Preparation of Garlic Extract Containing Allicin using Different Extraction Methods

Tipparat Saejung[1, a] and Thitiphan Chimsook[1, b]
[1]Applied Chemistry Program, Faculty of Science, Maejo University,
Chiangmai, 50290, Thailand
[a]< Numtip_tipparat@hotmail.com >, [b]<thitiphan.cs@gmail.com>

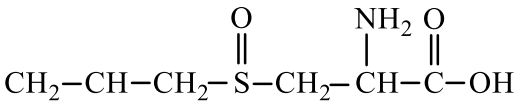
Abstract

Garlic has various pharmacological activities such as antibacterial, anticancer and anti-inflammatory activities. In this work, fresh pearl garlic or single clove garlic was used the raw material to prepare the garlic extracts. The aim of this work is to prepare the garlic extracts by using chemical and non-chemical extraction. For chemical extraction, different garlic/95% ethanol (w/v) ratios were 1:10, 1:15 and 1:20 using extraction methods including maceration, soxhlet, and microwave assisted extraction, respectively. For non-chemical extraction, the garlic extract was prepared by mixing the garlic and honey in the ratio of 1:1 (w/v). All garlic extracts were evaluated the bioactivities and allicin contents. The antioxidant activity and total phenolic contents of each extract were investigated by DPPH radical scavenging assay and Folin Ciocalteu method, respectively. The results revealed that the garlic extract obtained from soxhlet extraction using a mixture of garlic and ethanol in the ratio of 1:15 (w/v) displayed the highest antioxidant activity and total phenolic contents of 17.95±1.05 µmol of trolox equivalents per gram of garlic and 26.88±0.08 mg of gallic acid equivalents per gram of garlic, respectively. Each garlic extract was determined the content of allicin by HPLC-UV analysis. The results revealed that garlic extract obtained from the mixture of garlic and honey displayed the highest content of allicin at 8.12 µg/ml.

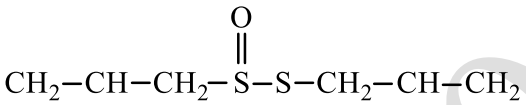
Keywords: *Pearl garlic*, *Allicin*, *Total phenolic contents*, *Antioxidant activity*

.1 Introduction

Garlic (*Allium sativum*) is one of the traditional herbals and utilized as flavoring [1]. It is well known to acquire various phytochemicals. Several evidences highlight its pharmacological potential such as antibacterial, antifungal, anti-inflammatory and antiviral activities [2]. *In vitro* studies verified its antiplatelet aggregation, anticancer and antioxidant activities [3]. Moreover, *in vivo* studies, both in human clinical trials and in animal modelling, have demonstrated beneficial aspects of garlic against various threats including hyperlipidaemia, hyperglycaemia, and atherosclerotic plaque formation, respectively [4]. Allicin and alliin is one of the main biologically active components of garlic (Figure 1). They have a potential to reduce various ailments [5-6]. Allicin is produced by an enzymatic reaction when raw garlic is either crushed or injured. The enzyme allinase combined with alliin and produces allicin [7]. In this work, fresh pearl garlic or single clove garlic (Figure 2) was used the raw material to prepare the garlic extracts.



Alliin



Allicin

Figure 1 Chemical structures o alliin and allicin.

Figure 2 The characterize of pearl garlic.

Many conventional extraction methods have been reported for extracting various bioactive compounds such as maceration, reflux, soxhlet, percolation etc. These methods which have been used for many decades are very time consuming and require relatively large quantities of solvents. In general, those extraction methods will use the organic solvents or distilled water for eluting the active substances from the plant or animal tissue. Microwave assisted extraction (MAE) is non-conventional methods that has many advantages including high extraction efficiency, low solvent consumption, high-purity extracts and shortened extraction time which make it well-suited for the extraction of bioactive compounds from

plant materials because it can result in a yield increase in shorter time using less solvent [8]. Moreover, the new extraction was adjusted by using only honey as which can determined as one of non-chemical extraction methods.

The aim of this work is to prepare the garlic extracts by using chemical and non-chemical extraction. For chemical extraction, 95% ethanol was used as organic solvent in a mixture of garlic and 95% ethanol in different ratios. For non-chemical extraction, the garlic extract was prepared by mixing the garlic and honey in the ratio of 1:1 (w/v). All garlic extracts were studied the antioxidant activity, total phenolic contents and allicin contents, respectively.

.2 Materials and Methods

The pearl garlic was obtained from Yunnan, China. All solvent was purchased from Merck. The ethanolic extracts were obtained from a mixture of garlic and 95% ethanol in the ratio of 1:10, 1:15 and 1:20 (w/v) using extraction methods including maceration, soxhlet and microwave-assisted extraction (MAE), respectively. Moreover, the garlic extract was prepared by mixing the garlic and honey in the ratio of 1:1 (w/v). The antioxidant activity and total phenolic contents of each extract were analysed. The allicin contents of all garlic extracts were investigated by HPLC-UV.

2.1 Extraction of garlic extracts

The ethanolic extracts were obtained from a mixture of garlic and 95% ethanol in different ratios. Three extraction methods including maceration, soxhlet and ultrasound-assisted extraction were used to perform the garlic extracts. Moreover, the garlic extract was prepared by mixing the garlic and honey in the ratio of 1:1 (w/v). The outer skin or transparent coverings of the pearl garlic was peeled off, washed and cut into small pieces. The garlic samples were prepared immediately before extraction.

For maceration, a mixture of garlic and ethanol in the ratio of 1:10, 1:15 and 1:20 (w/v) were carried out using 95% ethanol. 1 g of the sample was soaked in 200 ml of 95% ethanol for 48 hrs at room temperature. For soxhlet extraction, 1 g of fresh garlic was performed using a mixture of garlic and 95% ethanol in the same ratio of solvent to solid as maceration technique. The soxhlet condition was done for 1.5 hours at 70°C using soxhlet apparatus. For MAE, the microwave oven (Figure 3) was applied for extracting all samples. In brief, domestic microwave system (Toshiba) was used for the extraction of garlic extract. A mass of 1 g of crushed fresh garlic was used in the extraction process. The microwave power was set 400 W [9-12] and extraction time for 3 min. An ethanol aqueous solution was added in the microwave processed mixture for extraction. For every 15 seconds of microwave

processing the oven was stopped for another 15 seconds in order to prevent from the material overheat. The optimized parameters of extraction duration were varied the ratios of ethanol and garlic of 1:10, 1:15 and 1:20 (w/v), respectively in order to investigate the effect of solvent to solid ratio to percentage yield, bioactivities and allicin contents in garlic extracts. After microwave heating, the mixture in the extraction vessel was allowed to cool down to room temperature.

All extracts were centrifuged and then combined the supernatants. All extracts from each extraction technique were later filtered using filter paper and the filtrate was evaporated the residue solvent using rotary evaporator before freezing dry for 48 hours to obtain the powder of garlic extract. For special non-solvent extraction, fresh garlic was extracted with honey in the ratio of 1:1 (w/v) in vacuum system and called "NChem".

Figure 3 MAE apparatus for extraction.

.22 Determination of antioxidant activity

The radical scavenging activity was examined by the reduction of 1,1-diphenyl-2-picrylhydrazyl (DPPH) in methanol and trolox as standard. Aliquots (0.1 ml) of diluted extracts were added to 1 ml of DPPH solution, and the absorbance of the DPPH solution was determined at 515 nm after 30 min of incubation at room temperature. methanolic solutions of trolox in a range of 0-500 µmol/l were used for calibration to compare the antioxidant activity of garlic extracts. The antioxidant activity of the sample was expressed as µmol trolox equivalents/gram sample (µmol trolox/g sample) [13-17].

.23 Determination of total phenolic contents

Total phenolics of extracts were quantified colorimetrically using Folin-Ciocalteu method [13, 18] and gallic acid as standard with minor changes. In brief, Folin-Ciocalteu reagent was diluted with deionized water. The methanolic garlic extracts (0.1 ml) were mixed with 0.75 ml of the diluted Folin-Ciocalteu reagent and incubated for 10 min at room temperature. Then, 0.80 ml of 2% Na_2CO_3 (w/v) solution was added. The mixture was kept in the dark for 45min before measuring the absorbance at 765 nm using UV/Visible spectrophotometer against a blank, containing deionized water instead of sample extract. The total phenolic contents values were determined from a calibration curve prepared with a series of gallic acid standards Results are expressed as mg of gallic acid equivalents/g sample (mg GAE/g sample).

.24 Determination of allicin

Allicin in the garlic extracts was quantified by a HPLC method a UV detector with 25 µl injection. Above parts were included in the Agilent-1100 HPLC system. Allicin or garlic extracts were applied onto the column and eluted by the gradient elution of water/methanol at a flow rate of 1.0 ml/min. The total running time was 25 min. The absorbance of allicin or garlic extract was monitored at 240 nm. Quantification of allicin in garlic extract was made by comparing its peak area with the calibration curve of the standard allicin with known concentrations [18-19].

3. Results and discussion

3.1 Extraction of garlic extracts

Aqueous ethanol is a preferred extraction solvent system for the garlic extract because it was safe than other organic solvents and used in the food industry. In this work, fresh pearl garlic were prepared the garlic extracts by using solvent extraction and non-solvent extraction. The solvent extraction methods were performed using 95% ethanol in different ratios of garlic:ethanol. Three solvent extraction methods composed of maceration, soxhlet

and MAE. For non-solvent extraction, fresh garlic was extracted with honey in the ratio of 1:1 (w/v) in vacuum system called NChem. The characteristic of garlic extract was brown and fine powder as shown in Figure 4. The percentage yields of garlic extract in the ratio of 1:10, 1:15 and 1:20 (w/v) using maceration, soxhlet and MAE were shown in Table 1. For maceration, the preliminary studies indicated that the optimised parameters generated by using extraction time for 48 hrs. at room temperature and ratios of garlic: ethanol of 1:10 w/v gave the highest yield of garlic extract. In addition, the preliminary studies for extracting the garalic extract using soxhlet achieved the highest yield of garlic extract from the extraction time for 1.5 hrs, 70°C and ratios of garlic:ethanol of 1:10 w/v. For MAE, the previous studies indicated that variables including microwave power, solid to solvent ratio and extraction cycles had little effect on the bioactivities of extracts. Therefore, the preliminary studies of garlic extraction using domestic microwave were performed by studying the effect of microwave power, extraction time to percentage yield of garlic extracts. The results revealed that optimised parameters generated by using extraction time for 3.0 mins at medium microwave power of 400 W by fixed the ratio of garlic:ethanol of 1:10 w/v which gave the highest yield of garlic extract. Thus, the objective of this research is to determine the effect of solvent to solid ratio to percentage yield, bioactivities and allicin contents in garlic extracts.

The results showed that the highest percentage yield of 4.44±0.05 % was carried out using soxhlet at ratios of garlic:ethanol of 1:20 w/v. It can be seen from the results that the ratios of garlic:ethanol has a significant impact on the garlic extracts. Higher ratios of garlic:ethanol would be beneficial to the percentage yield of garlic extracts. When the solvent volume increases, the yield reaches a highest value and then decreases when higher volume of solvent (data not shown). It can be concluded that factors affecting the product yield for extraction of garlic extract was the solvent quantity used at optimised condition. Moreover, the garlic extract was prepared by mixing the garlic and honey in the ratio of 1:1 (w/v) and gave the percentage yields of garlic extract at 4.30±0.02%. All extracts were screened for their biological activities.

Figure 4 Garlic powder from extraction using honey as solvent.

Table 1 The percentage yields of garlic extract from different extraction methods and ratios of garlic to ethanol.

Extraction methods	Ratio of garlic: ethanol (w/v)	Percentage yields (%)
Maceration	1:10	3.88±0.03
	1:15	3.90±0.08
	1:20	3.84±1.08
Soxhlet	1:10	4.33±0.13
	1:15	4.39±0.03
	1:20	4.44±0.05
MAE	1:10	4.37±0.13
	1:15	4.42±0.14
	1:20	4.40±0.22
NChem	-	4.30±0.02

.32 Determination of antioxidant activity

All extracts were investigated the antioxidant activities. The results were shown in Table 2 and Figure 5. It was found that the garlic extract obtained from soxhlet extraction using a mixture of garlic and ethanol in the ratio of 1:15 (w/v) displayed the highest antioxidant activity at 17.95±1.05 µmol trolox equivalents/g. Moreover, garlic powder which extracted

using honey gave high antioxidant activity at 17.95±1.05 µmol of trolox equivalents/g. It was found that the antioxidant activities of garlic extracts prepared from soxhlet at different ratios of garlic:ethanol are slightly different from other extraction methods. For example, the optimal antioxidant activity from MAE (17.92±2.01 µmol trolox equivalents/g) was found to be at the ratios of garlic and ethanol at 1:15 (w/v), whereas the optimal antioxidant activity from maceration exhibited the highest value of 16.27±1.11 µmol trolox equivalents/g at the ratios of garlic and ethanol at 1:20 (w/v), respectively. However, antioxidant activity of garlic extract obtained from maceration was less than that of obtained from other extraction methods. Many researches have reported the antioxidant activity of garlic extract which prepared from different methods and also solvents such as water, methanol. For example, Sultan *et al* worked-on methanol crude extract of garlic by DPPH assay [20-21]. Chung *et al* studied the garlic and garlic extracts and reported that they have high antioxidant activities and also total phenolic contents [22]. However, Wangcharoen *et al* revealed that heating causes the decrease in antioxidant activity due to decomposition of some phenolics and S-containing compounds [23]. This suggests that the ratios of garlic:ethanol (w/v) had less effective in extracting antioxidant phenolic compounds from garlic by using two extraction methods both soxhlet and MAE.

Table 2 Antioxidant activity of garlic extract from different extraction methods and ratios.

Extraction method	**Ratio of garlic: ethanol (w/v)**	**Antioxidant activity (µmol trolox equivalents/g)**
Maceration	1:10	16.20±0.03
	1:15	16.24±1.03
	1:20	16.27±1.11
Soxhlet	1:10	17.79±0.41
	1:15	17.95±1.05
	1:20	17.89±1.01
MAE	1:10	17.90±2.13
	1:15	17.92±2.01
	1:20	17.89±0.88
NChem	-	17.91±0.08

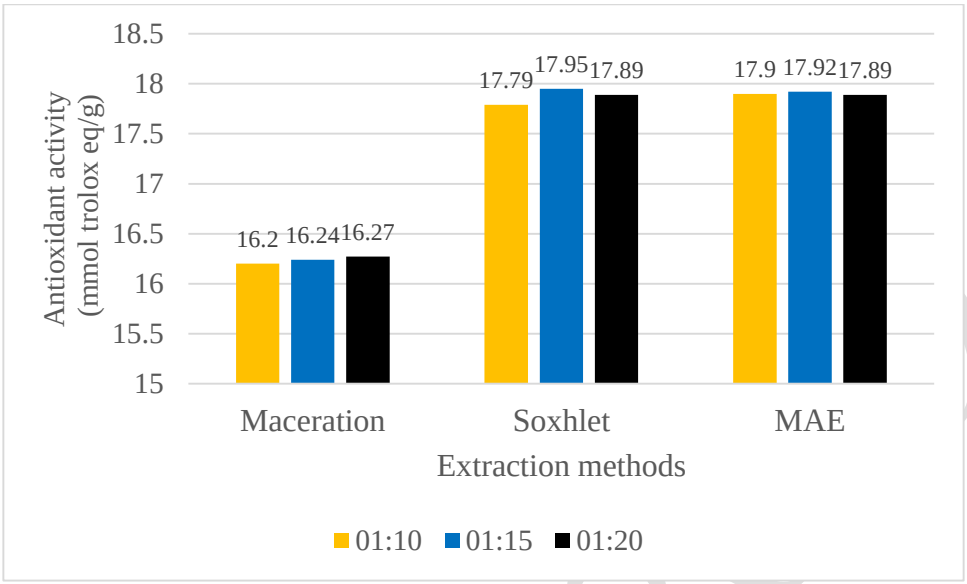


Figure 5 Antioxidant activity of garlic extract from maceration, soxhlet and MAE.

.33 Determination of total phenolic contents

The results of total phenolic contents of each garlic extract were shown in Table 3 and Figure 6. Total phenolic contents of garlic extract as measured by the Folin-Ciocalteu method were from 22.27-22.47 mg of gallic acid equivalents/g for maceration, 25.84-26.88 mg of gallic acid equivalents/g for soxhlet and 26.43-26.79 mg of gallic acid equivalents/g for MAE. The results showed that the garlic extract obtained from soxhlet extraction using a mixture of garlic and ethanol at the ratio of 1:15 (w/v) displayed the highest total phenolic contents (26.88±0.08 mg of gallic acid equivalents/g). For garlic extract which extracted using honey gave the total phenolic contents at 26.75±0.21 mg of gallic acid equivalents/g. The total phenolic contents of extracts obtained from soxhlet were slightly higher than that from MAE. This finding is consistent with those reported in the antioxidant activities of garlic extract. However, MAE can reduce extraction time significantly as compared to conventional extraction methods. As mention in antioxidant activity, the optimized conditions were determined by varying the ratio of solvent to solid in order to evaluate the effect of extraction method to the total phenolic contents of garlic extracts. In previous study, they have reported that the total phenolic contents of the 43 garlic cultivars varied from 17.16 to 42.53 mg of gallic acid equivalents/g. Variability of total phenolic contents in bulbs of different cultivars could be attributed to various cultivar characteristics. Clove size must be considered, as it indirectly affects the final concentration of phenolic compounds. In agreement with our

results, previous reports have shown that different garlic cultivars had different yields, polyphenolic contents and allicin content [24-26].

Table 3 Total phenolic contents of garlic extract from different extraction methods and ratios.

Extraction method	Ratio of garlic: ethanol (w/v)	Total phenolic contents (mg of gallic acid equivalents/g)
Maceration	1:10	22.27±0.14
	1:15	22.43±0.33
	1:20	22.47±0.91
Soxhlet	1:10	25.84±0.04
	1:15	26.88±0.08
	1:20	26.53±0.21
MAE	1:10	26.43±0.08
	1:15	26.79±0.11
	1:20	26.68±1.12
NChem	-	26.75±0.21

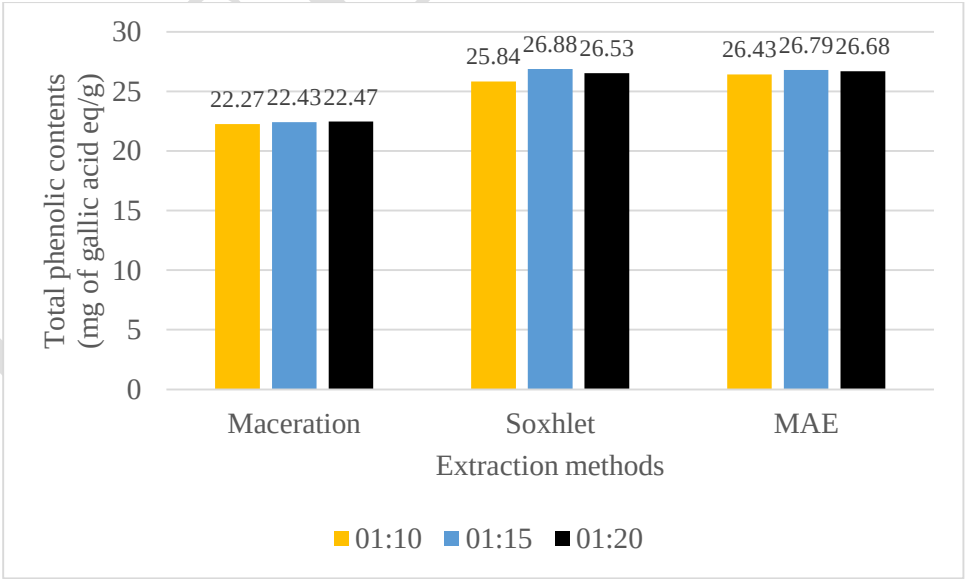


Figure 6 Total phenolic contents of garlic extract from maceration, soxhlet and MAE.

.34 Determination of allicin

Each garlic extract was determined the content of allicin by HPLC-UV analysis. The results of allicin contents in each extract were shown in Table 4. The results revealed that garlic extract obtained from the mixture of garlic and honey displayed the highest content of allicin at 8.12 μg/ml whereas the obtained allicin from three solvent extraction methods was less than 2.27 μg/ml. However, from this experiment MAE can be extracted the powder of garlic extract which had higher allicin than the other solvent extraction methods. It can be concluded that MAE can enhance the allicin recovery. In general, allicin is nonstable compound and easy to decompose when garlic is cut or chopped into small pieces. Thus, it must use the special extraction technique or equipment help to preserve the allicin from garlic in process of extraction.

Table 4 Allicin contents in each extract from maceration, soxhlet and MAE.

Extraction method	Ratio of garlic: ethanol (w/v)	Allicin contents (μg/ml)
Maceration	1:10	1.99
	1:15	1.21
	1:20	1.21
Soxhlet	1:10	2.22
	1:15	2.23
	1:20	2.23
MAE	1:10	2.27
	1:15	2.28
	1:20	2.27
NChem	-	8.12

4. Conclusions

This work studied and reported about the effect of solvent extraction and non-solvent extraction methods of fresh pearl garlic to extraction yield, antioxidant activity, total phenolic contents and quantity of allicin. The results revealed that soxhlet is the best extraction method for prepared the garlic extract using 95% ethanol because the garlic extract had highest antioxidant activity and total phenolic contents. However, the quantity of allicin were found in non-solvent extraction method.

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Thitiphan Chimsook received her Ph.D. degree at Chulalongkorn University, Bangkok, Thailand in 2010. She worked as a lecturer at Maejo University, Chiangmai, Thailand. She now holds an assisted professor position at Meajo University. Her main research interests are in natural product chemistry and biological chemistry.

Tipparat Saejung has been studied Ph.D's degree of Applied Chemistry at Faculty of Science, Maejo University, Chiangmai, Thailand. Her main research interests are in natural product chemistry and cytotoxicity.