

Influence of extraction method and solvent system on the antioxidant activity of *Scenedesmus parvus* extract

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Abstract

Scenedesmus parvus, a Malaysian freshwater microalga, has garnered attention as a promising source of secondary metabolites with applications in various industries, including nutraceuticals, pharmaceuticals, food, and cosmetics. This study aimed to evaluate the extraction yield, total phenolic and flavonoid content, and antioxidant potential of *S. parvus* extracts obtained through different extraction methods (sonication, non-sonication, and soxhlet). Additionally, the fatty acid profile and key chemical constituents of *S. parvus* were analysed using GC-FID. Results revealed that the sonication method utilizing methanol as the extraction solvent yielded the highest extraction yield ($14.5 \pm 1.5\%$), whereas the non-sonication method employing hexane yielded the lowest ($1.34 \pm 0.2\%$). 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 demonstrated superior antioxidant activity in both DPPH (IC₅₀: 0.48 ± 0.035 mg mL⁻¹) and ABTS+ (IC₅₀: 0.13 ± 0.003 mg mL⁻¹) scavenging assays compared to the others. These findings highlight the potential of different extraction methods from *S. parvus*, particularly those obtained with methanol as the extraction solvent, as valuable sources of natural antioxidants for application in nutraceuticals, pharmaceuticals, food, and cosmetics.

1. Introduction

Over the last few years, consumers are increasingly seeking 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 neutralising free radicals, which helps to avoid oxidative stress and lower the probability of some 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; 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). There are several microalgae 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; Wali et al. 2020; Kumar et al. 2021; Montoya-Arroyo et al. 2022; Trentin et al. 2022; Ganeson et al. 2024). Within 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 significant levels of polyphenols, flavonoids, and other bioactive compounds associated with 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 2019; Coulombier et al. 2021a; Coulombier et al. 2021b).

Extracting these valuable antioxidants from *S. parvus* is essential for determining the yield, purity, and bioactivity of the extracts. Different techniques like maceration, ultrasonic-assisted extraction (UAE), and soxhlet extraction have been employed for the extraction of these compounds (Vongsak et al. 2013; Ameer et al. 2017; Łabowska et al. 2019; Mirzadeh et al. 2020; Monteiro et al. 2020). All methods have its own advantages and limitations affecting the performance of the extraction process and the stability of the identified bioactive compounds. Furthermore, the selection of solvent system is critical for the extraction process. It has been established that commonly used solvents such as water, ethanol, methanol, and their mixtures significantly affect the solubility and extraction efficiency of various antioxidant compounds. (Do et al. 2014; Chigayo et al. 2016; Lim et al. 2019; Muhamad et al. 2019; Nawaz et al. 2020). Hence, attributes like the polarity of the solvent, extraction temperature, and time also significantly affect the extracts' overall antioxidant activity. Thus, factors such as solvent polarity, extraction temperature, and duration play a crucial role in modulating the overall antioxidant activity of the extracts, as they directly influence the efficiency of bioactive compound isolation and stability. Optimizing these parameters is a key to maximizing the yield and potency of antioxidants while preserving their functional integrity. This study aims to comprehensively investigate the influence of various extraction methods and solvent systems on the antioxidant activity of *S. parvus* extracts. Through a comprehensive comparative analysis of various extraction parameters, this study aims to determine the optimal conditions for maximizing the antioxidant potential of *S. parvus*. Understanding these optimal conditions is essential for enhancing the practical applications of this microalga in developing natural antioxidant products, which can significantly contribute to both scientific knowledge and industrial advancements in the field of natural antioxidants.

2. Materials and Methods

2.1 Microalgae cultivation

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

The microalgae biomass was produced following a modified protocol adapted from a previous study (Hui et al. 2023a). 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 ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), 0.17 g L^{-1} sodium nitrate (NaNO_3), 0.035 g L^{-1} di-potassium phosphate (K_2HPO_4), and 0.029 g L^{-1} calcium chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$). Sterilization of the medium was achieved using a $0.22 \text{ }\mu\text{m}$ 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 $0.03\text{--}0.05 \text{ g L}^{-1}$ ($\text{OD}_{680} = 1.0$). 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 $450 \text{ }\mu\text{mol m}^{-2} \text{ s}^{-1}$, and a temperature of $24 \pm 0.5^\circ\text{C}$. Both microalgal cultures were grown under identical conditions and harvested at the late logarithmic

growth phase. The harvested cells were centrifuged at 4500 rpm for 15 minutes, rinsed twice with distilled water, and dried at 70°C for 24 hours.

2.2 Metabolite extraction methods

2.2.1 Ultrasound-assisted extraction (UAE)

The extraction procedure was developed based on the previous study (Hui et al. 2023a) 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 minutes at 60°C. The power and frequency of the ultrasonic bath were set at 100 watts and 40 kHz, respectively. 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 minutes. 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.

2.2.2 Soxhlet extraction.

Approximately 10g sample of *S. parvus* biomass was extracted with 400 mL of methanol over a period of 12 hours using a standard Soxhlet apparatus with a 500 mL boiler. After the extraction, the liquid extract was filtered and then evaporated to dryness under vacuum at 60°C using a rotary evaporator. The resulting dried extract was subsequently stored at 4°C for further analyses.

2.2.3 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) non-ultrasound-assisted extraction. The detailed test conditions are presented in Table 1. A comparative analysis of all evaluated parameters will be 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)
	Non-Ultrasound-assisted extraction method.

2.2.4 Crude Yield Calculation for Extraction

The crude yield was determined by weighing the mass of the crude product obtained. The crude extract was subsequently then transferred into a pre-weighed aluminium plate, and the solvent was evaporated at 60°C in the dry oven until a constant weight was obtained. 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. 2023a):

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

2.3 Antioxidant capacity

2.3.1 Determination of total phenolic content (TPC)

The total phenolic content assay was included in this study as it served as a standard measure for phenolics and was often utilised to optimize the experimental conditions. 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–Ciocâlteu reagent. After approximately 6 minutes 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 minutes 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.

2.3.2 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} NaNO_2 , and the mixture was vortexed for 5 minutes. Subsequently, 15 μL of 100 g L^{-1} AlCl_3 was added. The solution was allowed to stand for 6 minutes, followed by adding 50 μL of 1 mol L^{-1} NaOH and another 50 μL of DDW. The mixture was shaken for 30 seconds, 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 $\mu\text{g mL}^{-1}$ range to generate a calibration curve ($R^2 < 0.9900$).

2.4 Antioxidant activity

2.4.1 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). 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 minutes at 25°C, the absorbance at 520 nm was measured against a blank. The free radical scavenging activity of each solution was determined as a percentage of inhibition using the following formula:

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

The antioxidant activity of the 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.

2.4.2 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. 2019). 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 hours 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 μL of ascorbic acid or extracts) were then mixed with 280 μL of fresh ABTS + solution, and absorbance was measured 6 minutes 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%.

2.5 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, California, USA). Separation of fatty acids was achieved utilizing an Agilent DB-Fast FAME column (60m × 250 µm × 0.25 µm). A sample volume of approximately 1 µL 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 minute, followed by a ramp to 165°C at a rate of 40°C min⁻¹, maintained for 1 minute, and concluded with an increase to 230°C at 4°C min⁻¹, held for 15 minutes. 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.

2.6 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, California, USA). To analyse the collected data, one-way ANOVA was employed, supplemented with Tukey's multiple comparison tests to determine statistical significance.

3. Results

3.1 Effect of sonication and solvent system on the extraction yield

The extraction yields of *S. parvus* using various solvents under sonicated and non-sonicated conditions are shown in Fig. 1. The results demonstrate significant variations in extraction efficiency depending on the types of solvents used and the implementation of sonication. Furthermore, the findings suggest that extracts produced via sonication result in considerably greater yields compared to those extracted without sonication, irrespective of the solvent employed.

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

For future assessments of the antioxidant potential of *S. parvus*, this study will focus solely on the crude extracts of methanol and ethanol. These extracts were selected due to their higher yields compared to the other solvents tested in the study. This study believes higher yield extracts will likely contain a broader range of bioactive compounds, facilitating more comprehensive antioxidant evaluation. This increased diversity may enhance the sensitivity of assays, thereby yielding more accurate and reliable results. The summarised results in Table 2 and Fig. 2 show that extraction is significantly influenced by the method and solvent used.

Table 2

Assessment of the total phenolic and flavonoid content and the antioxidant activity of *S. parvus* extract.

Extract	TPC (mg GAE g ⁻¹ DW)	TFC (mg QE g ⁻¹ DW)	DPPH (mg mL ⁻¹)	ABTS+ (mg mL ⁻¹)
Non-sonicated MeOH	23.58 ± 2.168	270.01 ± 19.174	0.57 ± 0.023	0.16 ± 0.002
Sonicated MeOH	14.82 ± 0.660	148.04 ± 43.758	0.84 ± 0.083	0.17 ± 0.002
Non-sonicated EtOH	20.15 ± 1.007	191.83 ± 17.646	0.69 ± 0.049	0.21 ± 0.009
Sonicated EtOH	66.32 ± 0.818	684.45 ± 28.928	1.57 ± 0.025	0.18 ± 0.004
Soxhlet	5.05 ± 0.632	39.96 ± 13.751	0.48 ± 0.035	0.13 ± 0.003

3.3 Effect of different extraction methods on the antioxidant activity

The antioxidant activity of the *S. parvus* extract was evaluated using a radical scavenging assay targeting DPPH and ABTS + free radicals. The IC₅₀ values, which represent the concentration required to inhibit 50% of the free radical activity, were used as indicators of antioxidant potency, with lower values corresponding to higher antioxidant activity. A summary of all data on antioxidant activity is summarized in Table 2 and Fig. 3.

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

The data in Table 3 shows a detailed comparison of the fatty acid composition of *S. parvus* extracts obtained using two different methods: soxhlet extraction and non-sonicated methanol extraction. The values are expressed in grams per 100 grams of extract sample.

Table 3
Fatty acid profile of soxhlet and non-sonicated methanol *S. parvus* extract.

Fatty Acid	Soxhlet extract (g/100g)	Non-sonicated MeOH extract (g/100g)
Dodecanoic Acid (C12:0)	0.5	2.1
Tridecanoic Acid (C13:0)	0.7	2.8
Myristic Acid (C14:0)	0.5	1.7
Myristoleic Acid (C14:1)	0.9	3.3
Palmitic Acid (C16:0)	37.6	38.5
Palmitoleic Acid (C16:1)	1.0	1.7
Stearic Acid (C18:0)	4.1	10.2
Oleic Acid (C18:1 cis)	36.8	23.9
Linoleic Acid (C18:2 cis)	13.2	9.6
Linolenic Acid (C18:3n3)	4.8	4.6
Eicosanoic Acid (C20:0)	-	1.7

4. Discussions

4.1 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 yielding approximately $10.3 \pm 0.919\%$ for the non-sonicated 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* (Ren et al. 2021; Tavakoli et al. 2021; Hui et al. 2023a). Chloroform, being a less polar solvent than methanol and ethanol, yielded approximately $6.05 \pm 0.474\%$ for the non-sonicated samples and about $9.3 \pm 0.354\%$ for the sonicated samples. This indicates that chloroform extracts have lesser numbers of compounds 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, the extraction yields were very low, ranging from 1–2%, 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 non-sonicated ones. Methanol and ethanol showed higher yield compared to the control, suggesting that sonication increases the efficiency of extraction by breaking down the cell walls and releasing the internal compound, as reported in many previous studies (Das and Eun 2018; Ren et al. 2021; Tavakoli et al. 2021; Hui et al. 2023b). 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 non-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.

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

In the comparison between non-sonicate and sonicate methods for methanol extraction, the non-sonicate method yielded a higher phenolic content of 23.58 ± 2.168 mg GAE g⁻¹ DW and flavonoid content of 270.01 ± 19.174 mg QE g⁻¹ DW. In contrast, the sonicate method yielded a lower phenolic content of 14.82 ± 0.660 mg GAE g⁻¹ DW and a flavonoid content of 148.04 ± 43.758 mg QE g⁻¹ DW. These results are inconsistent with the previous studies that suggest sonication with methanol should enhance the TPC and TFC values rather than lowering the compound extracted from the *S. parvus* (Upadhyay et al. 2015; Kim and Lee 2017; Agarwal et al. 2018; Özcan et al. 2020; Ahmed et al. 2020). Therefore, further study is needed to elucidate this phenomenon.

On the other hand, ethanol extraction depicted a different pattern of results. The sonication method was significantly more effective than the non-sonicate method, where the phenolic content increased from 20.15 ± 1.007 GAE g⁻¹ DW to 66.32 ± 0.818 mg GAE g⁻¹ DW, and the flavonoid content increased from 191.83 mg QE g⁻¹ DW to 684.45 ± 28.928 mg QE g⁻¹ DW. This significant increment further supports the effectiveness of sonication with ethanol in the disruption of the cell walls of the *S. parvus* to extract the phenolic and flavonoid compounds. This finding is consistent with previous studies showing that using sonication and ethanol during extraction enhances the recovery of phenolic and flavonoid compounds (Gogoi et al. 2019; Mousavi et al. 2022; Irfan et al. 2022). In contrast, the soxhlet extraction method,

which has the longest extraction time and continuous solvent circulation, yielded the lowest yields of phenolic (5.05 ± 0.632 mg GAE g⁻¹ DW) and flavonoid (39.96 ± 13.751 mg QE g⁻¹ DW) contents. This reduced efficiency could be due to the degradation of bioactive compounds due to more prolonged exposure to heat compared to the other methods.

In conclusion, this study demonstrates that selecting a proper extraction method and solvent will directly influence the quantity of phenolic and flavonoid compounds extracted from *S. parvus*. The results showed that sonication in ethanol was the most effective method for enhancing the extraction yield of these bioactive compounds. These findings could improve extraction processes in both research and industry, where maximizing the recovery of phenolic and flavonoid compounds is essential. Additionally, the results suggest that conventional Soxhlet extraction may not be ideal for such applications, particularly when targeting heat-sensitive bioactive compounds.

4.3 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 non-sonicated 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 non-sonicated 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 is 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. On the other hand, no significant differences in antioxidant activity were observed among different extraction methods and solvents for the ABTS + radical.

These findings indicate that the choice of extraction method and solvent is crucial in determining the antioxidant activity of *S. parvus* extracts. Methanol emerged as a more effective solvent than ethanol, especially in non-sonicated extracts. Notably, the Soxhlet extraction method demonstrated significantly higher antioxidant activity against the DPPH radical (0.48 ± 0.035 mg mL⁻¹) compared to other methods, except for the non-sonicated methanol extract, despite having lower TPC and TFC values. These insights are essential for refining extraction techniques to maximize antioxidant content and are of great value to researchers and industries involved in the production of functional foods and nutraceuticals.

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

The dodecanoic acid in the non-sonicated methanol extraction yielded a significantly higher concentration ($2.1\text{g } 100\text{g}^{-1}$) compared to the soxhlet extraction ($0.5\text{g } 100\text{g}^{-1}$), indicating that the non-

sonicated methanol method is more effective for extracting shorter-chain saturated fatty acids. Similarly, the concentration of tridecanoic acid was higher in the non-sonicated methanol extract ($2.8\text{g } 100\text{g}^{-1}$) than in the soxhlet extract ($0.7\text{g } 100\text{g}^{-1}$). For myristic acid, both methods resulted in relatively low concentrations, but the non-sonicated methanol extraction method extracted slightly more ($1.7\text{g } 100\text{g}^{-1}$) than the soxhlet method ($0.5\text{g } 100\text{g}^{-1}$). Myristoleic acid concentrations were also higher in the non-sonicated methanol extract ($3.3\text{g } 100\text{g}^{-1}$) compared to the soxhlet extract ($0.9\text{g } 100\text{g}^{-1}$).

Palmitic acid was the most abundant fatty acid in both extracts, with similar concentrations in both methods ($37.6\text{g } 100\text{g}^{-1}$ in soxhlet and $38.5\text{g } 100\text{g}^{-1}$ in non-sonicated methanol), suggesting that both methods are equally effective for extracting this fatty acid. The concentration of palmitoleic acid was higher in the non-sonicated methanol extract ($1.7\text{g } 100\text{g}^{-1}$) than in the soxhlet extract ($1.0\text{g } 100\text{g}^{-1}$). The non-sonicated methanol extract had more than double the concentration of stearic acid ($10.2\text{g } 100\text{g}^{-1}$) compared to the soxhlet extract ($4.1\text{g } 100\text{g}^{-1}$), highlighting the significant advantage of the non-sonicated methanol method for extracting this fatty acid. Conversely, oleic acid was more effectively extracted using the soxhlet method ($36.8\text{g } 100\text{g}^{-1}$) than the non-sonicated methanol method ($23.9\text{g } 100\text{g}^{-1}$), indicating that soxhlet extraction may be better for extracting monounsaturated fatty acids like oleic acid.

Linoleic acid concentration was also higher in the soxhlet extract ($13.2\text{g } 100\text{g}^{-1}$) compared to the non-sonicated methanol extract ($9.6\text{g } 100\text{g}^{-1}$), similar to the trend observed with oleic acid. Both methods showed similar concentrations of linolenic acid ($4.8\text{g } 100\text{g}^{-1}$ in soxhlet and $4.6\text{g } 100\text{g}^{-1}$ in non-sonicated methanol), indicating no significant difference between the methods for this fatty acid. Eicosanoic acid was detected only in the non-sonicated methanol extract ($1.7\text{g } 100\text{g}^{-1}$), suggesting that the soxhlet method may not be suitable for extracting longer-chain saturated fatty acids.

The fatty acid profile of *S. parvus* extracts varies significantly depending on the extraction method used. The non-sonicated methanol method generally yielded higher shorter-chain and saturated fatty acids concentrations. In contrast, the soxhlet method was more effective for extracting certain monounsaturated and polyunsaturated fatty acids. These findings suggest that the extraction method should be tailored to the specific fatty acids of interest in *S. parvus* extracts.

5. Conclusion

In conclusion, this study concludes that sonication is crucial in maximizing for optimizing the extraction yield and bioactive compound content of *Scenedesmus parvus*. Methanol and ethanol, particularly especially when combined with sonication, proved to be the most effective solvents for extracting phenolic and flavonoid compounds and enhancing antioxidant activity. Therefore, future research should explore the specific compounds extracted by each solvent and evaluate their associated biological activities.

Declarations

Competing interests

The authors declare no conflict of interest.

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Author Contribution

I.R., S.M., and M.K. made significant contributions throughout this study, including writing the manuscript, conducting experiments, and analyzing the data to its conclusion.

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References

1. Agarwal C, Máthé K, Hofmann T, Csóka L (2018) Ultrasound-Assisted Extraction of Cannabinoids from *Cannabis Sativa* L. Optimized by Response Surface Methodology. J Food Sci 83(3):700–710. <https://doi.org/10.1111/1750-3841.14075>
2. Ahmed M, Ramachandraiah K, Jiang G-H, Eun JB (2020) Effects of Ultra-Sonication and Agitation on Bioactive Compounds and Structure of Amaranth Extract. Foods Basel Switz 9(8):1116. <https://doi.org/10.3390/foods9081116>
3. Ameer K, Shahbaz H, Kwon J-H (2017) Green Extraction Methods for Polyphenols from Plant Matrices and Their Byproducts: A Review: Polyphenols extraction by green methods . . . Compr Rev Food Sci Food Saf 16. <https://doi.org/10.1111/1541-4337.12253>
4. Atta E, Mohamed N, Abdelgawad A (2017) Antioxidants: An Overview on the Natural and Synthetic Types. Eur Chem Bull 6:365–375. <https://doi.org/10.17628/ecb.2017.6.365-375>
5. Banskota A, Sperker S, Stefanova R, McGinn P, O'Leary S (2019) Antioxidant properties and lipid composition of selected microalgae. J Appl Phycol 31. <https://doi.org/10.1007/s10811-018-1523-1>
6. Chigayo K, Mojapelo PEL, Mnyakeni-Moleele S, Misihairabgwi JM (2016) Phytochemical and antioxidant properties of different solvent extracts of *Kirkia wilmsii* tubers. Asian Pac J Trop Biomed

- 6(12):1037–1043. <https://doi.org/10.1016/j.apjtb.2016.10.004>
7. Coulombier N, Jauffrais T, Lebouvier N (2021a) Antioxidant Compounds from Microalgae: A Review. *Mar Drugs* 19(10):549. <https://doi.org/10.3390/md19100549>
 8. Coulombier N, Jauffrais T, Lebouvier N (2021b) Antioxidant Compounds from Microalgae: A Review. *Mar Drugs* 19(10):549. <https://doi.org/10.3390/md19100549>
 9. Das PR, Eun J-B (2018) A comparative study of ultra-sonication and agitation extraction techniques on bioactive metabolites of green tea extract. *Food Chem* 253:22–29. <https://doi.org/10.1016/j.foodchem.2018.01.080>
 10. Del Mondo A, Smerilli A, Ambrosino L, Albini A, Noonan DM, Sansone C, Brunet C (2021) Insights into phenolic compounds from microalgae: structural variety and complex beneficial activities from health to nutraceuticals. *Crit Rev Biotechnol* 41(2):155–171. <https://doi.org/10.1080/07388551.2021.1874284>
 11. Didier AJ, Stiene J, Fang L, Watkins D, Dworkin LD, Creeden JF (2023) Antioxidant and Anti-Tumor Effects of Dietary Vitamins A, C, and E. *Antioxidants* 12(3):632. <https://doi.org/10.3390/antiox12030632>
 12. Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, Ju Y-H (2014) Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *J Food Drug Anal* 22(3):296–302. <https://doi.org/10.1016/j.jfda.2013.11.001>
 13. Ganeson Y, Paramasivam P, Palanisamy KM, Govindan N, Maniam GP (2024) LCMS and FTIR profiling of microalga *Chlorella* sp. for cosmetics and skin care applications. *Clean Water* 2:100028. <https://doi.org/10.1016/j.clwat.2024.100028>
 14. Gogoi P, Chutia P, Singh P, Mahanta CL (2019) Effect of optimized ultrasound-assisted aqueous and ethanolic extraction of *Pleurotus citrinopileatus* mushroom on total phenol, flavonoids and antioxidant properties. *J Food Process Eng* 42(6):e13172. <https://doi.org/10.1111/jfpe.13172>
 15. Gu C, Howell K, Dunshea FR, Suleria HAR (2019) LC-ESI-QTOF/MS Characterisation of Phenolic Acids and Flavonoids in Polyphenol-Rich Fruits and Vegetables and Their Potential Antioxidant Activities. *Antioxidants* 8(9):405. <https://doi.org/10.3390/antiox8090405>
 16. Gupta RK, Patel AK, Shah N, Choudhary AK, Jha KJ, Yadav UC, Gupta PK, Pakuwal U (2014) Oxidative Stress and Antioxidants in Disease and Cancer: A Review. *Asian Pac J Cancer Prev* 15(11):4405–4409
 17. He L, He T, Farrar S, Ji L, Liu T, Ma X (2017) Antioxidants Maintain Cellular Redox Homeostasis by Elimination of Reactive Oxygen Species. *Cell Physiol Biochem Int J Exp Cell Physiol Biochem Pharmacol* 44(2):532–553. <https://doi.org/10.1159/000485089>
 18. Herald TJ, Gadgil P, Tilley M (2012) High-throughput micro plate assays for screening flavonoid content and DPPH-scavenging activity in sorghum bran and flour. *J Sci Food Agric* 92(11):2326–2331. <https://doi.org/10.1002/jsfa.5633>
 19. Hui GT, Meng TK, Kassim MA (2023a) Green ultrasonication-assisted extraction of microalgae *Chlorella* sp. for polysaturated fatty acid (PUFA) rich lipid extract using alternative solvent mixture.

- Bioprocess Biosyst Eng 46(10):1499–1512. <https://doi.org/10.1007/s00449-023-02917-x>
20. Hui GT, Meng TK, Kassim MA (2023b) Green ultrasonication-assisted extraction of microalgae *Chlorella* sp. for polysaturated fatty acid (PUFA) rich lipid extract using alternative solvent mixture. Bioprocess Biosyst Eng 46(10):1499–1512. <https://doi.org/10.1007/s00449-023-02917-x>
21. Irfan S, Ranjha MMAN, Nadeem M, Safdar MN, Jabbar S, Mahmood S, Murtaza MA, Ameer K, Ibrahim SA (2022) Antioxidant Activity and Phenolic Content of Sonication- and Maceration-Assisted Ethanol and Acetone Extracts of *Cymbopogon citratus* Leaves. Separations 9(9):244. <https://doi.org/10.3390/separations9090244>
22. Kim I, Lee J (2017) Comparison of different extraction solvents and sonication times for characterization of antioxidant activity and polyphenol composition in mulberry (*Morus alba* L.). Appl Biol Chem 60(5):509–517. <https://doi.org/10.1007/s13765-017-0303-y>
23. Kumar N, Banerjee C, Jagadevan S (2021) Identification, characterization, and lipid profiling of microalgae *Scenedesmus* sp. NC1, isolated from coal mine effluent with potential for biofuel production. Biotechnol Rep 30:e00621. <https://doi.org/10.1016/j.btre.2021.e00621>
24. Łabowska MB, Michalak I, Detyna J (2019) Methods of extraction, physicochemical properties of alginates and their applications in biomedical field – a review. Open Chem 17(1):738–762. <https://doi.org/10.1515/chem-2019-0077>
25. Lim S, Choi A-H, Kwon M, Joung E-J, Shin T, Lee S-G, Kim N-G, Kim H-R (2019) Evaluation of antioxidant activities of various solvent extract from *Sargassum serratifolium* and its major antioxidant components. Food Chem 278:178–184. <https://doi.org/10.1016/j.foodchem.2018.11.058>
26. Lobo V, Patil A, Phatak A, Chandra N (2010) Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev 4(8):118–126. <https://doi.org/10.4103/0973-7847.70902>
27. Lourenço SC, Moldão-Martins M, Alves VD (2019) Antioxidants of Natural Plant Origins: From Sources to Food Industry Applications. Molecules 24(22):4132. <https://doi.org/10.3390/molecules24224132>
28. Mahmood WMAW, Theodoropoulos C, Gonzalez-Miquel M (2017) Enhanced microalgal lipid extraction using bio-based solvents for sustainable biofuel production. Green Chem 19(23):5723–5733. <https://doi.org/10.1039/C7GC02735D>
29. Malekmohammad K, Sewell RDE, Rafieian-Kopaei M (2019) Antioxidants and Atherosclerosis: Mechanistic Aspects. Biomolecules 9(8):301. <https://doi.org/10.3390/biom9080301>
30. Martin GJO, Hill DRA, Olmstead ILD, Bergamin A, Shears MJ, Dias DA, Kentish SE, Scales PJ, Botté CY, Callahan DL (2014) Lipid Profile Remodeling in Response to Nitrogen Deprivation in the Microalgae *Chlorella* sp. (Trebouxiophyceae) and *Nannochloropsis* sp. (Eustigmatophyceae). PLOS ONE 9(8):e103389. <https://doi.org/10.1371/journal.pone.0103389>
31. Mirzadeh M, Arianejad MR, Khedmat L (2020) Antioxidant, antiradical, and antimicrobial activities of polysaccharides obtained by microwave-assisted extraction method: A review. Carbohydr Polym 229:115421. <https://doi.org/10.1016/j.carbpol.2019.115421>

32. Monteiro M, Santos RA, Iglesias P, Couto A, Serra CR, Gouvinhas I, Barros A, Oliva-Teles A, Enes P, Díaz-Rosales P (2020) Effect of extraction method and solvent system on the phenolic content and antioxidant activity of selected macro- and microalgae extracts. *J Appl Phycol* 32(1):349–362. <https://doi.org/10.1007/s10811-019-01927-1>
33. Montoya-Arroyo A, Lehnert K, Muñoz-González A, Schmid-Staiger U, Vetter W, Frank J (2022) Tocochromanol Profiles in *Chlorella sorokiniana*, *Nannochloropsis limnetica* and *Tetraselmis suecica* Confirm the Presence of 11'- α -Tocomonoenol in Cultured Microalgae Independently of Species and Origin. *Foods* 11(3):396. <https://doi.org/10.3390/foods11030396>
34. Moreira DC (2019) ABTS decolorization assay – in vitro antioxidant capacity v1
35. Mousavi SA, Nateghi L, Javanmard Dakheli M, Ramezan Y, Piravi-Vanak Z (2022) Maceration and ultrasound-assisted methods used for extraction of phenolic compounds and antioxidant activity from *Ferulago angulata*. *J Food Process Preserv* 46(3):e16356. <https://doi.org/10.1111/jfpp.16356>
36. Muhamad SHA, On S, Sanusi SNA, Hashim AA, Zai MHA (2019) Antioxidant activity of Camphor leaves extract based on variation solvent. *J Phys Conf Ser* 1349(1):012102. <https://doi.org/10.1088/1742-6596/1349/1/012102>
37. Nawaz H, Shad MA, Rehman N, Andaleeb H, Ullah N (2020) Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (*Phaseolus vulgaris*) seeds. *Braz J Pharm Sci* 56:e17129. <https://doi.org/10.1590/s2175-97902019000417129>
38. Özcan MM, Al Juhaimi F, Uslu N, Ahmed IAM, Babiker EE, Osman MA, Gasseem MA, Alqah HAS, Ghafoor K (2020) Effect of sonication process of terebinth (*Pistacia terebinthus* L.) fruits on antioxidant activity, phenolic compounds, fatty acids and tocopherol contents. *J Food Sci Technol* 57(6):2017–2025. <https://doi.org/10.1007/s13197-019-04235-1>
39. Pantami HA, Ahamad Bustamam MS, Lee SY, Ismail IS, Mohd Faudzi SM, Nakakuni M, Shaari K (2020) Comprehensive GCMS and LC-MS/MS Metabolite Profiling of *Chlorella vulgaris*. *Mar Drugs* 18(7):367. <https://doi.org/10.3390/md18070367>
40. Ranjha MMAN, Amjad S, Ashraf S, Khawar L, Safdar MN, Jabbar S, Nadeem M, Mahmood S, Murtaza MA (2020) Extraction of Polyphenols from Apple and Pomegranate Peels Employing Different Extraction Techniques for the Development of Functional Date Bars. *Int J Fruit Sci*
41. Ren X, Wei C, Yan Q, Shan X, Wu M, Zhao X, Song Y (2021) Optimization of a novel lipid extraction process from microalgae. *Sci Rep* 11(1):20221. <https://doi.org/10.1038/s41598-021-99356-z>
42. Safafar H, Van Wagenen J, Møller P, Jacobsen C (2015) Carotenoids, Phenolic Compounds and Tocopherols Contribute to the Antioxidative Properties of Some Microalgae Species Grown on Industrial Wastewater. *Mar Drugs* 13(12):7339–7356. <https://doi.org/10.3390/md13127069>
43. Sarian MN, Ahmed QU, Mat So'ad SZ, Alhassan AM, Murugesu S, Perumal V, Syed Mohamad SNA, Khatib A, Latip J (2017) Antioxidant and Antidiabetic Effects of Flavonoids: A Structure-Activity Relationship Based Study. *BioMed Res Int* 2017:1–14. <https://doi.org/10.1155/2017/8386065>
44. Shalaby E (ed) (2019) Antioxidants. IntechOpen

45. Sharma DK, Gautam K, Jueppner J, Giavalisco P, Rihko-Struckmann L, Pareek A, Sundmacher K (2015) UPLC-MS analysis of *Chlamydomonas reinhardtii* and *Scenedesmus obliquus* lipid extracts and their possible metabolic roles. *J Appl Phycol* 27(3):1149–1159. <https://doi.org/10.1007/s10811-014-0407-2>
46. Tan Y, Lin J (2011) Biomass production and fatty acid profile of a *Scenedesmus rubescens*-like microalga. *Bioresour Technol* 102(21):10131–10135. <https://doi.org/10.1016/j.biortech.2011.07.091>
47. Tavakoli S, Hong H, Wang K, Yang Q, Gahruie HH, Zhuang S, Li Y, Liang Y, Tan Y, Luo Y (2021) Ultrasonic-assisted food-grade solvent extraction of high-value added compounds from microalgae *Spirulina platensis* and evaluation of their antioxidant and antibacterial properties. *Algal Res* 60:102493. <https://doi.org/10.1016/j.algal.2021.102493>
48. Trentin R, Custódio L, Rodrigues MJ, Moschin E, Sciuto K, da Silva JP, Moro I (2022) Total Phenolic Levels, In Vitro Antioxidant Properties, and Fatty Acid Profile of Two Microalgae, *Tetraselmis marina* Strain IMA043 and Naviculoid Diatom Strain IMA053, Isolated from the North Adriatic Sea. *Mar Drugs* 20(3):207. <https://doi.org/10.3390/md20030207>
49. Upadhyay R, Nachiappan G, Mishra HN (2015) Ultrasound-assisted extraction of flavonoids and phenolic compounds from *Ocimum tenuiflorum* leaves. *Food Sci Biotechnol* 24(6):1951–1958. <https://doi.org/10.1007/s10068-015-0257-y>
50. Vongsak B, Sithisarn P, Mangmool S, Thongpraditchote S, Wongkrajang Y, Gritsanapan W (2013) Maximizing total phenolics, total flavonoids contents and antioxidant activity of *Moringa oleifera* leaf extract by the appropriate extraction method. *Ind Crops Prod* 44:566–571. <https://doi.org/10.1016/j.indcrop.2012.09.021>
51. Wali AF, Al Dhaheri Y, Ramakrishna Pillai J, Mushtaq A, Rao PGM, Rabbani SA, Firdous A, Elshikh MS, Farraj DAA (2020) LC-MS Phytochemical Screening, In Vitro Antioxidant, Antimicrobial and Anticancer Activity of Microalgae *Nannochloropsis oculata* Extract. *Separations* 7(4):54. <https://doi.org/10.3390/separations7040054>
52. Wang J, Hu S, Nie S, Yu Q, Xie M (2016) Reviews on Mechanisms of *In Vitro* Antioxidant Activity of Polysaccharides. *Oxid Med Cell Longev* 2016:1–13. <https://doi.org/10.1155/2016/5692852>
53. Xu J (2017a) DPPH radical scavenging capacity measurement. <https://doi.org/10.17504/protocols.io.jiqckdw>
54. Xu J (2017b) ABTS radical scavenging capacity measurement v1. <https://doi.org/10.17504/protocols.io.jirckd6>

Figures

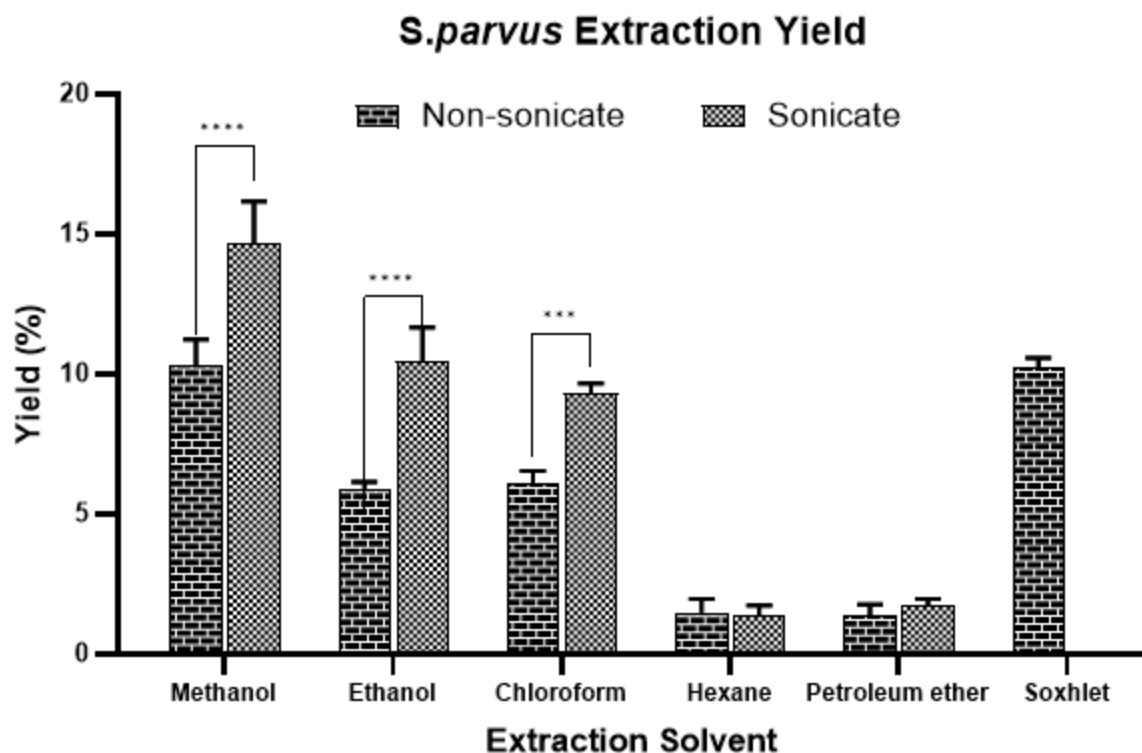


Figure 1

Extraction yield produced from different extraction procedures

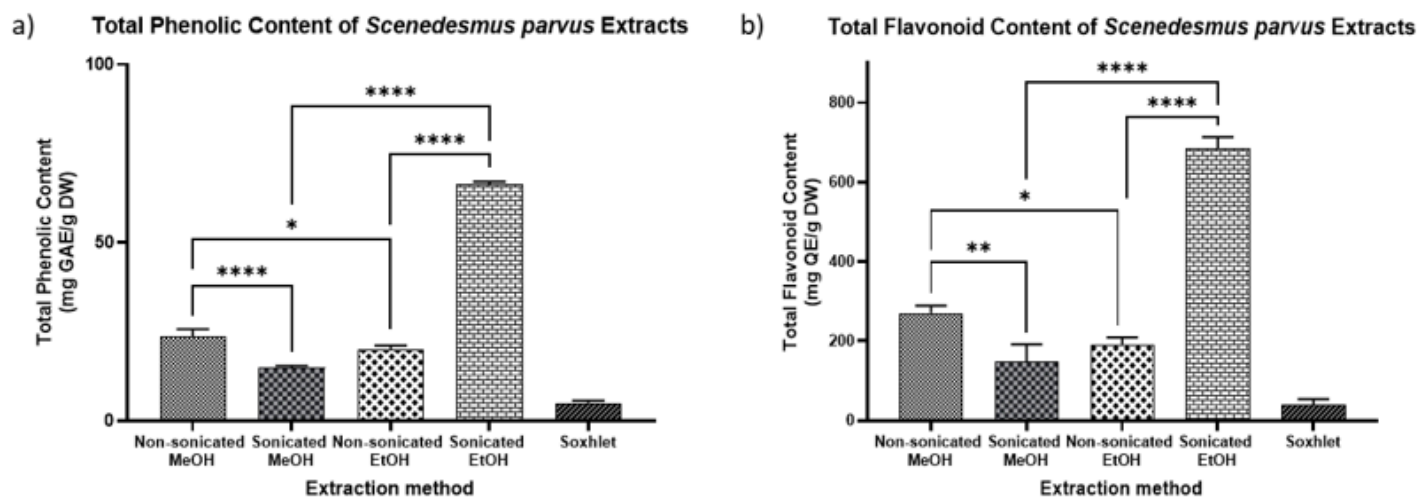


Figure 2

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

Extract IC50 Against Free Radical



Figure 3

Extract IC50 against DPPH and ABTS+ free radical.