



Impact of Extraction Techniques and Solvent Systems on the Antioxidant Activity and Fatty Acid Composition of *Scenedesmus parvus* Extracts

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Abstract

Scenedesmus parvus, a Malaysian freshwater microalga, has emerged as a promising source of secondary metabolites with potential applications in nutraceutical, pharmaceutical, food, and cosmetic industries. This study aimed to assess the extraction yield, total phenolic and flavonoid content, and antioxidant potential of *S. parvus* extracts obtained using different extraction methods, including sonication, maceration, and Soxhlet extraction methods. Additionally, the fatty acid profile and key chemical constituents of *S. parvus* were analyzed using gas chromatography-flame ionization detection (GC-FID). To date, this study represents one of the first comprehensive investigations into the synergistic interaction between sonication and solvent systems on the *S. parvus*, concurrently evaluating effects on both antioxidant activity and lipid profile. The results indicate that the sonication method with methanol as the extraction solvent yielded the highest extraction efficiency ($14.65 \pm 0.503\%$), whereas the maceration method with hexane resulted in the lowest yield ($1.34 \pm 0.371\%$). Furthermore, the sonicated ethanol extract exhibited the highest total phenolic content (66.32 ± 0.818 mg GAE g⁻¹ DW) and total flavonoid content (684.45 ± 28.928 mg QE g⁻¹ DW). Soxhlet extraction with methanol as solvent demonstrated the highest antioxidant activity, as evidenced by its superior performance in both DPPH (IC₅₀, 0.48 ± 0.035 mg mL⁻¹) and ABTS⁺ (IC₅₀, 0.13 ± 0.003 mg mL⁻¹) radical scavenging assays. These findings highlight the impact of extraction methods on the yield of bioactive compounds and the antioxidant properties of *S. parvus*. In particular, methanol-based extraction demonstrated significant potential for obtaining high antioxidant activity and an optimal fatty acid profile, underscoring its suitability for applications in nutraceutical, pharmaceutical, food, and cosmetic formulations.

Keywords Microalgae · *Scenedesmus parvus* · Fatty acid · Extraction · Antioxidant activity

Introduction

In recent years, consumers have increasingly sought natural antioxidants due to their associated health benefits and their applications across the food, cosmetic, and pharmaceutical

sectors (Atta et al. 2017; Lourenço et al. 2019). Antioxidants play a crucial role in neutralizing free radicals, which helps mitigate oxidative stress and reduce the risk of certain diseases, including cancer, autoimmune diseases, and neurodegenerative disorders (Lobo et al. 2010; Gupta et al. 2014; He et al. 2017; Malekmohammad et al. 2019; Lourenço et al. 2019; Didier et al. 2023). Microalgae have emerged as a significant natural source, recognised for their high concentrations of bioactive compounds exhibiting potent antioxidant activities (Safafar et al. 2015; Banskota et al. 2019; Del Mondo et al. 2021). Several microalgal species, including *Chlorella* sp., *Tetraselmis* sp., *Nannochloropsis* sp., and *Scenedesmus* sp., have been reported to possess significant potential for producing secondary metabolites that can be utilised in the pharmaceutical industry (Tan and Lin 2011; Martin et al. 2014; Sharma et al. 2015; Pantami et al. 2020;

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Wali et al. 2020; Kumar et al. 2021; Montoya-Arroyo et al. 2022; Trentin et al. 2022; Ganeson et al. 2024).

Among the diverse array of microalgae species, *Scenedesmus parvus* has garnered significant attention in Malaysia, particularly those isolated from acidic environments, because it can be considered a resilient strain. This extremophile microalga has generated substantial local interest because of its potential applications across various industries, especially the nutraceutical sector. It is postulated to have substantial levels of polyphenols, flavonoids, and other bioactive compounds known for their antioxidant properties. These compounds act as reducing agents, hydrogen donors, singlet oxygen quenchers, and metal chelators and give multiple protection against oxidative damage in the human body (Wang et al. 2016; Sarian et al. 2017; Shalaby 2013; Coulombier et al. 2021; Coulombier et al. 2021).

Optimizing extraction methods is critical for maximizing yield, purity, and bioactivity of microalgal compounds. Techniques like ultrasonication, maceration, and Soxhlet extraction each present unique advantages and limitations affecting compound stability and recovery (Vongsak et al. 2013; Ameer et al. 2017; Łabowska et al. 2019; Mirzadeh et al. 2020; Monteiro et al. 2020). Furthermore, solvent selection such as water, ethanol, methanol, or mixtures significantly influences extraction efficiency, with parameters like polarity, temperature, and duration directly modulating antioxidant activity (Do et al. 2014; Chigayo et al. 2016; Lim et al. 2019; Muhamad et al. 2019; Nawaz et al. 2020). Given the lack of comparative data on extraction methods for *S. parvus*, this study aims to (1) evaluate extraction yield, total phenolic content and total flavonoid content, and antioxidant potential across sonication, non-sonication (maceration), and Soxhlet methods; (2) characterize the fatty acid profile via GC-FID; and (3) identify the optimal method for maximizing bioactive compound recovery.

Materials and Methods

Microalgae Cultivation

For this study, the chosen microalgae species was *Scenedesmus parvus*, which was identified and isolated by the Bioprocess Technology Division, School of Industrial Technology, Universiti Sains Malaysia.

The microalgae biomass was produced following a modified protocol adapted from a previous study (Hui et al. 2023). The modified algae growth (MLA) medium was used as both the seed culture and biomass production medium. The medium composition consisted of 0.49 g L⁻¹ magnesium sulphate (MgSO₄·7H₂O), 0.17 g L⁻¹ sodium nitrate (NaNO₃), 0.035 g L⁻¹ di-potassium phosphate (K₂HPO₄), and 0.029 g L⁻¹ calcium chloride (CaCl₂·2H₂O). Sterilization of

the medium was achieved using a 0.22-μm Millipore filter. Microalgal seeds were cultivated in 1-L Scott bottles containing 700 mL of the modified MLA medium. The initial microalgal cell concentration was set at 10% (v/v), equivalent to 0.03–0.05 g L⁻¹ (OD₆₈₀=1.0). The cultures were incubated in an illuminated incubator with a controlled environment: a 0.3-L min⁻¹ flow of compressed air, light intensity of 450 μmol m⁻² s⁻¹, and a temperature of 24±0.5 °C. Both microalgal cultures were grown under identical conditions and harvested at the late logarithmic growth phase. The harvested cells were centrifuged at 2000 g for 15 min, rinsed twice with distilled water, and dried at 70 °C for 24 h.

Metabolite Extraction Methods

Ultrasound-Assisted Extraction (UAE)

The extraction procedure was developed based on the previous study (Hui et al. 2023) with minor modifications. The microalgae biomass (2 g) was mixed with 20 mL of organic solvent in sealed conical flasks and sonicated in an ultrasonic bath (GT sonic P3, China) for 25 min at 60 °C. The ultrasonic bath was operated at 100 W and 40 kHz. After being treated with ultrasound, the mixture of solvent and biomass was placed in an incubation shaker set to 150 rpm and maintained at 35 °C for 15 min. After this period, the sample was filtered and dried in an oven. The dried extract was weighed and stored at 4 °C for the subsequent analyses.

Soxhlet Extraction

A 10-g sample of *S. parvus* biomass underwent exhaustive extraction using 400 mL methanol in a 500-mL Soxhlet apparatus (12 h). The extract was filtered, concentrated under vacuum at 60 °C (rotary evaporator), and stored at 4 °C. This Soxhlet-methanol protocol served as the conventional control for all comparative analyses, as it represents the industry-standard exhaustive extraction method (Azwanida 2015). All subsequent yields and bioactivities were calculated relative to this benchmark.

Determination of Microalgae Crude Extraction Method

The optimal extraction process for maximum yield and antioxidant activity was established based on different solvent systems and techniques. This study employed five solvents: methanol, ethanol, chloroform, hexane, and petroleum ether. Two extraction methods were assessed: (i) ultrasound-assisted extraction (UAE) and (ii) maceration extraction. Table 1 presents the detailed test conditions. A comparative analysis of all evaluated parameters was performed against the Soxhlet extraction method, which was regarded as the standard for extraction procedures.

Table 1 Details on tested conditions

Optimized parameter	Tested condition
Solvent system	100% methanol
	100% ethanol
	100% chloroform
	100% hexane
	100% petroleum ether
Extraction method	Ultrasound-assisted extraction method (UAE)
	Maceration

Crude Yield Calculation for Extraction

The crude yield was quantified by measuring the mass of the obtained crude product. The crude extract was then transferred into a pre-weighed aluminium plate, and the solvent was evaporated at 60 °C in a drying oven until a constant weight was achieved. The two solvents that resulted in the highest yields were selected for antioxidant analysis. The yield of the crude extract The crude extract yield was then calculated using Eq. (1), as described in a previous study (Mahmood et al. 2017; Hui et al. 2023):

Crude yield (%) = $\frac{\text{Extracted crude (mg)}}{\text{Microalgae dry weight (mg)}} \times 100\%$

Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

Determination of Total Phenolic Content (TPC)

The total phenolic concentration was estimated using a modification of the method described in the previous study (Herald et al. 2012). Specifically, 25 µL of the extract or standard was mixed with 75 µL of distilled deionized water (DDW) and 25 µL of Folin–Ciocalteu reagent. After

Free radical inhibition (%) = $\frac{\text{Blank Absorbance} - \text{Sample Absorbance}}{\text{Blank Absorbance}} \times 100$

The antioxidant activity of the liquid extracts was expressed as IC50, which represents the concentration necessary to achieve a 50% reduction in the initial DPPH free radical concentration. IC50 values were calculated using linear regression, with ascorbic acid serving as the reference standard.

approximately 6 min of reaction time, 100 µL of 75 g L⁻¹ Na₂CO₃ was added to the mixture. The solution was kept in the dark at room temperature for 90 min for the reaction to occur, after which the absorbance was measured at 765 nm against a reagent blank. Gallic acid was used as a standard within the 0 – 1000 µg mL⁻¹ range to create a calibration curve (R² < 0.9900). The total phenolic concentration was expressed as mg gallic acid equivalent (GAE) per gram of dried sample.

Determination of Total Flavonoid Content (TFC)

Total flavonoid concentration was quantified using the spectrophotometric method described by the previous study (Herald et al. 2012). In detail, 25 µL of the extract was combined with 100 µL of distilled deionized water (DDW) and 10 µL of 50 g L⁻¹ NaNO₂, and the mixture was vortexed for 5 min. Subsequently, 15 µL of 100 g L⁻¹ AlCl₃ was added. The solution was allowed to stand for 6 min, followed by adding 50 µL of 1 mol L⁻¹ NaOH and another 50 µL of DDW. The mixture was shaken for 30 s, and the absorbance was measured at 510 nm against a reagent blank. Total flavonoid concentration was expressed as mg quercetin equivalent (QE) per gram of sample. Quercetin was used as a standard in the 0 – 1000 µg mL⁻¹ range to generate a calibration curve (R² < 0.9900).

Antioxidant Capability

DPPH Scavenging Activity

The antioxidant scavenging activity was assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical method, adhering to protocols established in prior studies with some adjustments (Herald et al. 2012; Xu 2017a; Tibebe et al. 2024). Different dilutions of the samples (50 µL of ascorbic acid or extracts) were mixed with 195 µL of a 0.2 mM methanolic DPPH solution. After incubating for 30 min at 25 °C, the absorbance at 520 nm was measured. The free radical scavenging activity of each solution was determined as a percentage of inhibition using the following formula:

ABTS + Scavenging Activity

The radical scavenging ability of the samples for ABTS + (2,2'-azinobis-3-ethylbenzo-thiazoline-6-sulfonate) was evaluated using the method described in previous studies (Xu 2017b; Moreira 2019; Gu et al. 2019b). The preparation of the ABTS + solution began by mixing 5 mL of a 7 mM ABTS solution adjusted to pH 7.4 with 88 µL of a 140 mM potassium

persulfate solution. This mixture was allowed to react in a dark environment at room temperature for 16 h to ensure the complete formation of the ABTS+ radical cation. A 0.5-mL portion of this mixture was diluted with 45 mL of ethanol to achieve an absorbance of 0.70 ± 0.05 units at 734 nm, measured by spectrophotometry. Different dilutions of the samples (20 μ L of ascorbic acid or extracts) were then mixed with 280 μ L of fresh ABTS+ solution, and absorbance was measured 6 min after the initial mixing. Ascorbic acid served as a reference. The free radical scavenging activity was determined as IC₅₀ (mg mL⁻¹), representing the concentration that reduced the ABTS+ by 50%.

Fatty Acid Composition Analysis

The analysis of fatty acids in the microalgae extract was performed using a gas chromatography with flame ionization detector (GC-FID) system, using the Agilent model 7880B (Agilent Technologies, Santa Clara, CA, USA). Separation of fatty acids was achieved utilizing an Agilent DB-Fast FAME column (60 m $\times$ 250 μ m $\times$ 0.25 μ m). A sample volume of approximately 1 μ L was injected into the GC column. Hydrogen gas served as the carrier gas, flowing at a rate of 40 mL min⁻¹. The temperature program was initiated at 80 °C for 1 min, followed by a ramp to 165 °C at a rate of 40 °C min⁻¹, maintained for 1 min, and concluded with an increase to 230 °C at 4 °C min⁻¹, held for 15 min. The detector temperature was set at 260 °C, while the injector operated at 250 °C with a split ratio 100:1. The identification of each fatty acid within the extract was accomplished by comparing their retention times against a Supelco® standard. Quantification was performed using GC Solution® software; results were presented as percentages relative to the total identified fatty acids.

Statistical Analysis

The results are expressed as mean values accompanied by their respective standard deviations. Data compilation and analysis were facilitated by Microsoft Excel 2016 and GraphPad Prism version 9.1.0 for Windows (GraphPad Software based in San Diego, CA, USA). To analyze the collected data, one-way and two-way ANOVA were employed, supplemented with Tukey's multiple comparison tests to determine statistical significance.

Results

Effect of Sonication and Solvent System on the Extraction Yield

The extraction yields of *S. parvus* obtained using various solvents under sonication and maceration method conditions are presented in Fig. 1. The results indicate significant variations in extraction efficiency depending on the solvent type

and the application of sonication. Notably, extracts obtained through sonication exhibited a significantly higher yield ($p < 0.01$) compared to those extracted without sonication, except for hexane and petroleum ether. Among the solvents tested, methanol demonstrated the highest extraction yield ($p < 0.001$), followed by ethanol and chloroform.

The Impact of Different Extraction Methods on TPC and TFC

For antioxidant potential assessment in *S. parvus*, this study focused solely on methanol and ethanol crude extracts. These solvents were prioritized due to their substantially higher extraction yields compared to other solvents tested (Table 1). We hypothesized that higher-yield extracts would contain a broader spectrum of bioactive compounds, enabling more comprehensive antioxidant profiling. This enhanced compositional diversity was expected to improve assay sensitivity and result reliability.

Extraction efficiency was significantly influenced by both method and solvent (Fig. 2), with sonication with ethanol emerged as the most effective approach, maximizing phenolic contents and flavonoids recovery from *S. parvus* biomass.

Effect of Different Extraction Methods on the Antioxidant Activity

Soxhlet extraction demonstrated the highest antioxidant activity (the lowest IC₅₀) in *S. parvus* extracts, significantly outperforming other methods in scavenging both DPPH (0.48 ± 0.035 mg mL⁻¹) and ABTS+ (0.13 ± 0.003 mg mL⁻¹) free radicals. This superiority was consistent across solvent systems, with methanol-based Soxhlet extracts showing the strongest radical inhibition. A comprehensive summary of the antioxidant activity is presented in Fig. 3.

Fatty acid composition of the *S. parvus* extract.

The data in Table 2 shows a comparison of the fatty acid composition of *S. parvus* extracts obtained using two different methods: Soxhlet extraction and maceration extraction with methanol, with palmitic and oleic acids constituted the major fatty acids in both extracts. The values are expressed in grams per 100 g of dry extract sample.

Discussions

Effect of Sonication and Solvent System on the Extraction Yield

Methanol and ethanol produced the highest extraction yields among all the solvents tested in the study, with methanol

Scenedesmus parvus Extraction Yield

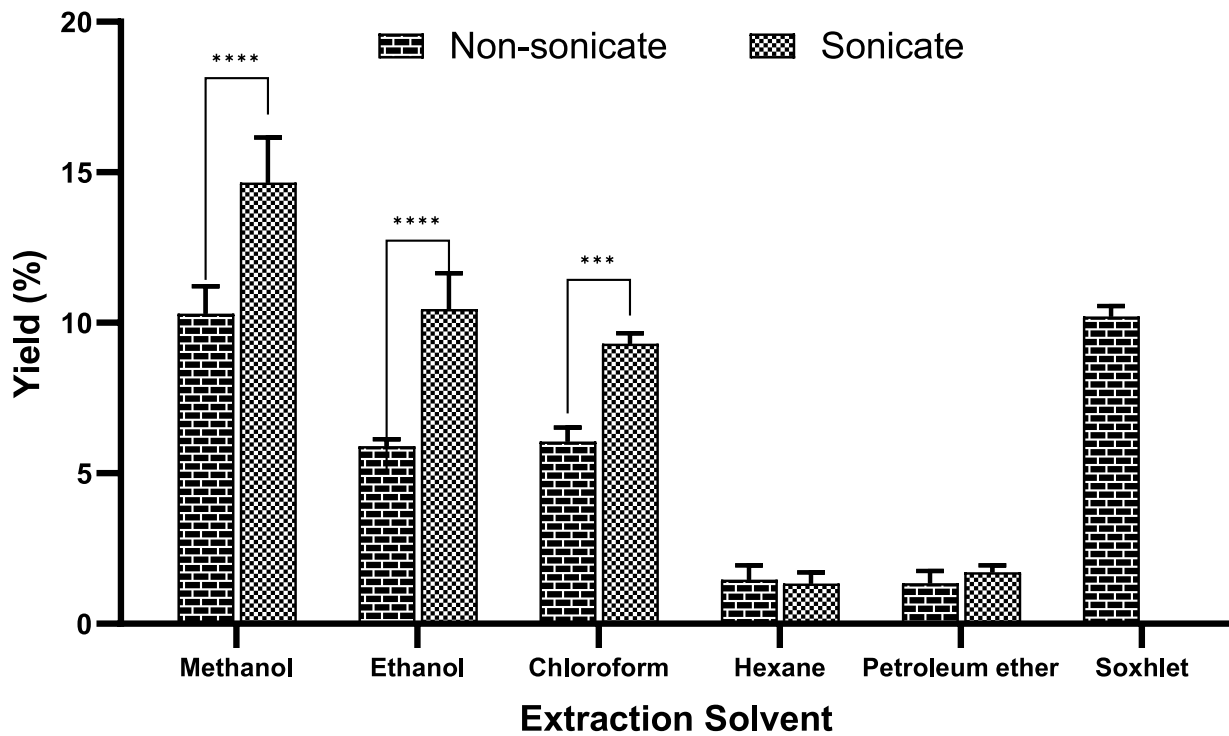


Fig. 1 Extraction yield produced from different extraction procedures

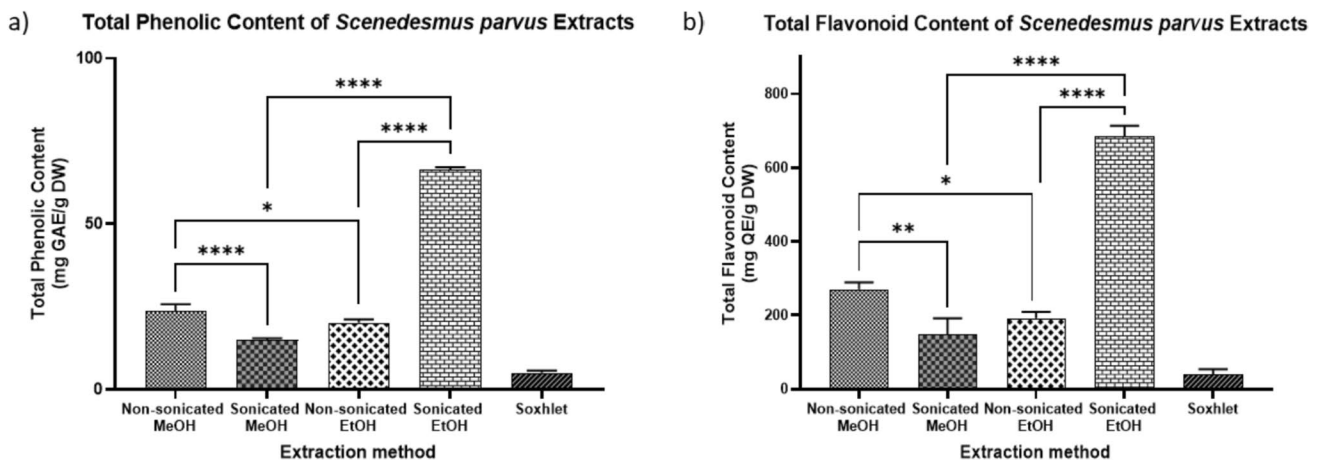
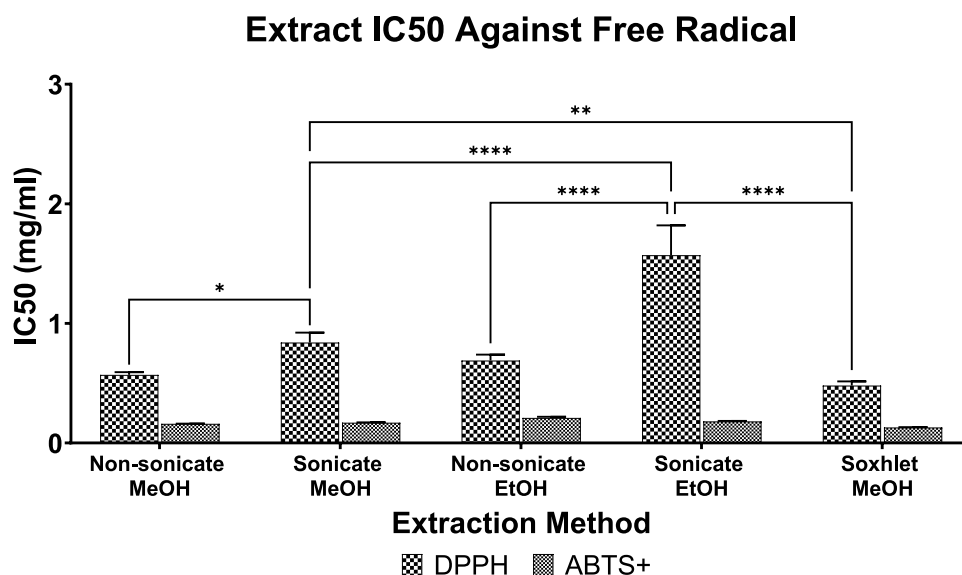


Fig. 2 The total phenolic and flavonoid content and the antioxidant activity of *S. parvus* extracts

yielding approximately $10.3 \pm 0.919\%$ for the maceration samples and around $14.65 \pm 0.503\%$ for the sonicated samples. Ethanol yielded $5.9 \pm 0.230\%$ in the absence of sonication, whereas the yield increased to $10.45 \pm 1.202\%$ with sonication. These findings aligned with previous findings that reported both methanol and ethanol as the most efficient

solvents for the extraction the bioactive compounds from *S. parvus* due to their high polarity (Ren et al. 2021; Tavakoli et al. 2021; Hui et al. 2023). Chloroform, being a less polar solvent than methanol and ethanol, yielded approximately $6.05 \pm 0.474\%$ for the maceration extract and about $9.3 \pm 0.354\%$ for the sonicated samples. This indicates that chloroform extracts have lesser numbers of compounds

Fig. 3 Extract IC₅₀ against DPPH and ABTS + free radical**Table 2** Fatty acid profile of Soxhlet and non-sonicated methanol *S. parvus* extract

Fatty acid	Soxhlet extract (g/100 g)	Non-sonicated MeOH extract (g/100 g)	P value
Dodecanoic acid (C12:0)	0.5 ± 0.18	2.1 ± 0.34	> 0.05
Tridecanoic acid (C13:0)	0.7 ± 0.14	2.8 ± 0.91	< 0.01
Myristic acid (C14:0)	0.5 ± 0.05	1.7 ± 0.15	> 0.05
Myristoleic acid (C14:1)	0.9 ± 0.09	3.3 ± 0.72	< 0.01
Palmitic acid (C16:0)	37.6 ± 1.62	38.5 ± 0.89	> 0.05
Palmitoleic acid (C16:1)	1.0 ± 0.29	1.7 ± 0.12	> 0.05
Stearic acid (C18:0)	4.1 ± 0.33	10.2 ± 0.56	< 0.0001
Oleic acid (C18:1 cis)	36.8 ± 1.67	23.9 ± 0.78	< 0.0001
Linoleic acid (C18:2 cis)	13.2 ± 1.21	9.6 ± 0.45	< 0.0001
Linolenic acid (C18:3n3)	4.8 ± 0.45	4.6 ± 0.67	> 0.05
Eicosanoic acid (C20:0)	-	1.7 ± 0.23	> 0.05

compared to methanol and ethanol, but the application of sonication significantly increases the extraction yield. In the case of hexane and petroleum ether, both non-polar solvents and the extraction yields were very low, ranging from 1 to 2%, even with sonication. This suggests that the compounds in *S. parvus* have limited solubility in non-polar solvents, resulting in low extraction yields.

Sonication also influenced the extraction yield of most solvents, as demonstrated by the relatively higher extraction yields for sonicated samples compared to maceration extract.

Methanol and ethanol showed higher yield compared to the control, indicating that sonication enhances extraction efficiency by disrupting cell walls and facilitating the release of intracellular compounds, as reported in many previous studies (Das and Eun 2018; Ren et al. 2021; Tavakoli et al. 2021; Hui et al. 2023). Sonication with chloroform also increased significantly, demonstrating its effectiveness in improving solvent's penetration and extraction capability. Interestingly, the extraction yield using Soxhlet extraction, despite being a lengthy process, was approximately $10.2 \pm 0.362\%$, slightly lower than the sonicated methanol and ethanol extracts. This suggests that, while Soxhlet extraction is effective, the combination of an appropriate solvent and sonication can yield better results, potentially outperforming conventional Soxhlet extraction.

Based on these findings, it can be concluded that solvent selection and the application of sonication are critical factors in the extraction of *S. parvus*. In comparison to the other solvents, methanol and ethanol used with sonication were the most effective solvents, producing significantly better results than conventional Soxhlet extraction and the less polar solvents. These results demonstrate that the extraction method and the type of solvent employed have a significant impact on the yield. The findings also support the sonication to enhance bioactive compound yield in microalgal research and related fields. Future studies should investigate the type of compounds extracted by each solvent and the biological activities associated with these extracts to further elucidate the benefits of sonication-assisted extraction.

The Impact of Different Extraction Methods on Total Phenolic and Flavonoid Content

Notable solvent-dependent effects emerged when comparing extraction methods for *S. parvus* bioactive compounds. For

methanol extraction, maceration yielded significantly higher phenolic (23.58 ± 2.168 mg GAE g⁻¹ DW) and flavonoid content (270.01 ± 19.174 mg QE g⁻¹ DW) than sonication (14.82 ± 0.660 mg GAE g⁻¹ DW; 148.04 ± 43.758 mg QE g⁻¹ DW). This contrasts with literature reporting sonication-enhanced yields (Upadhyay et al. 2015; Kim and Lee 2017; Agarwal et al. 2018; Özcan et al. 2020; Ahmed et al. 2020), suggesting methanol-specific degradation mechanisms. This study believed this happened due to the localized thermal spikes (> 80 °C) during cavitation bubble collapse (Cherepanov et al. 2015), where methanol's low boiling point (64.7 °C) exacerbates vapour pressure effects, intensifying collapse temperatures and degrading thermolabile phenolics.

Conversely, ethanol-based sonication dramatically increased phenolic content (66.32 ± 0.818 vs. 20.15 ± 1.007 mg GAE g⁻¹ DW) and flavonoids (684.45 ± 28.928 vs. 191.83 mg QE g⁻¹ DW) compared to maceration, which was consistent with its established cell-disruption efficacy (Gogoi et al. 2019; Mousavi et al. 2022; Irfan et al. 2022). This divergence likely stems from ethanol's higher boiling point (78.4 °C) reducing cavitation intensity and its hydrogen-bonding capacity stabilizing compounds (Izadiyan and Hemmateenejad 2016; Kim and Lee 2017). Notably, Soxhlet extraction yielded the lowest values (5.05 ± 0.632 mg GAE g⁻¹ DW phenolics; 39.96 ± 13.751 mg QE g⁻¹ DW flavonoids), confirming prolonged thermal exposure degrades heat-sensitive bioactive compounds regardless of solvent.

These results establish that sonication efficacy in *S. parvus* is fundamentally governed by solvent properties and operational parameters. Where ethanol excels in phenolic preservation through hydrogen-bond stabilization and attenuated radical formation, methanol achieves complementary advantages for better non-polar bioactive recovery, superior cell wall penetration, and selective concentration of thermostable nutraceutical that is unaffected by cavitation-derived heat. Consequently, methanol maintains industrial utility for applications prioritizing total extract mass or cost-efficiency in large-scale processing.

Effect of Different Extraction Methods on the Antioxidant Activity

The results of this study revealed significant variations in antioxidant activity depending on the extraction method and solvent used. For the methanol extract, the maceration method yielded lower IC₅₀ values for DPPH (0.57 ± 0.023 mg mL⁻¹), indicating greater antioxidant activity compared to the sonicated method, which had IC₅₀ values of 0.84 ± 0.083 mg mL⁻¹. A similar pattern was observed with the ethanol extract, where the maceration method showed higher antioxidant activity against the DPPH

free radical, with an IC₅₀ value of 0.69 ± 0.049 mg mL⁻¹, compared to 1.57 ± 0.025 mg mL⁻¹ for the sonicated method. These findings contrast with previous studies that reported increased antioxidant activity with sonication (Kim and Lee 2017; Özcan et al. 2020; Ranjha et al. 2020; Tavakoli et al. 2021; Mousavi et al. 2022; Irfan et al. 2022). It was hypothesized that the decrease in the antioxidant activity in sonicated extracts may result from the high energy during the sonication process affecting the structural integrity of antioxidant compounds, thereby reducing their activity.

These findings highlight the critical role of extraction methods and solvent selection in determining the antioxidant activity of *S. parvus* extracts. Methanol proved to be a more effective solvent than ethanol, particularly in maceration extracts, despite the higher phenolic and flavonoid content observed in ethanol extracts. A similar trend was observed in the Soxhlet extraction method, where the extract exhibited significantly higher antioxidant activity against the DPPH radical (IC₅₀: 0.48 ± 0.035 mg mL⁻¹) compared to other methods, except for the maceration methanol extract, despite having lower total phenolic content (TPC) and total flavonoid content (TFC). The results further indicate no significant correlation between the phenolic contents and flavonoids and the antioxidant activity against DPPH and ABTS⁺ free radicals. This suggests that other bioactive compounds, such as polysaccharides, carotenoids, amino acids, and other secondary metabolites, may contribute to the observed antioxidant activity (Fitriansyah et al. 2018; Salachna et al. 2021).

Additionally, all *S. parvus* extracts demonstrated good antioxidant activity against the ABTS⁺ radical, with no significant differences observed among different extraction methods and solvents. Despite variations in phenolic and flavonoid content, the extracts exhibited similar efficacy in scavenging ABTS⁺ radicals. This may be attributed to the higher sensitivity of ABTS⁺ radicals to a broader range of bioactive compounds, making them more readily neutralized compared to DPPH radicals (Fidrianny et al. 2015; Untea et al. 2018; Lins et al. 2018; Gu et al. 2019a; Minarti et al. 2024).

Therefore, these insights are essential for refining extraction techniques to maximize antioxidant activity and are of great value to researchers and industries involved in the production of functional foods and nutraceuticals.

Fatty Acid Composition of the *S. parvus* Extract

From the results, a significant difference between the methods can be observed in the fatty acid profile of *Scenedesmus parvus* extracts. Maceration methanol extraction demonstrated superior efficacy for most saturated fatty acids (SFAs), yielding substantially higher concentrations of dodecanoic acid (2.1 ± 0.34 vs

$0.5 \pm 0.18 \text{ g} \cdot 100 \text{ g}^{-1}$; $p > 0.05$), tridecanoic acid (2.8 ± 0.91 vs $0.7 \pm 0.14 \text{ g} \cdot 100 \text{ g}^{-1}$; $p < 0.01$), and myristic acid (1.7 ± 0.15 vs $0.5 \pm 0.05 \text{ g} \cdot 100 \text{ g}^{-1}$; $p > 0.05$) compared to Soxhlet extraction. This trend extended to longer-chain SFAs, with stearic acid recovery 2.5-fold higher under maceration methanol extract (10.2 ± 0.56 vs $4.1 \pm 0.33 \text{ g} \cdot 100 \text{ g}^{-1}$; $p < 0.0001$), while eicosanoic acid was exclusively detected in these extracts ($1.7 \pm 0.23 \text{ g} \cdot 100 \text{ g}^{-1}$). The sole exception was palmitic acid, which is the most abundant SFA and also showed a comparable yield between methods (38.5 ± 0.89 vs $37.6 \pm 1.62 \text{ g} \cdot 100 \text{ g}^{-1}$; $p > 0.05$).

For monounsaturated fatty acids (MUFAs), maceration methanol extracts outperformed Soxhlet in extracting myristoleic acid (3.3 ± 0.72 vs $0.9 \pm 0.09 \text{ g} \cdot 100 \text{ g}^{-1}$; $p < 0.01$) and palmitoleic acid (1.7 ± 0.12 vs $1.0 \pm 0.29 \text{ g} \cdot 100 \text{ g}^{-1}$; $p > 0.05$). Conversely, Soxhlet extraction yielded significantly higher concentrations of oleic acid (36.8 ± 1.67 vs $23.9 \pm 0.78 \text{ g} \cdot 100 \text{ g}^{-1}$; $p < 0.0001$). Among polyunsaturated fatty acids (PUFAs), Soxhlet provided superior recovery of linoleic acid (13.2 ± 1.21 vs. $9.6 \pm 0.45 \text{ g} \cdot 100 \text{ g}^{-1}$; $p < 0.0001$), whereas linolenic acid showed no significant between the methods (4.8 ± 0.45 vs. $4.6 \pm 0.67 \text{ g} \cdot 100 \text{ g}^{-1}$; $p > 0.05$).

These results demonstrate that maceration methanol preferentially extracts SFAs and shorter-chain MUFAs, while Soxhlet excels in recovering nutritionally critical longer-chain unsaturated acids.

Conclusion

In conclusion, these findings confirm that *S. parvus* compound recovery is highly dependent on extraction method and solvent. Sonicated ethanol yielded the most phenolics and flavonoids, while non-sonicated methanol gave optimal DPPH activity. Notably, this study focused exclusively on yield-optimizing methods and solvents, excluding practical considerations like cost or scalability. Thus, selection should target specific objectives: sonicated ethanol for phenolics and flavonoids or non-sonicated methanol for antioxidant scavenging activity.

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Author Contribution IIR coordinated this research and wrote the main manuscript, MAK providing isolated *S. parvus* and extraction technique, SNMS and SM prepared the experimental design.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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