Sample Preparation

S. macrophylla seeds were obtained from a local seller in Malaysia and subsequently authenticated and verified at the Forest Research Institute Malaysia (FRIM), Kepong, Selangor. As an authentic representative of the Meliaceae family, the species was verified with voucher specimen PID270818-25, as shown in Figure 1. The seeds were carefully selected to ensure they were free from physical damage or contamination. Following collection, they were dried at 60±5°C in a hot air oven for 24h to reduce moisture content and preserve their quality. After drying, the seeds were finely ground and passed through a 0.75 mm mesh sieve to achieve a uniform particle size. The resulting powder was stored in airtight containers under cool, dry conditions to maintain its stability and prevent degradation until further analysis.

Figure 1. The appearance of *S. macrophylla* seeds

Supercritical Fluid Extraction (SFE) of *S. macrophylla* Seed Oil

To maximize the extraction of total phenolic content (TPC) and total flavonoid content (TFC) from *S. macrophylla* seeds, single-factor experiments were conducted to determine the ideal CO_2 flow rate, temperature, and pressure. In this preliminary phase, extraction parameters were initially set to a CO_2 flow rate of 2 mL/min, a temperature of 50°C, and a pressure of 30 MPa, with ethanol maintained at a fixed concentration of 5% (v/v) as a polar co-solvent and a total extraction duration of 180 minutes based on recommendation by Hasmida *et al*. [14]. Approximately 5 g of ground seed material was loaded into an extraction vessel, sealed, and kept at the designated operating temperature. In each experimental run, one parameter was varied while the others remained constant. Liquid CO_2, supplemented with ethanol, was pumped into the vessel, and after the extraction period, the system was depressurized to collect the oil yield. The collected extract was then transferred to a vial and subjected to solvent evaporation at 40°C, after which the resulting oil was sealed and stored at 4°C to prevent degradation. The SFE apparatus for *S. macrophylla* seeds oil extraction is illustrated as in Figure 2, and the facility is located at the Centre of Lipid Engineering and Applied Research (CLEAR), Faculty of Chemical and Energy Engineering, Universiti Teknologi Malaysia, Skudai, Johor.

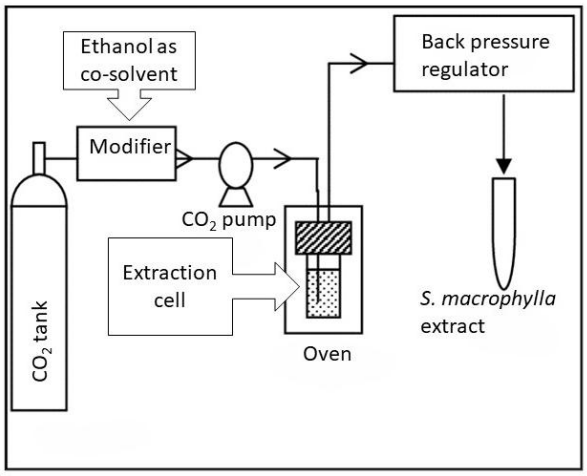

Figure 2. Supercritical fluid extraction (SFE) workflow setup

Determination of Total Phenolic Content

The total phenolic content (TPC) of the *S. macrophylla* seed extract was measured using a modified Folin-Ciocalteu reagent protocol [15]. For this assay, 2000ppm of the extract and a series of Gallic acid standards were diluted with absolute ethanol. To 1 mL of each diluted sample and standard, 5 mL of a 10% Folin-Ciocalteu reagent was added. After allowing the reaction to proceed for 10 minutes, 4 mL of a 7.5% sodium carbonate solution was introduced, and the mixtures were incubated in the dark at ambient temperature for 60 minutes. The resulting colour change was measured at 760 nm using a UV-visible spectrophotometer (Shimadzu UV-Vis 1800, Japan). The TPC was quantified using the Gallic acid calibration curve and expressed as milligrams of Gallic acid equivalents per gram of extract (mg GAE/g).

Determination of Total Flavonoid Content

A modified aluminium chloride ($AlCl_3$) colorimetric assay, adapted from Zhishen *et al*. [16], was employed to quantify the total flavonoid content (TFC) in *S. macrophylla* seeds extract. Calibration standards were prepared using quercetin dissolved in absolute ethanol at various concentrations, while the *S. macrophylla* extract was diluted to 2000 ppm in the same solvent. In each assay, an aliquot of 0.5 mL of the diluted extract or quercetin standard was pipetted into a 10 mL volumetric flask preloaded with 2 mL of distilled water. Then, 0.15 mL of sodium nitrite ($NaNO_2$) was introduced and allowed to react at ambient temperature for 5 minutes. This was followed by the addition of 0.15 mL of $AlCl_3$, and after another 5 minutes, 1 mL of 1 M sodium hydroxide (NaOH) was added. The mixture was then vortexed and left to stand at room temperature for an additional 15 minutes. Absorbance was measured at 415 nm using a Shimadzu UV-1900i spectrophotometer (Shimadzu Corporation, Kyoto, Japan) [17]. The TFC was calculated using the quercetin calibration curve and expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g extract).

Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

The *S. macrophylla* extract was examined using an Agilent 6890 gas chromatograph coupled to an Agilent 5973 mass spectrometer, operated with Agilent Chemstation software (Agilent Technologies, Palo Alto, CA, USA). Separation was carried out on an HP-5MS capillary column (30 m length, 0.25 mm internal diameter, coated with a 0.25-µm film). The oven was initially held at 70°C for 2 minutes, then programmed to ramp up to 285°C over 20 minutes. Helium served as the carrier gas at a flow rate of 1.2 mL/min, with the injector and detector maintained at 280°C and 250°C, respectively. Mass spectra were recorded in scan mode over a range of 20–500 m/z using an ionization energy of 70 eV. Compound identification was performed by comparing the obtained spectra with the NIST/Wiley Library and verifying GC retention indices against literature data [5].

Antioxidant Analysis

The total antioxidant capacity of the *S. macrophylla* seeds extract and linoleic acid standard was determined using the Ferric Reducing Ability of Plasma (FRAP) assay to evaluate their antioxidant power [18]. This assay is based on the ability of antioxidants to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), which subsequently form a blue Fe^{2+}-TPTZ complex. The FRAP reagent was freshly prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3$ in a 10:1:1 (v/v/v) ratio. For each reaction, FRAP reagent was mixed with 100 µL of the sample solution in a microplate well and incubated for 30 minutes at room temperature. The absorbance was measured at 593 nm using a spectrophotometer. A standard calibration curve was constructed using varying concentrations of Trolox, and results were expressed as mg Trolox equivalent per gram of dry weight (mg TE/g). All analyses were conducted in triplicate, and all solutions were prepared fresh on the day of the experiment to ensure accuracy and reproducibility.

Statistical Analysis

The experimental data for the optimum process were statistically analysed using a paired-sample t-test with IBM SPSS Statistics (Version 25.0.0.0, IBM, New York, USA). All experiments were conducted in triplicate, and the results are expressed as the mean ± standard deviation.

Results and Discussion

Effect Of CO_2 Flow Rate

Figure 3 illustrates the impact of CO_2 flow rate variations on the TPC and TFC in the extracts. The flow rate was adjusted between 2 and 4 mL/min while maintaining a constant pressure of 30 MPa and a temperature of 50°C. This flow rate range was selected based on a previous study. Rochfort *et al*. [19] reported that an excessively low flow rate prevents the system from functioning properly, whereas an excessively high flow rate leads to system shutdown due to overpressurization. Additionally, a high flow rate may reduce the residence time, causing the solvent to exit the extractor unsaturated, which can lower extraction efficiency [20]. Therefore, a flow rate of 2–4 mL/min was chosen for this study, ensuring sufficient contact time between supercritical CO_2 and the sample matrix for effective solubilization and extraction of target compounds.

The data revealed that the optimal extraction conditions were achieved at a flow rate of 2 mL/min, where the TPC reached 69.4 mg GAE/g and the TFC peaked at 63.23 mg QE/g. Conversely, as the flow rate was increased to 3 and 4 mL/min, both TPC and TFC levels declined, with the lowest values observed at 4 mL/min, measuring 44.79 mg GAE/g for TPC and 37.75 mg QE/g for TFC. A strong negative correlation was observed between CO_2 flow rate and both TPC ($r = -0.98$) and TFC ($r = -0.99$).

De Silva *et al*. [21] reported that using a lower CO_2 flow rate with ethanol as a co-solvent yielded higher phenolic content compared to higher flow rates. Findings by Hasmida *et al*. [14] found that the highest yield was obtained at a CO_2 flow rate of 2 mL/min (0.37%), compared to 4 mL/min, which resulted in a lower yield of 0.29% TPC in *Quercus infectoria* extract. The higher yield at lower flow rates can be attributed to improved solute-solvent saturation due to increased residence time. In addition, SFE with a low solvent flow rate is preferred for extracting seeds with high oil content, as it helps prevent the sample from becoming compacted in the vessel, which could block the complete extraction of the oil [22]. Furthermore, the combination of SFE with ethanol significantly improves the extraction efficiency compared to single-solvent systems, indicating that the use of binary solvent systems can extract a greater variety of phenolic and flavonoid compounds than single-solvent systems [23].

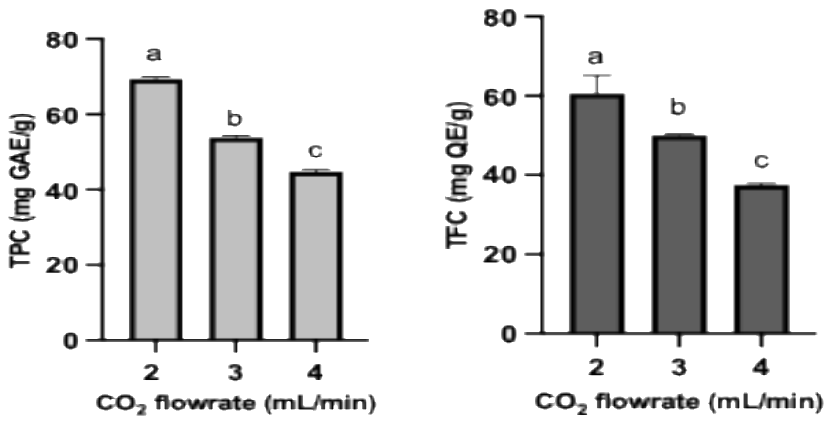

Figure 3. Effect of different CO_2 flow rates on TPC and TFC in *S. macrophylla* seeds extract. Values are mean±SD of triplicate analysis; Means with different letters denote significant differences of TPC and TFC values ($p<0.05$) within the ranges of CO_2 flowrate

Effect of Temperature

In the next evaluation, a series of extractions were conducted across a range of 40 °C to 70 °C, while maintaining a constant CO_2 flow rate of 2 mL/min and a pressure of 30 MPa. The initial temperature of 40°C was selected because it is slightly above the critical temperature of CO_2 (31°C). Operating at a slightly higher temperature ensures that CO_2 remains in its supercritical state, where it exhibits both liquid-like density and gas-like diffusivity. This enhances its solvent properties, optimizing the extraction of valuable compounds from the crude extract [24]. However, temperatures above 70°C may cause thermal degradation of the compounds, potentially reducing their bioactivity [25].

Figure 4 shows the effect of varying temperatures on TPC and TFC after extraction. Figure 4 presents the influence of temperature variations on TPC and TFC following extraction. The data indicate that an extraction temperature of 60°C yielded the highest TPC value. Under these conditions, the TPC was measured at 66.98 mg GAE/g, representing the highest peak value achieved in the study. Additionally, the TFC also reached its maximum at the same temperature, with a recorded value of 42.07 mg QE/g. The lowest TPC values were observed at temperatures 40°C and 50°C with no significant difference between them ($p<0.05$). These were followed by a further decline in TPC at 70°C. In contrast, TFC values showed a decreasing trend when extraction began at 40°C. As the temperature increased to 60°C, the TFC reached its highest value of 42.07 mg QE/g. However, a sharp decline was noted at 70°C, where the TFC dropped to 22.83 mg QE/g. TPC exhibited a weak positive correlation with temperature ($r =0.22$), indicating that temperature had little effect on its extraction yield. In contrast, TFC showed a moderate negative correlation with temperature ($r =-0.50$), suggesting that higher temperatures led to a noticeable decline in flavonoid content.

Research indicates that phenolic and flavonoid compounds are thermolabile, making them prone to degradation under elevated temperatures [26]. As the temperature increased, a decline in polyphenol content was observed, likely due to the reduced extraction efficiency and the thermal breakdown of these bioactive compounds. Tyskiewicz *et al*. [26] demonstrated that low temperatures are sufficient for extracting phenolic compound using SFE with ethanol as the modifier. This finding is supported by Valadez-Carmona *et al*. [27], who reported that the optimal conditions for *Theobroma cacao* extraction achieved both the highest extraction efficiency and the maximum TPC value at 60°C and 300 bar. Additionally, Hossein *et al*. [28] have found that increasing extraction temperatures positively affected TPC up to a certain point, but further increases will lead to diminishing returns and potential degradation of the compounds. These findings emphasize the significance of temperature optimization in SFE to enhance the yield of bioactive compounds effectively.

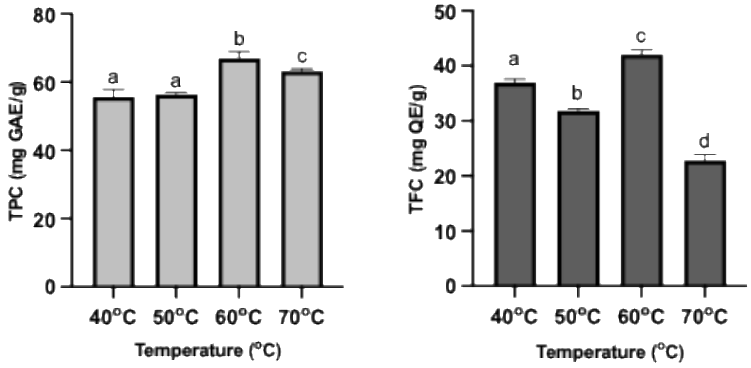

Figure 4. Effect of different temperatures on TPC and TFC in *S. macrophylla* seeds extract. Values are mean±SD of triplicate analysis; Means with different letters denote significant differences of TPC and TFC values ($p<0.05$) within the ranges of temperature

Effect of Pressure

The influence of pressure on the extraction of TPC and TFC from *S. macrophylla* seeds was examined to determine the optimal conditions for maximizing their yield. Pressure levels of 15 MPa and 20 MPa were evaluated, while CO_2 flow rate and temperature were maintained at constant values. Figure 5 illustrates the effect of pressure variation on TPC and TFC following extraction. The results demonstrated a consistent upward trend for both parameters as pressure increased from 15 MPa to 20 MPa. Notably, the TPC exhibited a significant rise from 56.77 mg GAE/g to 74.00 mg GAE/g, while the TFC increased from 34.94 mg QE/g to 42.34 mg QE/g. Both TPC and TFC showed strong positive correlation ($r = 1$) against pressure. These findings suggest that higher pressure enhances the extraction efficiency of phenolic and flavonoid compounds. The data further underscore the crucial role of pressure in improving compound recovery, likely due to increased mass transfer efficiency at elevated pressure levels.

This observation aligns with the findings of Avilés-Betanzos *et al*. [25], who reported that increasing pressure enhances the density of both the solvent and co-solvent, thereby improving extraction efficiency. Likewise, Pellicanò *et al*. [29] demonstrated that higher pressure positively influences the extraction of phenolic compounds, including catechin, chlorogenic acid, quercetin, naringenin, and luteolin, during the extraction process of tomato peel, by using SFE. Elevated pressure will facilitates deeper solvent penetration into the plant matrix, leading to an improved recovery of bioactive compounds. Pressures ranging from 20 MPa to 30 MPa were considered for the optimization study, however further increases beyond 30 MPa were not feasible due to the maximum operational capacity of the SFE system.

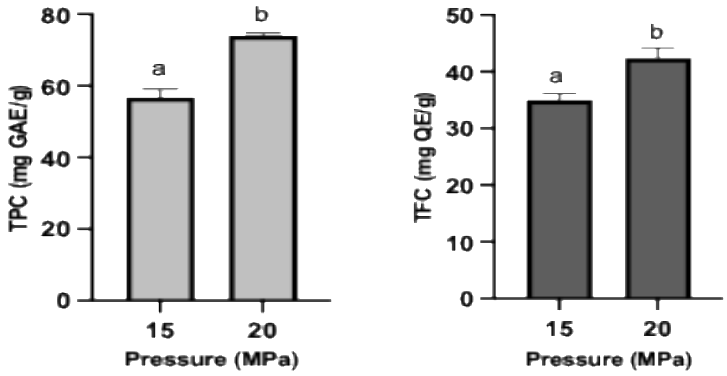

Figure 5. Impact of pressure variation on TPC and TFC in *S. macrophylla* seeds extract. Values are mean±SD of triplicate analysis; Means with different letters denote significant differences of TPC and TFC values ($p<0.05$) within the ranges of pressure

GCMS Analysis

Gas chromatography-mass spectrometry (GCMS) identification of the optimized *S. macrophylla* seeds extracts at a CO_2 flow rate of 2 mL/min, a temperature of 60°C, and a pressure of 20 MPa identified eight key bioactive compounds, as illustrated in Figure 6, with their respective concentrations are summarized in Table 1. The concentration of these compounds ranged from 0.15±0.00% to 62.88±0.71%. As shown in Figure 6, the GCMS spectra indicated that linoleic acid (62.88±0.71%) and oleic acid (15.24±0.90%) were the dominant fatty acids in the *S. macrophylla* extract obtained via SC-CO_2 extraction with the addition of ethanol as a polar modifier. The levels of linoleic acid and oleic acid observed in this study were notably higher compared to previous studies, where oleic acid (18.56%) and linoleic acid (17.72%) were reported as the primary components in *S. macrophylla* seeds oil extracted via methanol maceration [30]. The higher linoleic acid content in our study is likely due to the selective and efficient nature of SFE, which helps to preserve sensitive compounds like linoleic acid by preventing thermal degradation. SC-CO_2 was effectively utilized as a solvent in this extraction method, owing to its relatively low critical temperature, which protects bioactive compounds from thermal decomposition, a limitation often observed in traditional extraction methods [31].

Interestingly, the detection of eugenol, indicates that the inclusion of ethanol enhances the extraction of a wide range of both hydrophilic and lipophilic compounds from *S. macrophylla* seed oil. The presence of eugenol, although in smaller quantities (0.81%), is also noteworthy as shown in Table 1. Eugenol, a phenolic compound, is known for its potent antioxidant, anti-inflammatory, and antimicrobial effects. It has demonstrated significant potential in regulating blood glucose levels and enhancing insulin sensitivity. Studies suggest that eugenol can alleviate oxidative stress, a key factor in the onset and progression of diabetes [32]. The GCMS profile of the *S. macrophylla* seeds extract reveals the identification of multiple compounds with promising therapeutic potential for managing diabetes and enhancing lipolysis activity. Specifically, linoleic acid and oleic acid ethyl ester offer significant potential for improving insulin sensitivity and promoting fat metabolism, while eugenol adds further benefits with its antioxidant and anti-inflammatory properties [32-33].

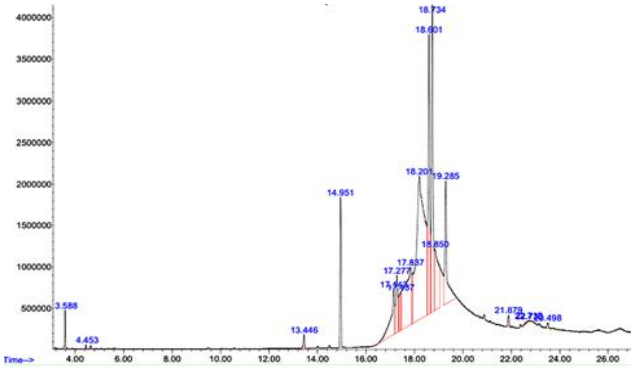

Figure 6. GCMS spectra of *S. macrophylla* seed oil extract

Table 1. GCMS analysis for *S. macrophylla* seed oil extract

No	Name	Common name	RT (min)	Relative abundance (%)
1	9,12-Octadecadienoic acid (Z,Z)	Linoleic acid (cis-cis isomer)	18.20	62.88±0.71
2	(E)-9-Octadecenoic acid ethyl ester	Oleic acid ethyl ester	18.73	15.24±0.90
3	Linoelaidic acid	Linoelaidic acid	18.85	7.85±0.53
4	Hexadecanoic acid, methyl ester	Methyl palmitate	14.95	7.39±0.06
5	Octadecanoic acid, ethyl ester	Stearic acid ethyl ester	19.28	4.76±0.31
6	Eugenol	Eugenol	3.58	0.79±0.03
7	E-11-Hexadecen-1-ol acetate	Hexadecen-1-ol acetate	20.87	0.15±0.00
8	Other natural compound			0.93±0.00

Antioxidant Analysis

The antioxidant activity of optimized *S. macrophylla* seed extract at a CO_2 flow rate of 2 mL/min, a temperature of 60°C, and a pressure of 20 MPa was evaluated by FRAP assay. The extract exhibited a scavenging activity of 21.062 ± 1.45 mg TE/g based on the FRAP assay. The relatively lower FRAP value, despite a high TPC, suggests that not all phenolic compounds contribute equally to reducing power. The FRAP assay measures the ability of antioxidants to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), forming a blue Fe^{2+}-TPTZ complex that indicates the sample's reducing potential [18-19]. While TPC quantifies total phenolics, it does not differentiate between those with strong and weak antioxidant capacities. Some phenolics, such as simple phenols, may be abundant yet possess low redox potential, resulting in a lower FRAP value [34]. Furthermore, FRAP specifically measures ferric ion reduction capacity, which depends on the structural characteristics of individual phenolic compounds [19]. The presence of eugenol, a polar phenolic compound, at only 0.81% in the extract may also influence the observed antioxidant activity [32]. Although eugenol is known for its antioxidant properties due to its free hydroxyl group, its low concentration suggests a minimal contribution to the overall FRAP value. Additionally, linoleic acid, the major compound in the extract, exhibited a FRAP value of only 1.285 ± 0.77 mg TE/g. While linoleic acid is an unsaturated fatty acid with some antioxidant properties, its reducing power is considerably weaker than that of phenolic compounds. Its limited direct radical scavenging ability and high susceptibility to oxidation may further reduce its antioxidant effectiveness [35]. To obtain a more comprehensive evaluation of the extract's antioxidant potential, future studies should employ multiple antioxidant assays, such as 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay (ABTS) and Oxygen Radical Absorbance Capacity assay (ORAC). Since *S. macrophylla* seed oil is known for its antidiabetic activity, alpha-amylase and alpha-glucosidase inhibition assays could also be used to assess the effectiveness of the polar modifier compared to SFE extraction without any modification.

Conclusion

This study emphasizes the crucial influence of CO_2 flow rate, temperature, and pressure on the efficient extraction of TPC and TFC from *S. macrophylla* seed oil through SFE, with the addition of ethanol as a polar modifier. The results identified the optimal conditions for maximizing both TPC and TFC, achieved at a CO_2 flow rate of 2 mL/min, a temperature of 60°C, and a pressure of 20 MPa. These findings provide valuable insights into the optimization of SFE parameters for improving the extraction yield of bioactive compounds. Despite the relatively low antioxidant activity observed, further investigations into alternative antioxidant assays and the potential effects of ethanol as a polar modifier are needed. Moving forward, to deepen the understanding of the extraction process, future research should incorporate a Design of Experiments (DOE) approach to systematically evaluate the intricate interactions among the key extraction parameters, thereby paving the way for more efficient and targeted extraction methodologies.

Conflicts of Interest

The authors declares that they have no conflict of interest in relation to the publication of this article.

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