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Comparison of Alliin Recovery from *Allium sativum* L. Using Soxhlet Extraction and Subcritical Water Extraction

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Abstract: Garlic (*Allium sativum* L.) is an herbaceous plant and is recognised for its numerous medicinal and culinary properties, and it is used in diverse food preparations for its characteristic flavour and aroma. High alliin content increases the formation of allicin, a bioactive compound of garlic. Therefore, this research aimed to compare different extraction methods for garlic (*Allium sativum* L.) between subcritical water extraction (SWE) and Soxhlet extraction to obtain a high extraction yield and alliin content. The SWE conditions were 120 °C and 180 °C temperatures and 2 mL/min and 6 mL/min flow rates at a constant pressure of 15 MPa for a 10 min extraction time, respectively. In the meanwhile, the extraction time for Soxhlet extraction with various solvents, namely, distilled water, ethanol–water (1:1), and 100% ethanol, was two hours. High-performance liquid chromatography (HPLC) was used to analyse alliin. Soxhlet extraction had the best yield (1.96 g) using ethanol–water (1:1) as the solvent in comparison to SWE extraction (1.28 g) at 180 °C and 6 mL/min. In contrast, SWE yielded a greater concentration of alliin (136.82 mg/g) at 120 °C and 2 mL/min than the Soxhlet method when using distilled water as the solvent (65.18 mg/g). Therefore, SWE may replace Soxhlet extraction as the conventional method for extracting alliin from garlic at a high concentration, and SWE has advantages that favour garlic extracts.

Keywords: *Allium sativum* L.; alliin; Soxhlet extraction; subcritical water extraction

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1. Introduction

The study of natural plants is a very thought-provoking subject for scientists to explore further. Garlic (*Allium Sativum* L.) is an herbaceous plant that belongs to the amaryllis family and is commonly known as 'bawang putih' in local Malaysia. Garlic is recognised for its numerous medicinal and culinary properties and is used in diverse food preparations for its characteristic flavour and aroma [1,2]. Raw garlic consumption is associated with reduced risks of cardiovascular diseases and gastrointestinal tract cancer, as well as anti-inflammatory, antidiabetic, immunomodulatory and antimicrobial activities [3]. Additionally, garlic contains a wide range of trace minerals. Garlic's bioactive components include an amino acid called alliin and the alliinase enzyme [2]. These compounds commix to form allicin, which is responsible for the strong smell of garlic when a garlic clove is masticated, chopped, bruised, or peeled. About 4–5 mg of allicin is found in one clove of fresh garlic, and its presence is easily detectable due to its unique odour [4]. Allicin-derived compounds, in turn, break down into other sulphur compounds within a few hours. These allyl sulphide compounds have a variety of overlapping rejuvenating properties [5].

Alliin is the major precursor of 2-propylene thiosulfate or diallyl thiosulfonate, and it is sulphur-containing non-protein amino acid. It is produced from intermediate compounds in the biosynthesis of S-alk(en)yl-L-cysteine sulphoxides (ACOs) [6] and has a variety of biological activities, including antioxidant, antimicrobial, and lipid-lowering activities, so it may be used in nutritional health and medical applications [7]. According to reports, with ammonia, pyruvate, and carbon dioxide as by-products, the conversion rate from alliin to allicin is 3:1. One gram of fresh and dry garlic contains about 10 and 30 mg of alliin, respectively [8]. Therefore, a high concentration of alliin will lead to the formation of allicin as a bioactive compound of garlic.

Soxhlet extraction is commonly applied by researchers because it has many benefits for solid–liquid extraction: for example, smaller amounts of solvents are used, and it does not require a further separation process since the cellulose thimble of the filter prevents direct contact between the solvent and samples [9]. To enhance the solvation power of Soxhlet extraction, binary-mixture solvents such as ethanol–water are applied for the extraction of garlic. The combination of ethanol–water (50% ethanol) can alter the polarity of the solvent mixture itself as well as attract the target compound to be extracted [10]. Previous studies revealed that Soxhlet extraction results in the highest recovery of global yield compared with ultrasound-assisted extraction (UAE), SWE, and supercritical fluid extraction (SFE) [9–11].

In this study, SWE, as a green extraction method, was applied due to its numerous benefits. The application of water as a solvent is economical, as there is no limitation on the source of water, resulting in reduced operating costs. It also enables high selectivity to extract different classes of compounds, especially polar compounds. Other than that, in SWE, the pH level of the solvent can be regulated by modifying the temperature. Thus, this method is suitable for extracting alliin from garlic [12]. This is because adjusting the temperature in the range of the subcritical phase can alter the polarity of water and manipulate the water behaviour to act as an acid or base catalyst as well as regulate the pH level, providing an advantage for the stability of alliin extraction, as alliin is an unstable compound [13].

Due to the paucity of research on garlic using this technique, it is vital to investigate the extraction of garlic by SWE. Thus, the main aim of this research was to compare the extraction of garlic using SWE and Soxhlet extraction in order to obtain the maximum amounts of garlic extract and alliin compound.

2. Materials and Methods

2.1. Chemicals

The chemicals used were 100% ethanol (analytical grade) and methanol (HPLC grade), which were purchased from Merck, Malaysia. Distilled water was obtained from the Centre of Lipids Engineering and Applied Research (CLEAR) laboratory, UTM, Malaysia.

2.2. Materials and Sample Preparation

In this study, the raw materials consisted of garlic obtained from a local market in Johor, Malaysia. The garlic was peeled, cleaned, and rinsed before being chopped. To preserve the garlic's freshness, it was promptly weighed and put in a teabag. To preserve the volatile sample and retain the freshness of garlic, the samples were placed in an airtight container and kept in a freezer (Model Liebherr, Malaysia) at approximately −20 °C. Due to budget constraints, neither method requires duplication of the extraction process.

2.3. Soxhlet Extraction

Soxhlet extractions were performed using 10 g of sliced fresh garlic loaded into a teabag and placed in the extraction chamber. The Soxhlet parameters in this study were water, ethanol–water (1:1), and 100% ethanol as solvents with a 2 h extraction time. Two hundred millilitres of each solvent was poured into a round-bottom flask. A 1:20 ratio of sliced garlic and solvent was selected for this extraction process. Then, the solvents were heated slightly above the boiling point of the solvent for a specified time interval using a heating mantle. After the completion of the extraction process, a rotary vacuum evaporator (Model Heidolph, Malaysia) in conditions below atmospheric pressure and temperature in the range 35–40 °C was used to recover the solvent from the extract.

2.4. Subcritical Water Extraction

A 50 mL extraction vessel, high-pressure water pump (Supercritical 24, Japan), back pressure regulator (Swagelok, Cleveland, OH, USA), and oven constitute the SWE system (Memmert, Germany). The schematic diagram of SWE is shown in Figure 1. The extraction process was performed at temperatures of 120 °C and 180 °C with flow rates of 2 and 6 mL/min and a pressure of 15 MPa. The extraction time throughout the experiment was 10 min. A total of 5 $\pm$ 0.005 g of minced garlic was placed in a 10 mL extraction vessel, followed by deionised water to fill up the empty space in the vessel. Then, the deionised water was continuously pumped using a metering pump (Eldex Optos, Napa, CA, USA). The extraction yield was collected in a vial and stored at −20 °C to prevent any possible degradation before drying using a freeze dryer (Alpha 1–2 LDplus, Germany).

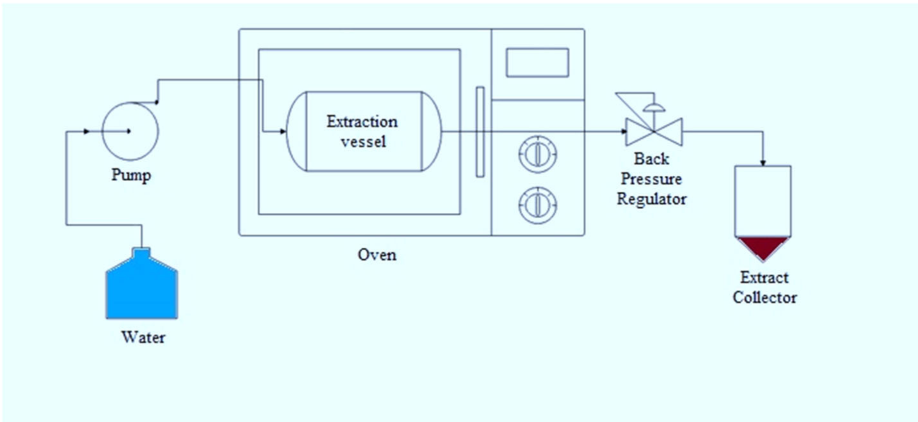


Figure 1. The SWE schematic diagram.

2.5. Analysis of Alliin by High-Performance Liquid Chromatography (HPLC)

The analysis of alliin was developed by Bose, et al. [14], where alliin was detected using an HPLC with ultraviolet–visible detection (Perkin Elmer Series 200, Waltham, MA, USA). The column temperature controller was set to 30 °C, and the wavelength detection was set to 254 nm. The injection volume and solvent flow rate were set to 10 µL and 0.5 mL/min, respectively. A mixture of HPLC grade methanol and pure water (50:50 MeOH:H_2O) was used as the mobile phase, along with a kromasil C18 column (250 $\times$ 4.6 mm i.d., 5 m particle size) and a rheodyne injector (Cotati, CA, USA, Model 7725i). The quantification of alliin was performed by using the slope of the calibration curve. Figure 2 shows the example of peak area of (a) alliin standard at concentration of 600 ppm and garlic extract consisting of alliin at 180 °C and 2 mL/min for a 10 min extraction time. To calibrate the alliin standard curve equation, the alliin peak region of the sample was substituted for the alliin concentration. The following equation was used:

$$\text{Peak Area } (mAU \times s) = 1530.1x + 6691.9 \tag{1}$$

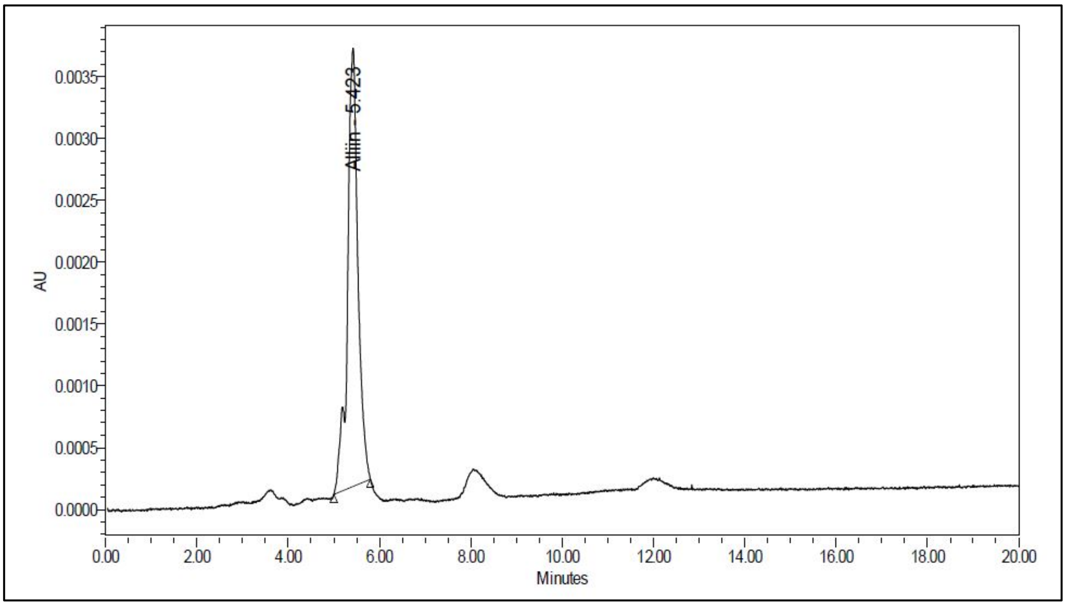
where x is the concentration of alliin (mg/g_{sample}).

2.6. Calculation of Extraction Yield

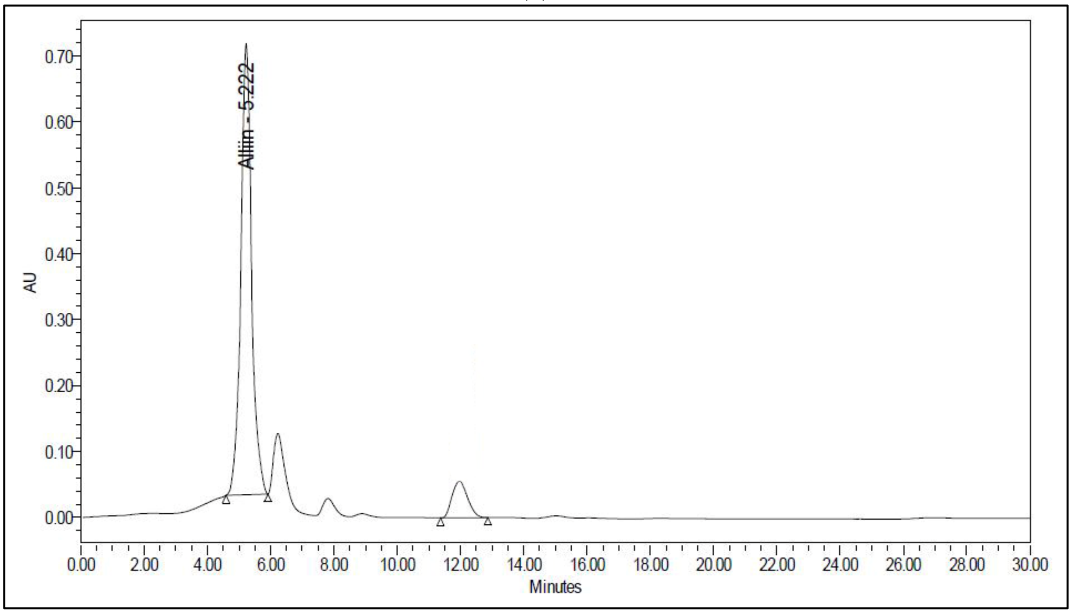
Equation (2) was used to quantify the extraction yield:

$$\text{Extract Yield } (\%) = \frac{M_x}{M_{xy}} \times 100 \tag{2}$$

where M_x is the mass of dry extract in grams, and M_{xy} is the mass of the sample in grams. The extraction yields from Soxhlet extraction and SWE were compared using the equation.



(**a**)



(**b**)

Figure 2. Peak area of (**a**) alliin standard at concentration of 600 ppm; (**b**) garlic extract consisting of alliin at 180 °C and 2 mL/min for a 10 min extraction time.

3. Results and Discussion

3.1. Soxhlet Extraction

In this study, 10 ± 0.005 of minced fresh garlic was used in Soxhlet extraction with 200 mL of distilled water, ethanol–water (1:1), and 100% ethanol, which were used as solvents. The extraction time was 2 h. Figure 3 illustrates that the garlic extract was obtained using the Soxhlet extraction with various solvents, i.e., distilled water, ethanol–water (1:1), and 100% ethanol. Generally, the used solvents yielded between 0.1 and 2 g of extract. Due to the polarity of the solvent, ethanol yielded the highest amount of extract (1.96 ± 0.07 g) as opposed to deionised water and 100% ethanol.

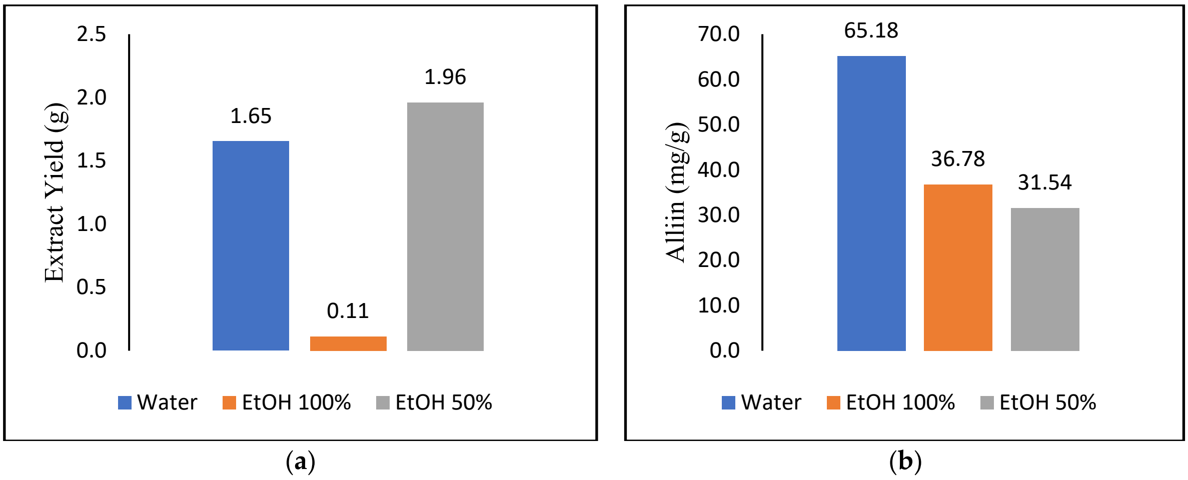


Figure 3. The results of garlic extraction by Soxhlet: (**a**) yield and (**b**) alliin recovery using distilled water, ethanol–water (1:1), and 100% ethanol.

Distilled water and 100% ethanol have polarities of 1.0 and 0.65, respectively, which are not favourable enough to extract and form bonds with polar and nonpolar compounds in a solute when compared with ethanol–water (1:1), which has a polarity of 0.71 [15]. As a result, the extraction yield using distilled water (1.65 ± 0.03 g) was lower than that using ethanol–water (1:1), and the lowest yield recovery was obtained with 100% ethanol (0.11 ± 0.005 g). In contrast, the concentration of alliin extract obtained with 100% ethanol was 36.78 mg alliin/g sample extract, slightly higher compared with ethanol–water (1:1), which yielded 31.54 mg/g. However, distilled water showed the highest yield, with 65.18 mg alliin/g sample extract. This is explained by the fact that the precursor of allicin (alliin) has low polarity, and it is often extracted using polar solvents at room pressure (0.1 MPa) due to its extreme instability in nonpolar organic solvents.

Besides alliin, organosulfur compounds such as S-allyl cysteine, diallyl disulphide (DAD), and diallyl trisulphide (DATS) have low polarity as well and are derived using polar solvents [16]. In addition, due to the heterogeneity of alliin, it has high volatility and is readily vaporised; hence, water, as a pure polar solvent, can be used to extract the vaporised compound. As for 100% ethanol and ethanol–water (1:1), these solvents are bipolar in nature and have a propensity to absorb both polar and nonpolar solutes, such as phenolic compounds, lowering the percentage of alliin recovered [17]. This result is consistent with the optimisation of an extraction and quantification technique for garlic's phenolic content utilising a variety of solvents [18]. The findings indicate that using Soxhlet with methanol as the solvent results in the highest concentrations of phenolics, including 15.27 ppm gallic acid, 85.24 ppm rutin, and 52.20 ppm quercetin [19]. Similarly, in the study by Ciric, et al. [20], the preliminary experimental results of Soxhlet extraction using ethanol as the solvent showed total phenolic contents and total flavonoid contents of 16.641 mg GAE/g fw and 1.419 mg RUT/g fw, respectively.

The variable character of solvent–solids should be examined. This is because an appropriate ratio of solvent to solids allows uniform and efficient heating. Since the heat radiation is absorbed by the solvent, excessive solvent results in inefficient Soxhlet heating. Due to the localised concentration of active chemicals, a low solvent-to-solid ratio causes mass transfer barriers that restrict the movement of these compounds out of the cell matrix [21].

3.2. Subcritical Water Extraction

The extraction yield of SWE, as shown in Figure 4a, was determined at different temperatures of 120 and 180 °C, flow rates of 2 and 4 mL/min, and constant pressure of 15 MPa for a total extraction time of 10 min throughout the experiment. The highest extraction yield obtained (1.28 ± 0.02 g) was at 6 mL/min and 180 °C, while the lowest extraction yield obtained (0.83 ± 0.03 g) was at 2 mL/min and 120 °C. In this work, the results clearly show that the extraction yield is linear with temperature due to extraction selectivity and efficiency. Increased temperatures can alter the dielectric constant of water and increase the solubility of less polar compounds in the solvent, hence increasing the efficiency of extraction. This condition is similar to the extraction of abalone viscera using SWE at increasing temperatures of 110 °C to 170 °C, where the extraction yield increased from 28% to 46%. However, when it was raised to 230 °C, the yield was reduced to 36% [22].

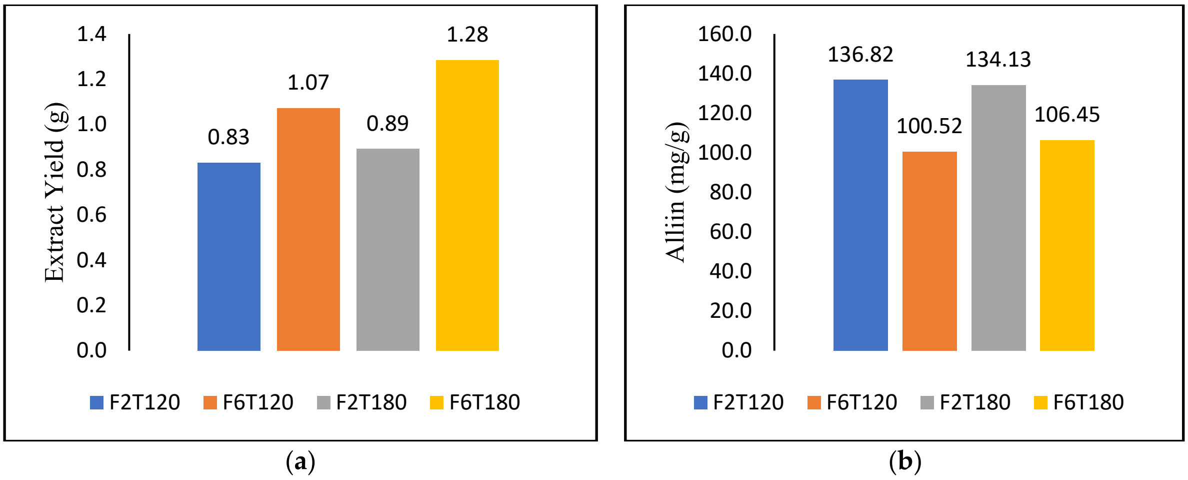


Figure 4. The responses of (**a**) extraction yield and (**b**) alliin using SWE at 2 mL/min and 6 mL/min, 120 °C and 180 °C, and 10 min extraction time.

However, a faster flow rate resulted in a lower concentration of alliin. As seen in Figure 4b, a flow rate of 2 mL/min at 120 °C yielded the highest concentration of alliin with 136.82 mg alliin/g sample extract, while only 100.52 mg alliin/g sample extraction yield was obtained when the sample ran at a flow rate of 6 mL/min at 120 °C. This is because a high flow rate of the extractant results in needless dilution of the extract, which may demand a concentration step after SWE [23]. A high flow rate will reduce the residence time of the solvent to extract the solute. Therefore, there is a minimum mass transfer process to separate alliin (the solute) from the garlic [11]. Additionally, recovery decreased when the flow rate exceeded 2 mL/min, owing to the shorter contact time between the solvent and the matrix. This scenario is similar to a prior study on the extraction of sesquiterpene lactones from Inula racemose, in which the recovery of AL&IAL could be improved by raising the SWE flow rate limit to 3 mL/min; otherwise, there was a loss in the recovery of the compounds [24].

Furthermore, the temperature in this study had a significant effect on the extraction yield. This is because high temperature will decrease the viscosity of water and the surface tension, and therefore, the water will easily penetrate the garlic to carry out the extract [25,26]. Furthermore, high-temperature conditions will lead to a decrease in pH, where low-pH conditions will disrupt the surface morphology of garlic. It will then be easy to penetrate the broken particles of garlic in order to recover the global extract [27,28]. High temperatures also increase the diffusivity of the solvent, and increasing the temperature will enhance the extraction rate of water [29,30].

However, a high temperature is not a suitable condition for thermolabile compounds, including alliin [25,31,32]. This was validated by the decreasing alliin concentration when the temperature increased from 120 °C to 180 °C. By increasing the temperature above 180 °C, the recoveries of alliin may be decreased due to the degradation of the target compounds. Reducing alliin, the compound of interest, will reduce the antioxidant activity of the extract [33,34]. Overall, the extraction of alliin using SWE resulted in the minimum amount of alliin, with 100 mg alliin/g sample extract. Increasing the temperature decreases the pH of water, and thus, bioactive compounds will be degraded by more acidic solvents [25]. However, low pH also has the advantage of breaking the particle surface of garlic, as low pH will react with lignin in garlic to produce phenolic compounds [11]. Similar results were obtained with peanut skin extraction with subcritical water, in which increased temperature decreased the catechin and epicatechin recovery. The high-temperature condition is not suitable for thermolabile compounds [25]. Therefore, the lower-temperature condition is suggested to obtain a high concentration of alliin.

3.3. Comparisons of Subcritical Water Extraction and Soxhlet Extraction

The total extraction yield achieved under optimal SWE conditions was compared to that obtained under Soxhlet extraction conditions. Moderate SWE conditions were chosen (2 mL/min and 120 °C) with a high concentration of alliin in order to compare its performance to Soxhlet extraction. As presented in Figure 5a, the highest extraction yield of 1.96 g was obtained with Soxhlet extraction with ethanol–water (1:1) as the solvent, followed by distilled water with a yield of 1.65 g, and SWE was the third best result, which yielded 0.83 g. Lastly, 100% ethanol had the lowest extraction yield with 0.11 g. Compared to other extraction media, Soxhlet extraction utilising ethanol–water (1:1) provided higher extraction yield. This explains why the majority of garlic's components are bipolar, with some contributing to its antioxidant function, as mentioned by Ahmad, Ahmad, Riaz, Al-tarouti, Aloufi, AlDarwish, Alalaq, Alhanfoush and Khan [19]. On the other hand, the study by Ramirez, et al. [35] successfully determined both the polar and nonpolar bioactive compound contents in garlic.

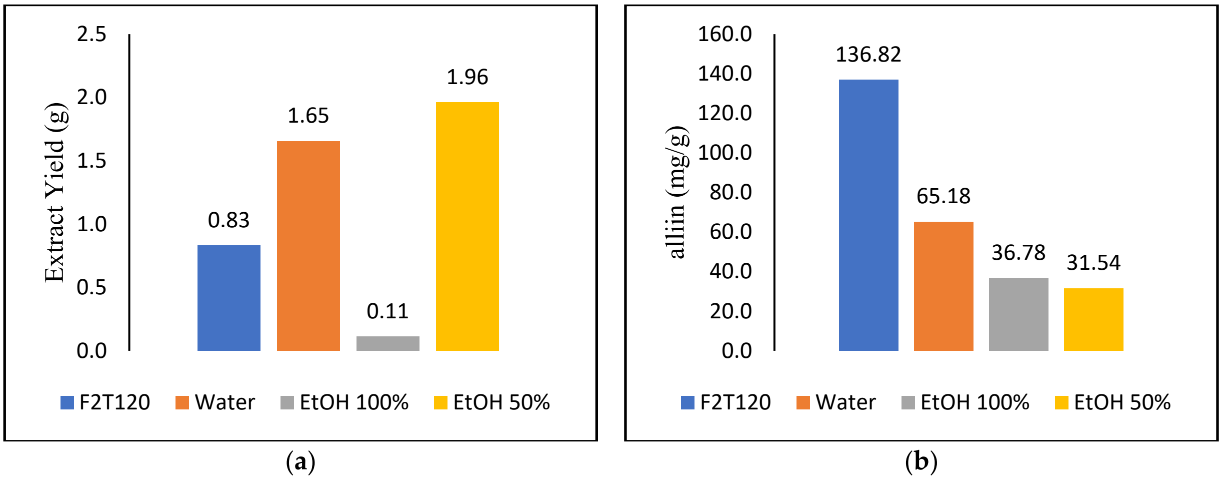


Figure 5. Comparison of (**a**) extraction yield and (**b**) alliin (**b**) between the optimum parameters of SWE and Soxhlet extraction.

Although Soxhlet extraction produced the highest garlic yield extract, the concentration of alliin in the extract was lower when compared to SWE. This is because Soxhlet extraction is capable of extracting the group of oleo resins and waxes, which consequently reduces the purity of alliin compounds. The same finding was reported by Arsad, et al. [36], where antioxidant activity was shown to be reduced when Soxhlet extraction was used, despite the increased oil yield recovered. Conversely, a prolonged extraction time is necessary to complete the extraction cycle in Soxhlet extraction, but it will decrease the concentration of alliin. Even though the sample was subjected to a high temperature when employing SWE, the dynamic system of this process permits one-time contact with the sample–solute throughout the experiment; hence, SWE is efficient in preventing the alliin compound from degrading [37]. According to Rodrigues, et al. [38], who reported on the recovery of bioactive compounds from Chaya, the antioxidant activity shown by Soxhlet was greater than that of SWE at 80 °C for 10 min. However, raising the temperature during SWE progressively lowered the value of EC50, indicating a rise in antioxidant activity. SWE was preferred since it allowed for the manipulation of the solvent's polarity to extract the desired compounds as well as needed a shorter extraction time. Additionally, the extracted quality was superior to that obtained through Soxhlet extraction.

4. Conclusions

In conclusion, the Soxhlet extraction technique yielded the highest extraction yield with 1.96 g when using 50% EtOH, compared to 1.28 g produced using SWE, at a flow rate of 6 mL/min and 180 °C. In contrast to Soxhlet extraction, which yielded 65.18 mg alliin/g of sample extract, SWE yielded the highest alliin concentration at 136.82 mg alliin/g of sample extract. In the SWE system, modifying the temperature can change the polarity of water, control the water's behaviour to operate as an acid or basic catalyst, and modulate the pH level, which is advantageous for the stability of alliin extraction since alliin is a volatile compound. In addition, SWE resulted in a shorter extraction time and concentrated the extracted alliin. Thus, SWE is a potential approach for extracting alliin from garlic, and the findings of this work may be used in future research. However, based on this study, the low-temperature condition is suitable for obtaining a high concentration of alliin because it is a thermolabile compound. It is suggested to extract garlic using supercritical carbon dioxide, where the lower-temperature condition can be used to prevent the degradation of alliin.

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