JOBIMB, 2025, Vol 13, No 2, 81-87
https://doi.org/10.54987//jobimb.v13i2.1141

JOURNAL OF BIOCHEMISTRY, MICROBIOLOGY AND BIOTECHNOLOGY
Website: http://journal.hibiscuspublisher.com/index.php/JOBIMB/index
JOBIMB VOL 13 NO 2 2025
AI and Biochemistry

Extraction of Phenolic Compounds from Banana Inflorescence (*Musa acuminata*) Using Supercritical Carbon Dioxide

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History

Received: 29th May 2025
Received in revised form: 25th July 2025
Accepted: 10th Aug 2025

Keywords

Antioxidants
Banana inflorescence
Ethanol
Flavonoids
Supercritical carbon dioxide

SDG Keywords

SDG 12 Responsible Consumption and Production
SDG 9 Industry, Innovation and Infrastructure
SFG 3 Good Health and Well-Being

Abstract

Banana inflorescence from Musa acuminata, frequently seen as agricultural waste, is a substantial source of bioactive compounds such as polyphenols and flavonoids. Efficient extraction methods can unlock its potential for functional food. This study aims to evaluate the impact of ethanol as a co-solvent concentration on extract yield, total flavonoid content (TFC), and antioxidant activity. Banana inflorescence was dried using an oven at 60 °C for 4 hours, ground into a fine powder, and stored in vacuum-sealed containers at -20 °C. For maceration, 10 g of the powder was combined with 100 mL of ethanol, agitated for 24 hours, and the ethanol was subsequently evaporated. For supercritical carbon dioxide ($scCO_2$) extraction, 10 g of powder was extracted using 50% and 100% ethanol as co-solvents under four conditions: 60 °C, 40 MPa; 60 °C, 25 MPa; 40 °C, 40 MPa; and 40 °C, 25 MPa. Extract yield, TFC, and antioxidant activity were measured, with flavonoid content quantified as quercetin equivalents (QE) and antioxidant capacity evaluated using IC50 values. The maximum yield (4.80%) was achieved using $scCO_2$ extraction with 100% ethanol at 60 °C and 40 MPa, attributed to enhanced solubility at elevated temperature and pressure. TFC was consistently higher with $scCO_2$ than maceration, reaching 552.94±0.84 mg QE/g using 50% ethanol at 60 °C and 40 MPa due to improved CO_2 density. Antioxidant activity peaked $scCO_2$ extraction using 100% ethanol at 40 MPa and 40 °C yielding the lowest IC50 (7.97±0.68), indicating superior radical scavenging. These results highlight $scCO_2$ extraction's efficacy in maximizing bioactive compounds recovery and antioxidant potential from the banana inflorescence.

INTRODUCTION

Banana (*Musa spp.*), particularly *Musa acuminata*, is one of the most extensively cultivated fruit crops in tropical and subtropical regions, with global production exceeding 119 million metric tons annually [1]. It is a vital staple and economic resource for millions, especially in countries across Asia, Africa, and Latin America. While bananas are primarily cultivated for their fruit, the plant also generates substantial biomass in the form of pseudostems, peels, and inflorescences. Unfortunately, these by-products are often discarded as agricultural waste despite their potential for value-added applications. Among these neglected parts, banana inflorescence, or banana blossom, has recently drawn attention for its remarkable nutritional and phytochemical profile [2]. Banana inflorescence is traditionally consumed in various Asian cuisines, particularly in India, the Philippines, Thailand, and Indonesia. It is known for its high dietary fiber, essential minerals (iron, potassium, calcium), and a diverse array of bioactive compounds such as flavonoids, tannins, and polyphenols [3]. These compounds contribute significantly to the antioxidant, anti-inflammatory, antidiabetic, and antimicrobial properties of banana blossom, making it a promising candidate for the development of functional foods and nutraceuticals [4]. Despite these benefits, its commercial use remains limited, largely due to a lack of awareness and underutilization in food processing industries.

In the era of sustainable development and increasing demand for health-promoting foods, there is a pressing need to explore plant-based, natural alternatives to synthetic antioxidants and supplements. Functional foods, defined as those that provide health benefits beyond basic nutrition, are a rapidly growing

segment of the global food market [5]. In parallel, the nutraceutical industry, where it comprising products that offer medical or health benefits including the prevention and treatment of disease, is projected to exceed USD 450 billion by 2027 [5]. These trends present a strong case for the exploration of underexploited plant materials like banana inflorescence as sustainable sources of bioactive compounds.

Polyphenols and flavonoids, the dominant phytochemicals in banana inflorescence, are known for their strong free radical scavenging activity and ability to modulate oxidative stress pathways. Their biological activity is closely linked to the prevention of chronic diseases such as cancer, cardiovascular disorders, and neurodegenerative conditions [6]. Total phenolic content (TPC) and total flavonoid content (TFC) are therefore widely used as indicators of the therapeutic potential of plant-based extracts. However, the actual recovery of these compounds from plant material is heavily influenced by the extraction method employed. Traditionally, methods such as maceration, Soxhlet extraction, and solvent percolation have been used to extract bioactive compounds from plant matrices.

These techniques rely on organic solvents like ethanol, methanol, and acetone to dissolve target compounds. While they are relatively simple and inexpensive, conventional extraction methods often involve long processing times, high solvent consumption, and the risk of thermal degradation of sensitive compounds [7] Additionally, the low selectivity and efficiency of these methods may result in suboptimal yields and reduced antioxidant activity in the final extracts. In contrast, supercritical fluid extraction (SFE), particularly using carbon dioxide (CO_2), offers a green, efficient, and highly tunable alternative. Supercritical CO_2 ($scCO_2$) behaves as both a gas and a liquid under conditions above its critical temperature (31.1°C) and pressure (7.38 MPa), enabling it to penetrate plant matrices and dissolve non-polar to moderately polar compounds with high efficiency [8]. The addition of co-solvents, such as ethanol, enhances the polarity of $scCO_2$, facilitating the extraction of more polar constituents like phenolics and flavonoids. This makes $scCO_2$ extraction a versatile technique for recovering a wide range of bioactive molecules while preserving their stability and bioactivity [6].

Moreover, $scCO_2$ extraction is regarded as an environmentally friendly and sustainable method. It uses CO_2, which is non-toxic, recyclable, and leaves no solvent residues in the final product. The low-temperature operating conditions help protect thermolabile compounds from degradation, which is particularly important when targeting antioxidants and polyphenols that are sensitive to heat [9]. The extraction parameters such as temperature, pressure, and co-solvent concentration make $scCO_2$ an ideal platform for optimizing yield and quality. Despite its advantages, the use of $scCO_2$ extraction for banana inflorescence remains underexplored in scientific literature.

Most studies on banana-based extracts have focused on peels, pulp, or pseudostems, leaving a significant research gap regarding the optimization of extraction protocols for banana blossoms. Furthermore, there is limited comparative analysis between conventional methods like maceration and advanced techniques like $scCO_2$ extraction in the context of banana inflorescence. Understanding how different ethanol concentrations, temperatures and pressures influence the extraction efficiency of phenolics, flavonoids, and antioxidants is crucial for the development of scalable and sustainable extraction processes. While the health benefits of banana inflorescence have been recognized in traditional medicine, the scientific literature on the bioactive properties and efficient extraction of these compounds is still in its infancy. A limited number of studies have investigated the chemical composition of banana inflorescence, with most focusing on antioxidant activities, antimicrobial properties, and nutritional profile [10,11]. These studies have primarily relied on conventional extraction methods such as maceration or Soxhlet extraction, with little emphasis on optimizing conditions to maximize the recovery of bioactive compounds such as polyphenols and flavonoids. Moreover, very few have compared the efficacy of different extraction techniques in terms of yield and bioactivity, especially with regard to $scCO_2$ extraction.

A key research gap in the field is the lack of systematic studies comparing $scCO_2$ extraction with traditional methods, particularly in the context of banana inflorescence. While $scCO_2$ has been extensively studied for its ability to extract bioactive compounds from a variety of plant materials, its application to banana inflorescence has not been fully explored. Specifically, the impact of different co-solvent concentrations, particularly ethanol, on the efficiency of $scCO_2$ extraction and the preservation of bioactive compounds has yet to be investigated. This gap is particularly important given that ethanol is a commonly used co-solvent in $scCO_2$ extraction due to its ability to enhance the solubility of polar compounds, such as phenolics and flavonoids, which are known for their antioxidant properties [12].

Additionally, while there is growing interest in the potential of banana inflorescence as a functional food ingredient, much of the current research focuses on the antioxidant and antimicrobial properties in isolation, without addressing the overall extraction efficiency, scalability, and sustainability of the process. Therefore, comprehensive studies that optimize extraction conditions for both yield and bioactivity are necessary to unlock the full potential of banana inflorescence in food and nutraceutical applications. Furthermore, the environmental sustainability of extraction processes remains a critical concern in the food industry. $scCO_2$ extraction stands out as a potential solution, being both eco-friendly and efficient. However, comprehensive comparisons between this method and traditional techniques in terms of energy consumption, solvent use, and overall sustainability have yet to be conducted. Given these research gaps, the current study aims to evaluate the extraction of bioactive compounds from banana inflorescence using both maceration and $scCO_2$ extraction methods.

This study will specifically investigate the impact of ethanol concentration as a co-solvent in $scCO_2$ extraction on the yield, total flavonoid content (TFC), and antioxidant activity of the extract. By doing so, this research seeks to identify the optimal extraction conditions (temperature, pressure, ethanol concentration) for maximizing bioactive compound recovery from banana inflorescence, to assess the antioxidant activity of the extracts using IC50 values, which provide a measure of the extract's ability to scavenge the free radicals, and finally contributing to the development of more sustainable and efficient extraction processes for bioactive compounds from agricultural waste, particularly banana inflorescence.

The expected outcome of this study is that the use of supercritical CO_2 extraction, in combination with ethanol as a co-solvent, will outperform traditional maceration methods in terms of extract yield, total phenolic and flavonoid content, and antioxidant activity. More specifically, it is hypothesized that $scCO_2$ extraction will yield higher concentrations of bioactive

compounds while preserving their antioxidant properties due to the ability to operate at lower temperatures and pressures. Furthermore, the study hypothesizes that varying ethanol concentrations will play a critical role in enhancing the solubility of phenolic and flavonoid compounds in the supercritical fluid, thereby optimizing the overall extraction process. Looking forward, the outcomes of this study could open doors to a range of applications in the food and nutraceutical sectors. Functional foods that promote antioxidant protection, anti-inflammatory effects, and immunomodulatory activity are gaining popularity among health-conscious consumers.

Banana inflorescence, with its rich bioactive compounds, could become an important ingredient in these formulations. For example, the extracts could be incorporated into beverages like smoothies, teas, and health tonics, or into snack foods designed to promote overall wellness. Furthermore, nutraceutical products such as dietary supplements or capsules containing concentrated extracts from banana inflorescence could serve as natural alternatives to synthetic antioxidants and pharmaceutical drugs. These products could be marketed as adjuncts to the management of chronic diseases such as diabetes, cardiovascular diseases, and cancer, where oxidative stress and inflammation play key roles in disease progression [7].

Preliminary studies on the antioxidant and anti-inflammatory properties of banana inflorescence have already shown promise, and this research will help confirm its broader potential. In the field of cosmetics and personal care, the antioxidant and anti-inflammatory properties of banana inflorescence could also lend themselves to topical applications [11]. Banana blossom extracts could be explored for use in skincare products such as creams, lotions, and serums aimed at reducing the signs of aging, protecting against UV damage, and soothing irritated skin. Given the global rise in demand for natural and plant-based cosmetic ingredients, this represents another promising avenue for the commercialization of banana inflorescence.

The extraction of bioactive compounds from plant materials is a complex process influenced by a variety of factors, including the nature of the plant matrix, solvent properties, extraction technique, and operational conditions such as temperature, pressure, and time. For banana inflorescence, which contains a diverse array of bioactive compounds such as polyphenols, flavonoids, and tannins, selecting an appropriate extraction method is crucial to maximizing both yield and bioactivity [8]. While traditional extraction methods like maceration and Soxhlet extraction are widely used, they often fail to achieve the high yields and retention of bioactivity that are necessary for commercial applications in functional foods and nutraceuticals. Therefore, advanced extraction techniques such as supercritical CO_2 ($scCO_2$) extraction are gaining popularity due to their efficiency, sustainability, and ability to preserve sensitive bioactive compounds [13].

One of the key advantages of $scCO_2$ extraction is its ability to operate under conditions that prevent the degradation of heat-sensitive compounds. Unlike conventional methods that require high temperatures and long extraction times, $scCO_2$ extraction uses carbon dioxide in its supercritical state, which behaves like both a gas and a liquid, allowing it to penetrate plant tissues efficiently and selectively extract target compounds. By adjusting the pressure and temperature conditions, it is possible to optimize the solubility of bioactive compounds and improve extraction efficiency [8]. The addition of ethanol as a co-solvent further enhances the ability of $scCO_2$ to extract polar compounds like polyphenols and flavonoids, making it a versatile method for recovering a wide range of bioactive molecules. However, while $scCO_2$ extraction offers numerous advantages, its efficiency is highly dependent on the optimization of operational parameters, particularly temperature, pressure, and co-solvent concentration. Studies on the extraction of bioactive compounds from other plant materials have shown that varying these parameters can significantly impact the yield, chemical composition, and antioxidant activity of the extracts [14].

Therefore, a key component of this study is to investigate the effect of different ethanol concentrations (50% and 100%) in combination with varying pressure (25 MPa and 40 MPa) and temperature (40 °C and 60 °C) on the extraction efficiency of banana inflorescence. The goal is to determine the optimal conditions that maximize the yield of polyphenols, flavonoids, and antioxidant activity while minimizing solvent usage and processing time. In addition to the $scCO_2$ extraction technique, the study will also evaluate traditional maceration as a baseline method. Maceration is a simple, low-cost extraction technique that involves soaking the plant material in a solvent for a period of time to allow the bioactive compounds to dissolve. While maceration is widely used in both laboratory and industrial settings, it often requires the use of large quantities of solvent and lengthy extraction times, which can lead to inefficient recovery of bioactive compounds.

Furthermore, maceration may result in the loss of volatile or thermolabile compounds due to prolonged exposure to solvent and ambient temperatures [7]. Moreover, optimizing extraction conditions for banana inflorescence requires careful consideration of various parameters that could influence the solubility and stability of the bioactive compounds. In particular, the interaction between the solvent (ethanol) and CO_2 plays a critical role in modulating the solubility of polar and non-polar compounds. Ethanol, when used as a co-solvent, can significantly alter the polarity of the supercritical fluid, enabling the extraction of a broader spectrum of bioactive compounds, including polyphenols, flavonoids, and alkaloids, which are known for their beneficial health effects [12]. The concentration of ethanol used in the extraction process will be varied to determine the optimal co-solvent concentration that enhances the recovery of bioactive compounds while minimizing solvent consumption and maximizing sustainability.

The extraction process will be conducted under different temperature and pressure conditions to explore how these factors influence the solubility of the target compounds and the overall efficiency of the extraction. For example, higher temperatures are generally expected to increase the solubility of certain compounds, while higher pressures improve the density of the supercritical CO_2, leading to higher extraction efficiency [15]. However, both parameters need to be carefully balanced to avoid degradation or transformation of sensitive compounds, such as polyphenols and flavonoids, which may be prone to thermal degradation at elevated temperatures [13]. By adjusting the temperature and pressure within the optimal range for banana inflorescence, this study will provide a comprehensive understanding of how these factors influence the yield and bioactivity of the extracts. Therefore, the focus on eco-friendly, sustainable extraction methods has the bigger potential to align with global trends in green chemistry and the growing demand for natural, plant-based products in the food and nutraceutical industries.

MATERIALS AND METHODS

Chemicals Preparation used for The Antioxidant Assay

The chemicals used in this study included potassium acetate, sodium carbonate, and Folin-Ciocalteu's reagent, which were obtained from Chemiz, UK. Other reagents such as aluminum chloride, quercetin hydrate, ethanol (95% and 99.8%), methanol (99.8%), and DPPH were purchased from Sigma Chemical Co., Malaysia. High-purity CO_2 (99.9%) was supplied by Alpha Gas Solution (AGS), Malaysia. Distilled water was used throughout the experiments and sourced from BERNAS Lab, Faculty of Food Science and Technology, UPM.

Supercritical Carbon Dioxide Extraction

The extraction was performed using a lab-scale supercritical fluid extraction system at Universiti Putra Malaysia (UPM). The setup included a CO_2 cylinder, chiller, high-pressure pump, stainless steel extractor, flowmeter, and back-pressure regulator. A 10 g sample of dried, sieved banana inflorescence powder (moisture <10%) was mixed with 10 g of ethanol (50% and 100% concentrations) and placed in a tea filter bag. This was inserted into the extractor chamber. CO_2 was cooled and pumped at a constant flow rate of 3.0 mL/min until the desired pressure was reached. Extraction was carried out continuously for 90 minutes. The resulting extracts were collected in aluminum-wrapped tubes and stored at -4 °C before analysis.

Separation of Polyphenols

Polyphenols were separated using a method adapted from Sungpud et al. [16]. Each extract was mixed with 5 mL of hexane and 5 mL of methanol/water (60:40, v/v), then vortexed for 2 minutes. The mixture was centrifuged at 3500 × g for 10 minutes to separate the layers. The hexane layer was re-extracted using another 5 mL of methanol/water in the same way. The methanol layer was then used for analysis of total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity.

Determination of Total Flavonoid Content (TFC)

The total flavonoid content (TFC) of the banana inflorescence extract was determined using a method adapted from Chang et al. [17]. A quercetin standard stock solution (0.01 g in 10 mL of 95% ethanol) was prepared. Solutions of 10% aluminum chloride and 1M potassium acetate were also prepared using distilled water. Five quercetin standard solutions (50–250 µg/mL) were made. For each sample, 0.5 mL of extract was mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M potassium acetate, and 2 mL of distilled water. The mixture was vortexed and incubated in the dark at room temperature for 40 minutes. Absorbance was measured at 415 nm using a spectrophotometer, and TFC was expressed as mg quercetin equivalent per gram of extract (mg QE/g).

Antioxidant Activity by DPPH Scavenging Assay

The antioxidant activity of the banana inflorescence extract was measured using the DPPH radical scavenging method, based on Marina et al. with slight modifications [18]. A 0.2 mM DPPH solution was prepared by dissolving 0.0039 g of DPPH in 50 mL of 60% methanol. For the assay, 2 mL of the DPPH solution was mixed with 2 mL of the extract at varying concentrations (0, 50, 100, 150, 200, and 250 µg/mL), vortexed, and incubated in the dark at room temperature for 30 minutes. The absorbance was then measured at 525 nm using a UV-Vis spectrophotometer. The radical scavenging activity (RSA%) was calculated as:

$$RSA\ (\%) = ((A_1 - A_2) / A_1) \times 100$$

A_1 = absorbance of control (DPPH only)
A_2 = absorbance of sample with DPPH after 30 minutes

The IC_{50} value, defined as the concentration of extract required to inhibit 50% of DPPH radicals, was determined from a calibration curve plotted using RSA values against sample concentrations, fitted to the linear equation $y = 0.0003x$ with a coefficient of determination (R^2) of 0.9366. All measurements were performed in duplicate.

Statistical Analysis

All data were reported as mean ± standard deviation from duplicate of data. One-way ANOVA and Tukey's Test were used for analysis using SPSS Software and Microsoft Excel 2021.

RESULT AND DISCUSSION

Total Yield Percentage

Total yield percentage refers to the efficiency of the extraction process and is calculated as the percentage ratio of the weight of the dried extract obtained to the initial weight of the dried banana inflorescence powder. It serves as an important parameter to assess the effectiveness of solvent extraction under varying conditions. A higher yield indicates more efficient extraction of soluble compounds from the plant matrix [20]. As shown in **Table 1**, the maceration method produced the highest total yield percentage (7.13% ± 0.004), significantly ($p<0.05$) greater than all supercritical CO_2 ($scCO_2$) extraction conditions. This high yield is attributed to the long extraction time, frequent agitation, and the use of ethanol at room temperature, which allows for better extraction of both polar and non-polar compounds [19,20].

Table 1. The total yield percentage of banana inflorescence extracts obtained under different extraction methods between maceration and $scCO_2$ and conditions with different pressures and temperatures for different concentrations of EtOH in $scCO_2$ extraction.

Extraction Methods	Total Yield Percentage (%)
Maceration	7.13 ± 0.004
60°C, 40 MPa	4.80
40°C, 40 MPa	4.30
60°C, 25 MPa	3.60
40°C, 25 MPa	3.40
$scCO_2$ (100% EtOH)	
60°C, 40 MPa	3.70
40°C, 40 MPa	3.20
60°C, 25 MPa	3.10
40°C, 25 MPa	2.70
$scCO_2$ (50% EtOH)	

Note: Values are expressed as means ± standard deviation.

In contrast, $scCO_2$ extraction using 100% ethanol gave lower yields, ranging from 4.80% at 60 °C and 40 MPa to 3.40% at 40 °C and 25 MPa. The highest yield occurred at higher temperatures and pressure due to improved solubility and CO_2 density [21]. At lower conditions, the lower yield suggests reduced efficiency for extracting polar compounds due to the non-polar nature of CO_2, even with ethanol as a co-solvent. When using 50% ethanol, yields were slightly lower but still showed similar trends. The highest yield (3.70%) was again at 60 °C and 40 MPa, while the lowest (2.70%) was at 40 °C and 25 MPa. Although 50% ethanol increases polarity and helps extract polar compounds, it may not be effective at lower pressure or temperature due to weaker solubility and mass transfer [22].

Previous studies have shown that $scCO_2$ requires optimal conditions, such as moderate temperature between 35 to 60 °C and higher pressure around 400 bar, to be effective, especially for heat-sensitive compounds. The $scCO_2$ method also offers better selectivity depending on the target compounds and may require modifiers like ethanol for extracting polar components [23]. Despite maceration showing higher overall yields, $scCO_2$ extraction has strong potential for more targeted and sustainable extraction when process conditions and solvent concentration are carefully optimized. For instance, 100% ethanol works better for less polar compounds, while 50% ethanol favors polar compounds [24]. In conclusion, maceration is an effective method for general extraction, while $scCO_2$ provides a more sustainable and efficient alternative for industrial applications, especially when aiming to extract specific bioactive compounds.

Total Flavonoid Content (TFC)

Flavonoids are important plant compounds with strong health benefits, such as anti-cancer, antioxidant, anti-viral, and anti-inflammatory effects [25]. Common flavonoids found in banana inflorescence include catechin, epicatechin, quercetin, and rutin [26]. **Table 2** shows the total flavonoid content (TFC) in banana inflorescence extracts using different extraction methods and conditions.

Table 2. The total flavonoid contents for banana inflorescence extracts were obtained under different extraction methods between maceration and $scCO_2$ and conditions with different pressures and temperatures for different concentrations of EtOH in $scCO_2$ extraction.

Extraction Methods		Total Flavonoid Contents (mg of quercetin/g of sample extract
Maceration		249.02 ± 16.98[a]
$scCO_2$ (100% EtOH)	60°C, 40 MPa	405.88 ± 0.78[a]
	40°C, 40 MPa	376.47 ± 1.05[b]
	60°C, 25 MPa	523.53 ± 1.10[b]
	40°C, 25 MPa	288.24 ± 0.86[a]
$scCO_2$ (50% EtOH)	60°C, 40 MPa	552.94 ± 0.84[b]
	40°C, 40 MPa	347.06 ± 0.76[a]
	60°C, 25 MPa	435.29 ± 0.65[b]
	40°C, 25 MPa	464.71 ± 0.71[b]

Note: Values ($p < 0.05$) are expressed as means ± standard deviation. Same subscript letters between the rows do not significantly differ from each other, while different subscript letters mean they have significant differences from the other values.

Maceration, a simple method using ethanol at room temperature, produced the lowest flavonoid content at 249.02 ± 16.98 mg quercetin/g. Supercritical carbon dioxide ($scCO_2$) extraction showed much higher TFC values, especially when 50% ethanol was used as a co-solvent. The highest flavonoid yield was obtained at 60 °C and 40 MPa, reaching 552.94 ± 0.84 mg quercetin/g, the lowest tested $scCO_2$ condition (40 °C and 25 MPa), the yield was still high at 464.71 ± 0.71 mg/g, showing that $scCO_2$ with 50% ethanol is highly effective.

When using 100% ethanol, $scCO_2$ with the best yield is 523.53 ± 1.10 mg/g at 60 °C and 25 MPa. However, lower temperatures and pressures, such as 40 °C and 25 MPa, gave a reduced yield (288.24 ± 0.86 mg/g), which is still better than maceration. Statistical analysis confirmed that $scCO_2$ extraction methods produced significantly more flavonoids than maceration ($p < 0.05$). The best results came from using high pressure and temperature, which help extract more compounds by improving solvent power.

Previous studies support these findings. For example, research on pine wood and turmeric showed that higher pressure and temperature, combined with polar solvents like ethanol, improved the extraction of flavonoids and other polar compounds [27,28]. The better performance of 50% ethanol compared to 100% ethanol can be explained by solvent polarity. A mix of ethanol and water increases the ability to dissolve polar compounds like flavonoids. Other studies found that 50:50 ethanol-water mixtures extracted more tannins and flavonoids than pure ethanol or water alone [29,30].

Thus, maceration is less efficient for extracting flavonoids, while $scCO_2$ extraction with 50% ethanol at 60 °C and 40 MPa gave the highest flavonoid content in this study. These results show that $scCO_2$ with ethanol-water mixtures is a powerful and sustainable option for extracting valuable compounds from plant materials, especially for industrial use, where high yield and low solvent usage are important.

DPPH Free Radical Scavenging Activity (RSA)

Antioxidants are essential in neutralising free radicals, thereby protecting cells from oxidative damage. A common method for evaluating antioxidant activity is the DPPH radical scavenging assay, which measures the ability of antioxidants to reduce DPPH radicals. The effectiveness of this reaction is expressed by the IC_{50} value, which refers to the concentration of extract required to inhibit 50% of the DPPH radicals. A lower IC_{50} value indicates stronger antioxidant activity, making it a reliable indicator for comparing extraction methods [31].

As shown in **Table 3**, the IC50 value for banana inflorescence extract obtained through maceration was 15.60 ± 0.82, indicating moderate antioxidant activity. In contrast, the supercritical CO_2 ($scCO_2$) extraction method showed greater efficiency, particularly at optimized conditions. The lowest IC50 value (7.97 ± 0.68), reflecting the highest antioxidant activity, was recorded at 40 °C and 40 MPa using 100% ethanol as a co-solvent. Higher temperatures, such as 60 °C, resulted in higher IC50 values, suggesting that elevated heat may degrade thermally sensitive antioxidant compounds.

Table 3. The half-maximal inhibitory concentration (IC_{50}) value for banana inflorescence extracts was obtained under different extraction methods, between maceration and $scCO_2$ and conditions with different pressures and temperatures for different concentrations of EtOH in $scCO_2$ extraction.

Extraction Methods		Half-maximal inhibitory concentration (IC_{50}) value
Maceration		15.597 ± 0.82[a]
$scCO_2$ (100% EtOH)	60°C, 40 MPa	44.14 ± 0.26[a]
	40°C, 40 MPa	7.97 ± 0.68[b]
	60°C, 25 MPa	27.89 ± 0.24[a]
	40°C, 25 MPa	19.94 ± 0.19[a]
$scCO_2$ (50% EtOH)	60°C, 40 MPa	68.00 ± 0.25[b]
	40°C, 40 MPa	10.39 ± 0.29[b]
	60°C, 25 MPa	46.55 ± 0.63[a]
	40°C, 25 MPa	88.02 ± 0.36[a]

Note: Values ($p < 0.05$) are expressed in means ± standard deviations. The same subscript letters between the rows do not significantly differ from each other while different subscript letters mean they have significant differences between the other values.

When 50% ethanol was used, the results varied. A high IC_{50} value of 88.02 ± 0.36 was observed at 40 °C and 25 MPa, indicating poor antioxidant activity under those conditions. However, under higher pressure (40 MPa) at the same temperature, the IC_{50} value dropped to 10.39 ± 0.29 as also shown in **Figure 1**, demonstrating that pressure plays a compensatory role in improving extraction performance, even with lower solvent polarity.

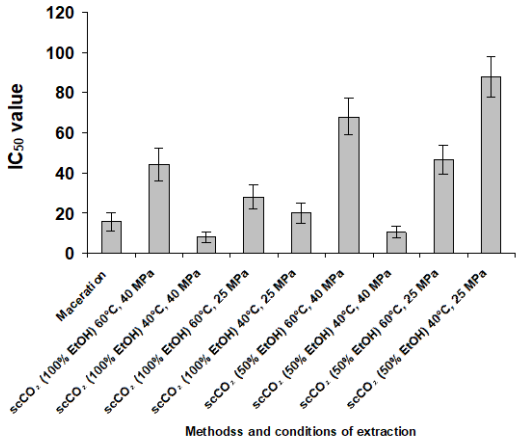

Fig. 1. Half-maximal inhibitory concentration (IC50) value of banana inflorescence extracts obtained under different extraction methods between maceration and $scCO_2$ and conditions with different pressure and temperature for different concentrations of EtOH in $scCO_2$ extraction { [A] 100% EtOH, 60 °C, 40 MPa; [B] 100% EtOH, 40 °C, 40 MPa; [C] 100% EtOH, 60 °C, 25 MPa; [D] 100% EtOH, 40 °C, 25 MPa; [E] 50% EtOH, 60 °C, 40 MPa; [F] 50% EtOH, 40 °C, 40 MPa; [G] 50% EtOH, 60 °C, 25 MPa; [H] 50% EtOH, 40 °C, 25 MPa }.

Based on the figure above, these findings highlight the importance of optimising extraction parameters such as temperature, pressure, and solvent composition. The superior performance of 100% ethanol compared to 50% ethanol may be due to its higher polarity, which enhances the solubility and recovery of antioxidant compounds from banana inflorescence. This observation is consistent with previous studies, such as Radzali et al. (2020), who reported improved antioxidant activity with higher ethanol concentrations during $scCO_2$ extraction [32]. Furthermore, higher pressures increased the solvating power of $scCO_2$, enhancing extraction efficiency, while lower temperatures helped preserve sensitive bioactives. In conclusion, $scCO_2$ extraction at 40 °C and 40 MPa with 100% ethanol was the most effective condition for maximizing antioxidant activity, yielding the lowest IC50 value among all methods tested. This highlights $scCO_2$ as a highly efficient and sustainable alternative to conventional extraction techniques for applications in the food and nutraceutical industries.

CONCLUSION

In conclusion, this study has demonstrated that ethanol concentration significantly influences total yield, total flavonoid content (TFC), and DPPH radical scavenging activity in banana inflorescence extraction using supercritical carbon dioxide ($scCO_2$). The optimal extraction conditions were identified at 40 MPa and 60 °C, which effectively maximized the recovery of bioactive compounds. The findings highlight the promising potential of banana inflorescence extract for applications in the pharmaceutical, food and beverage, and cosmetic industries. However, the study faced certain limitations, including the need to carefully control temperatures, particularly avoiding values above 80 °C to prevent thermal degradation, and time constraints that limited the ability to conduct kinetic studies for both ethanol concentrations.

CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

FUNDING

We express gratitude for the funding provided by Universiti Putra Malaysia (UPM) under the Geran Putra-Inisiatif Putra Muda (project code GP-IPM/2022/9718800), which enabled us to conduct the study. Furthermore, we extend our appreciation to the Department of Food Technology, Faculty of Food Science and Technology, UPM Serdang, for granting us access to their laboratory facilities. No data were used elsewhere to support this study, and it was a completely original dataset.

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