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Determination of synergic antioxidant activity of the methanol/ethanol extract of allicin in the presence of total phenolics obtained from the garlic capsule compared with fresh and baked garlic clove

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Abstract

This study presented the methanol/ethanol extraction of organosulfurs from a commercially available garlic capsule, commonly used as a herbal drug supplement, comparing with fresh and its baked garlic clove followed by its antioxidant activity evaluation by both 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 1,10-phenanthroline (Phen) methods. Using various ratios of methanol or ethanol in deionized water under stirring at ambient temperature, the highest antioxidant activities of the crude extract obtained from garlic (powder) capsule, fresh garlic clove and baked garlic powder were 224, 189 and 253 µg BHT/g DW for DPPH assay, and 9.5, 9.2 and 9.6 µg Fe/g DW for Phen assay, respectively. However, the garlic crude extract contained rather high contents of total phenolics, as determined by Folin-Ciocalteu reagent, of 554, 1866 and 536 µg GAE/g DW, respectively. When removal of total phenolics at least 10 folds using C18-SPE expecting only for an allicin, its antioxidant activity of the garlic capsule drastically decreased (88%) as the total phenolics decreased about 91%. Concerning the powder of the baked garlic clove, its antioxidant activity also decreased (82%) with removal of the phenolics (93%). While that of fresh garlic remained increase of 14% as its total phenolics decreased 92%. Therefore, the obtained antioxidant activity of which the organosulfurs in the garlic extract would be synergic results from the residual phenolics.

Introduction

Garlic (*Allium sativum* Linn.) is one of the oldest vegetables, and it has been used as a spice, food and medicine. Especially, garlic clove has been known to possess various biological functions, including antioxidant and antimicrobial activities (Park *et al*., 2010). These functions may be due to the presence of organosulfurs, including alliin, allicin and diallyl disulphide. Normally, allicin (diallyl thiosulfinate) is the main biologically active component of the garlic extracts, which is generated upon its interactions with the enzyme alliinase. It exerts various biological activities such as antimicrobial and anticancer activities in addition to the antioxidant capacity to lower serum lipid levels, particularly cholesterol levels, and ocular pressure (Ogita *et al*., 2007). At the present a variety of biological effects of allicin is attributed to both antioxidant activity and modification of SH-dependent activities (Miron *et al*., 2000).

The antioxidant activities are widely interested to apply in pharmaceuticals, foods, cosmetics and agricultures (Rodriguez *et al*., 2000; Park *et al.*, 2004; Bortolomeazzi *et al*., 2007). Scientists keep trying to validate the properties of garlic, especially in terms of identification of the active components, their mechanisms of action and explore the potential benefits as food supplements (Verma *et al.*, 2008). However, these functional compounds may be affected by the extraction or preparation methods (Banerjee and Sarkar, 2003). Among different chemical compounds found in garlic clove, an allicin has long been recognized as the main antioxidant agent of the crushed garlic clove. The antioxidant and antimicrobial effects of some garlic preparations such as fresh garlic, garlic powder, garlic oil in chicken sausage were reported and found that the fresh garlic form showed greater both antioxidant and antimicrobial effects than the others (Sallam *et al*., 2004).

The nature of some volatile compounds in garlic which is known to possess many beneficial activities for human health has been depicted. The importance of thiosulfinates in the flavor of garlic distillates is known, since the discovery of allicin as the responsible of fresh garlic flavor (Cavallito *et al*., 1945; Stoll and Seebeck, 1951; Cavallito and Bailey, 1994). In addition, the organosulfurs are identified for their unique pharmacological aspects associated with redox processes, metal binding and catalytic activities based on the antibiotic and anticancer activities (Abu-awwad, 2010). It is shown that the medicinal

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properties of *Allium* species, such as garlic, onion, or shallot, including their role as natural cures for various human viral, bacterial and fungal infections have been mainly attributed to their contents of the organosulfurs (Rabinowitch *et al*., 2002).

In analytical chemistry, an extraction is very important step to isolate allicin. Many solvent extraction methods were used. The effect of various solvents i.e. methanol, ethanol, acetone, diethyl ether, hexane and ethyl acetate on the antioxidant activity of garlic was studied and concluded that the methanol extract showed the highest yield and antioxidant activity than others (Iqbal and Bhanger, 2007). However, the antioxidant activity of garlic preparations can be changed by various factors such as thermal treatment and extraction solvent. In addition, many problems associated with the solvent extraction included the incomplete phase separation of allicin with other organosulfurs and usage of high ratio of organic solvents. Solid-phase extraction (SPE) was recently considered as an alternative extraction method, to clean-up the garlic extract.

The extraction of allicin from garlic clove depends on polarity of the solvent used. Thus, it is somewhat difficult to develop specific extraction procedure suitable for the extraction of all plants. Several solvent systems have been tried, including methanol, ethanol, ethyl acetate, dichloromethane for the extraction of allicin, often with different proportions of de-ionized water. Although various extraction methods can be used for plant materials and can be applied to garlic sample for subsequently determining their allicin content, the effect of heating on garlic property has been performed and found that microwave heating effectively destroys an allinase activity (Song *et al*., 1999). Due to the abundance of alliin, the main thiosulfinate formed upon crushing garlic is allicin. Traditionally, the extraction of allicin from the garlic clove was studied using simple magnetic stirring extraction or ultrasonication (Pedraza-Chaverri *et al*., 2004; Queiroz *et al*., 2009; Park *et al*., 2010). Then, qualitative and quantitative analysis of garlic compositions is usually carried out by chromatographic methods. According to the labile nature of such organosulfurs, certain improvements on isolation and analytical methods to minimize the effect of heat are necessary (Kimbaris *et al.*, 2006). Recently, high-performance liquid chromatography (HPLC) analysis of the allicin has been widely done.

Folin–Ciocalteu assay is commonly used for assessment of total phenolic compounds. The determination of total phenolics has relied colorimetric assays using phosphomolybdic acid reagents (Folin–Ciocalteu's reagent) reacts with phenolic compounds. A number of the synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been extensively added to foodstuffs instead, although their use has begun to be questioned because of their toxicity (Ito *et al.*, 1983; Canadanovic-Brunet *et al*., 2006). So, there is considerable interest in preventive medicine and food industry in the development of natural antioxidants obtained from botanical sources, especially herbal plants (Djilas *et al*., 2003). Therefore, the organosulfurs in some fruits and vegetables like garlic clove and/or their finished products are still interested for an evaluation of the antioxidant activity, particularly in the presence of total phenolics.

Therefore, the objective of this study was to test the antioxidant activity of garlic powder in its capsule by solvent extraction, and evaluated the effect of total phenolics on the antioxidant activity. An identification and characterization of the allicin extract after C18 SPE clean-up from the garlic samples were also attempted by HPLC-PDA/Fluorescence and Fourier Transform infrared (FTIR) spectrometry.

Materials and Methods

Chemicals

The analytical reagent (AR) grade of chemicals and reagents were used. De-ionized water used for the preparation of all solutions was purified by Milli-Q purification system (Millipore) (Massachusetts, USA). Gallic acid and butylated hydroxytoluene (BHT) were used as reference standard for phenolic content and for antioxidant activity, respectively, which obtained from Sigma-Aldrich (USA). Ferric chloride ($FeCl_3$), ferrous sulfate heptahydrates ($FeSO_4.7H_2O$), Folin-Ciocalteu reagent and sodium carbonate (Na_2CO_3) were purchased from Carlo Erba (Italy). 1,1-Diphenyl-2-picryhydrazyl radical (DPPH) and 1,10-phenanthroline (1,10-Phen) were obtained from Fluka (Switzerland). Ethanol (EtOH) and methanol (MeOH) for solvent extraction were purchased from Lab Scan (Ireland). Methanol of HPLC grade was also obtained from Lab Scan (Ireland).

Instruments

The experiments were carried out on a Liquid Chromatograph (LC-20A Shimadzu, Japan). It consists of an autosampler (SIL-20A, Shimadzu, Japan), pump (LC-20AD, Shimadzu, Japan), a Rheodyne injector with sample loop of 25 µL and a photodiode array coupled with fluorescence detector (SPD-M20A, Shimadzu, Japan). Empower software was used for data acquisition. A Vertisep AQS

C18 column (250 × 4.6 mm i.d., 5 µm) was used. A spectrophotometric measurement was performed using visible spectrophotometer (Spectronic 15, Thermo Scientific, USA). A low pressure rotary evaporator (Buchi R-114, Switzerland) was employed to eliminate the solvent. Centrifuge (Hettich EBA 20, Germany), vertex mixer (Genie2, Scientific Industries, USA), conical centrifuge tube with screw cap (Pyrex, Maxico), a micropipet (Pipetman, France) and membrane filter, 0.45 µm, (Whatman International Ltd., UK) were used. A magnetic stirrer (Barusteal Thermolyne SP 46920-26, Iowa, USA) was used.

Garlic capsule and garlic samples

The garlic capsule was commercially available as a branded product (Samutprakarn, Thailand) with the label amounts of approximately 2000 mg/L allicin. Fresh garlic clove was purchased from a local market in Khon Kaen, Thailand and processed as fresh garlic sample into two kinds: fresh (raw) garlic and its baked form, the fresh garlic was baked at 70°C for 24 h and milled in mortar with pestle to get fine powder. These samples were stored in a plastic box in dessicator before used.

Solvent extraction of garlic samples

About 1.5 g sample of fresh garlic/garlic powder were extracted with each 10 mL of two kinds of moderate polar organic solvents including 5-100% (v/v) MeOH, 5-100% (v/v) EtOH and de-ionized (DI) water under magnetic stirring at ambient temperature for 1 h. The garlic extract was centrifuged at 3000 rpm for 20 min, and the supernatant was filtered with filter paper Whatman No.1 and made up to final volume 25 mL with each of the solvents used.

Solid-phase extraction of the allicin extract

The garlic crude extract (5 mL) was added into C18 SPE cartridge (500 mg), which was conditioned with 5 mL MeOH and 5 mL DI water. Allicin was expected to adsorb onto the cartridge, while other water soluble compounds were removed by washing the cartridge with 10 mL DI water. The main fraction containing allicin was eluted with 5 mL MeOH. The solution was filtered through a 0.45 µm nylon filter membrane prior to analysis by HPLC or FTIR. The allicin extract was evaluated for its antioxidant activity comparing with its crude extract.

DPPH radical scavenging assay

Stock standard solution of 1000 mg/L of BHT was prepared by dissolving 0.0200 g BHT in methanol and then adjusted volume to 20 mL. The BHT standard solution was used for the calibration curve ranging from 2-50 mg/L, and each concentration was prepared in triplicates. A DPPH solution was prepared from 1,000 µM of DPPH, which dissolved 0.0086 g of DPPH in methanol and then adjusted volume to 100 mL. The DPPH solution was diluted to be 50, 100, 200, 250, 300, 400 and 500 µM by methanol. The series of the DPPH concentrations were used for the antioxidant activity assay. The absorbance of final concentrations of the DPPH solution was measured at 517 nm by UV-Visible spectrophotometry using methanol as a blank. The DPPH solution giving the absorbance in the range of 0.192-0.761 ABFS was selected.

The free radical scavenging activity was determined by using DPPH assay. Briefly, 2.0 mL of each extract was mixed with 3 mL of 300 µM DPPH solution. After that the solution was kept in the dark at ambient temperature for 45 min and the absorbance of the mixture was read at 517 nm. The control solution was prepared from 3 mL of the DPPH solution, 2 mL of methanol and the absorbance was measured at the same wavelength. The percentage of an inhibition was calculated according to following equation.

$$\% \text{ inhibition} = \frac{[Abs_{(t=0)} - Abs_{(t=45)}]}{Abs_{(t=0)}} \times 100$$

Where; $Abs_{(t=0)}$ is the absorbance of the control reaction (containing all reagents except the test extract) at 0 min. $Abs_{(t=45)}$ is the absorbance of the test extract at 45 min. The DPPH scavenging percentage of each sample was compared with the BHT calibration curve, which plotted the inhibition percentage against all BHT concentration. The antioxidant activity was expressed as µg of BHT per g dry weight (µg BHT/g DW). Data are reported as means ± standard deviation (SD) for three replicates.

Phen assay

Stock standard solution of Fe(II) 2000 µmol/L was prepared by dissolving 0.0556 g of ferrous sulfate heptahydrates ($FeSO_4.7H_2O$) in methanol and then adjusted the final volume to 5-mL volumetric flash. The Fe(II) stock solution was used to plot the calibration curve ranging from 0.1-1.0 µmol/L. 1,10-Phenanthroline (1,10-Phen) solution (0.5%, w/v) was prepared by dissolving 0.5 g of 1,10-phenanthroline in methanol and then adjusted final volume to 100-mL volumetric flash.

The ferric ion-based total antioxidant activity assay was used for determination of antioxidant activity of the phenolics in the garlic extract. The antioxidant activities were determined by coloration

of a methanolic solution of 1,10-Phen, proposed by Szydlowska-Czerniak *et al.* (2008), with slightly modified method. Briefly, 2.0 mL solution of the allicin extract, 1 mL 0.2% $FeCl_3$ and 1 mL 0.5% 1,10-Phen and made up to volume with solvent. The obtained solution was mixed and left at room temperature in a dark. After 20 min, the absorbance of an orange-red solution was measured at 510 nm. The calibration curve of Fe(II) complex was prepared in the range of 0.1-1.0 µmol/L. The absorbance that affected by the garlic extract was compared with Fe(II) calibration curve. The antioxidant activity was expressed as µmol Fe per g dry weight (µmol Fe/g DW). Data are also reported as means ± standard deviation (SD) for three replicates.

Determination of total phenolics of the garlic extracts by Folin-Ciocalteu assay

Stock standard solution of 1000 mg/L gallic acid (GA) was prepared by dissolving 0.0200 g of GA and adjusted volume to 20 mL with methanol. The GA standard solution was used for the calibration curve ranging from 1.0-32 µg/L, and each concentration was prepared in triplicates. 7.5% (w/v) Na_2CO_3 solution was prepared by dissolving 7.5 g of Na_2CO_3 and adjusted volume to 100 mL with DI water. 10% (v/v) Folin-Ciocalteu reagent was prepared by diluting the Folin-Ciocalteu reagent with DI water.

The total phenolic contents in the samples were estimated by colorimetric assays. Gallic acid was used as a reference compound (Queiroz *et al.*, 2009). Briefly, 1.0 mL of the extract was added into a 5-mL volumetric flask, and then followed by 1 mL 10% Folin–Ciocalteu's reagent. After that, 0.5 mL 7.5% Na_2CO_3 solution was added, and then adjusted to final volume by the solvent. The solution mixture was stored in the dark at room temperature for 30 min. The absorbance was measured at 765 nm. Total phenolic contents were expressed as µg of GA equivalents per g of the garlic extract.

Preliminary study of the garlic extract containing allicin using RP-HPLC-PDA/Fluorescence

Preliminary identification of chemical compositions of the garlic extract by using RP-HPLC with PDA/Fluorescence detection was carried out at ambient temperature with a flow-rate of 0.7 mL/min. The analysis of allicin was investigated using 50% (v/v) MeOH in DI water as a mobile phase (Rahman *et al.*, 2012). The mobile phase was filtered through a 0.45 µm nylon membrane and degassed before use. The HPLC column was equilibrated with the mobile phase for about 30 min prior to separation.

Characterization of allicin in the garlic extract by FTIR

In this study, three kinds of the garlic samples were defined as fresh garlic, garlic powder and baked garlic. They were identified to confirm the presence of allicin as the main component of the organosulfurs in these garlic samples by FTIR. The liquid sample was determined as liquid film (neat) and solid sample using potassium bromide (KBr) pellet.

Results and Discussion

Identification and characterization of allicin in the garlic extract

From HPLC-PDA chromatograms, the retention time of allicin in the garlic extract obtained from fresh garlic clove, garlic capsule and baked garlic was noticed by using the mobile phase described elsewhere. In this study, the organic solvent selected for the preliminary experiment was methanol due to the solubility of allicin. However, the mobile phases containing various percents of MeOH in DI water were also investigated. Under the optimal isocratic conditions, the allicin was separated within 5 min (data not shown). As PDA detector was used allowing an identification of compound using an absorption spectrum and peak purity. Identification of the expected allicin at 254 nm was achieved by comparing its retention time and absorption spectrum reported in literature (Bocchini *et al.*, 2001).

On the other hand, the chromatogram obtained from HPLC with fluorescence detector showed five main peaks with retention time (t_R) at 4.4, 5.2, 6.6, 12.6 and 29.1 min (Figure 1). The peak at t_R 6.6 min was traced to be allicin. However, it was noted that the chromatograms of the garlic extracts from fresh garlic clove, garlic capsule and baked garlic clove were very similarly with respect to the number of components.

The garlic extract was characterized by FTIR to indicate the main sulfhydryl group (SH) of allicin as shown in Figure 2. FTIR spectra of the allicin extract demonstrate similar pattern of their functional groups. The mode assignments of S=O occur at 1026-1030 cm^{-1}, S-H at 2382 cm^{-1}, C=C at 1648 cm^{-1} and C-C at 1465 cm^{-1}.

Determination of the antioxidant activity of the garlic crude extract

The antioxidant activity of the garlic extract was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and 1,10 phenanthroline (Phen) methods. Various concentrations of DPPH

Table 1. The antioxidant activities of the crude extracts of fresh garlic clove, garlic capsule, and baked garlic samples determined by DPPH assay (mean ± SD, n = 3)

Solvent (v/v)	Antioxidant activity (µg BHT/g DW)		
	Fresh garlic	Garlic powder	Baked garlic
5% MeOH	42.7±7.2	111.3±0.01	146.0±4.3
10% MeOH	94.8±0.11	124.3±1.3	174.4±1.9
20% MeOH	182.7±3.6	184.6±2.5	226.7±0.0
50% MeOH	162.7±0.0	223.9±0.21	253.0±0.16
75% MeOH	104.5±2.0	215.1±2.6	184.1±0.17
100% MeOH	64.9±6.2	39.4±3.8	62.2±0.02
5% EtOH	17.6±2.9	104.5±3.7	97.3±1.1
10% EtOH	47.6±6.4	140.8±2.9	125.8±0.14
20% EtOH	107.5±0.22	172.6±0.13	173.7±0.0
50% EtOH	189.2±3.9	223.4±0.0	199.1±2.5
75% EtOH	112.7±2.2	139.3±0.09	115.3±2.2
100% EtOH	35.2±0.29	34.9±1.1	52.4±0.34
Deionized water	92.6±3.2	103.0±18.3	86.9±2.0

Table 2. The antioxidant activities of the crude extracts of fresh garlic clove, garlic capsule, and baked garlic samples determined by Phen assay (mean ± SD, n = 3)

Solvent (v/v)	Antioxidant activity (µmol Fe^{2+}/g DW)		
	Fresh garlic	Garlic powder	Baked garlic
5% MeOH	5.55±0.10	8.22±0.14	7.07±0.01
10% MeOH	7.48±0.00	8.50±0.00	8.05±0.01
20% MeOH	8.35±0.01	9.52±0.01	8.84±0.03
50% MeOH	7.81±0.10	8.11±0.00	7.02±0.12
75% MeOH	5.10±0.01	6.80±0.09	5.60±0.04
100% MeOH	3.83±0.54	2.35±0.14	2.50±0.09
5% EtOH	3.84±1.1	8.82±0.26	8.75±0.22
10% EtOH	5.42±0.28	7.99±0.01	8.92±0.16
20% EtOH	6.96±0.01	7.13±0.04	8.10±0.02
50% EtOH	9.18±0.58	6.12±0.50	8.17±0.00
75% EtOH	7.90±0.14	7.69±0.00	7.12±0.08
100% EtOH	5.64±0.19	4.50±0.14	5.30±0.00
Deionized water	5.67±0.33	8.47±0.01	9.55±0.19

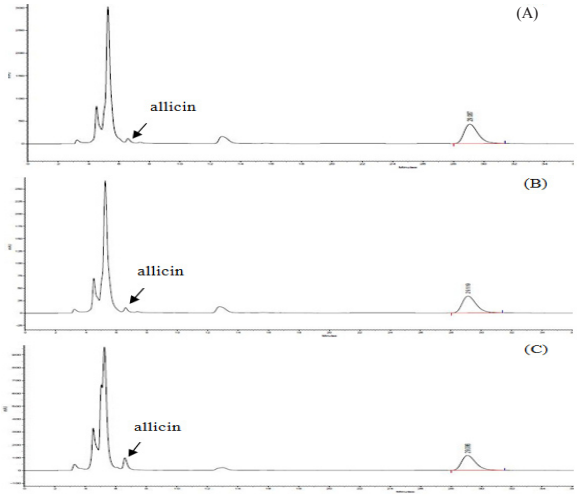

Figure 1. The chromatograms of allicin extract obtained from (A) fresh garlic clove, (B) garlic capsule and (C) baked garlic. Peak with arrow indicates allicin.

were optimized in this study. Higher concentrations of DPPH in the reaction mixture gave absorbance beyond the accuracy of spectrophotometric measurements. The calibration curve of DPPH was found in the range of 50-500 µM. It is noted that the absorbance of DPPH increased with an increasing of the DPPH concentration. The linear graph ranging of 50-400 µM, however, it was deviated at 300 µM. The accuracy for the spectrophotometry showed within an absorbance range of 0.256-1.038 ABFS. Therefore, this work selected a DPPH concentration of 300 µM, in consonance with the requirements of the accuracy

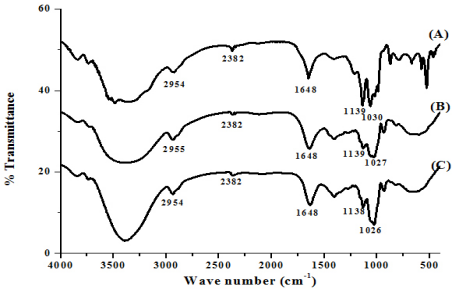

Figure 2. FTIR spectra of the allicin extract obtained from (A) fresh garlic clove, (B) garlic capsule and (C) baked garlic

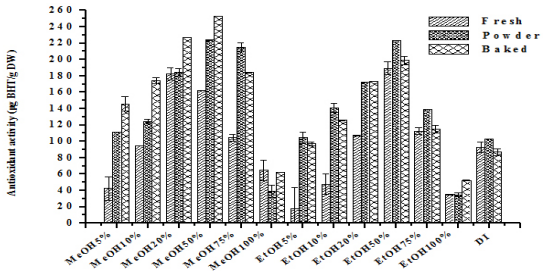

Figure 3. The comparison of the antioxidant capacities of various crude extracts of fresh garlic clove, garlic capsule, and baked garlic samples determined by DPPH assay

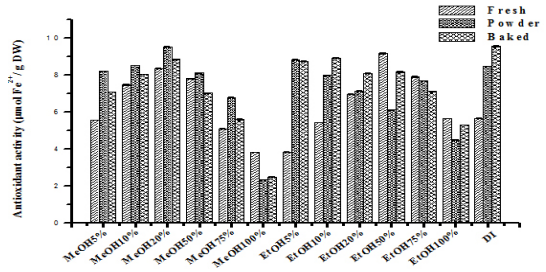

Figure 4. The comparison of the antioxidant capacities of various crude extracts of fresh garlic clove, garlic capsule, and baked garlic samples determined by Phen assay

of the instrument.

The reduction capability of DPPH radicals determined by the decreasing of its absorbance at 517 nm was induced by antioxidants. A linear calibration curve is plotted giving the percentage of an inhibition against the concentration of BHT standard in the range from 1-50 µg BHT; y = 1.43x + 6.24 with r^2 of 0.9961 was constructed. The inhibition of DPPH radical increased with an increasing of BHT concentration due to the scavenging ability of BHT standard. The antioxidant activity was expressed as µg BHT/g DW.

As the results shown in Table 1 and Figure 3, the organosulfurs exhibited significant scavenging effects on the DPPH radical and their effect increased with increasing of concentrations. Compared with BHT, the scavenging effect of the organosulfurs was higher. In garlic capsule, the antioxidant activity (224 µg BHT/g DW) of the crude extract with either 50% MeOH or 50% EtOH was significantly higher ($p < 0.05$) than other solvent compositions. While that of

Table 3. Total phenolic contents of the crude extracts of fresh garlic clove, garlic capsule and baked garlic samples (mean ± SD, n = 3)

Solvent (v/v)	Total phenolic (µg GAE/g DW)		
	Fresh garlic	Garlic powder	Baked garlic
5% MeOH	946.6±54.3	542.2±48.4	505.5±34.5
10% MeOH	1446.5±0.27	530.8±3.2	455.5±0.81
20% MeOH	1515.7±0.68	536.3±0.61	516.3±0.43
50% MeOH	1638.2±5.3	476.4±3.4	452.4±4.8
75% MeOH	1351.9±1.5	404.0±2.6	412.7±0.27
100% MeOH	1815.4±52.8	348.9±52.4	375.5±29.3
5% EtOH	1028.2±38.1	535.5±33.4	503.6±24.7
10% EtOH	1337.1±5.9	542.0±8.4	491.5±1.8
20% EtOH	1346.5±5.4	497.1±0.58	536.0±10.9
50% EtOH	1597.5±12.7	407.1±13.8	419.4±6.2
75% EtOH	1536.8±18.4	378.0±15.1	370.5±12.9
100% EtOH	1865.9±63.3	362.5±47.7	346.8±48.5
Deionized water	831.5±90.3	554.1±72.6	529.3±64.6

Table 4. The antioxidant activities of the allicin extracts of fresh garlic clove, garlic capsule and baked garlic samples determined by DPPH assay (mean ± SD, n = 3)

Solvent (v/v)	Antioxidant activity (µg BHT/g DW)		
	Fresh garlic	Garlic powder	Baked garlic
5% MeOH	130.6±0.10	19.4±0.39	24.9±0.04
10% MeOH	148.0±0.03	21.8±0.48	25.6±0.04
20% MeOH	191.0±0.03	22.1±0.19	38.8±0.57
50% MeOH	208.0±0.14	27.4±0.01	42.7±0.60
75% MeOH	212.7±0.02	20.8±1.2	40.7±0.30
100% MeOH	152.7±0.08	25.9±2.0	24.9±0.30
5% EtOH	131.6±0.04	19.1±0.31	23.8±0.0
10% EtOH	150.9±0.06	19.8±0.01	29.9±0.25
20% EtOH	215.9±0.12	22.4±0.62	36.5±0.02
50% EtOH	213.2±0.17	27.6±0.0	45.1±0.96
75% EtOH	199.2±0.24	23.6±0.01	27.8±1.2
100% EtOH	200.4±0.85	22.1±0.09	22.2±3.8
Deionized water	210.6±0.01	24.7±0.09	37.1±0.0

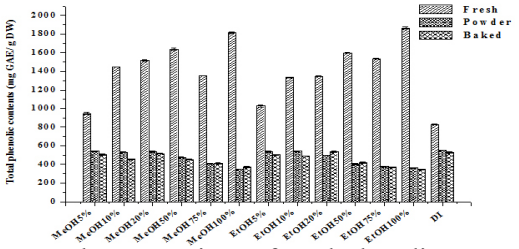

Figure 5. The comparison of total phenolic contents in various crude extracts of fresh garlic clove, garlic capsule and baked garlic samples

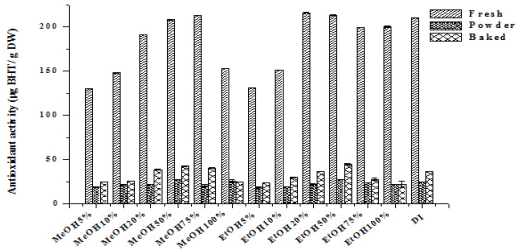

Figure 6. The comparison of the antioxidant capacities of the allicin extracts of fresh garlic clove, garlic capsule and baked garlic samples determined by DPPH assay

fresh garlic clove with 50% EtOH was about 189 µg BHT/g DW), and its baked garlic extracted with 50% MeOH showed its activity (253 µg BHT /g DW) in a similar trend.

For Phen assay, the antioxidant activity of sample was determined by the increasing of its absorbance at 510 nm. The calibration curve of ferrous ion in the range of 0.1-1.0 µmol Fe^{2+}; y = 0.68x + 0.12 with r^2 value of 0.9979 was used to calculate the concentration of ferrous ion in the sample solution. The antioxidant activity of the sample extract was expressed as µmol Fe/g DW.

The trend of antioxidant capacity of the garlic extract using Phen assay was similar to that using DPPH radical scavenging assay. The obtained results are presented in Table 2 and Figure 4. The antioxidant capacities were found to be 2.4-10 µmol Fe/g DW. It is noted that for garlic capsule, the crude extract of 20% MeOH gave significantly higher antioxidant activity (9.5 µmol Fe/g DW) ($p < 0.05$) than other extracts. While that of the crude extract from fresh garlic clove with 50% ethanol was found to be 9.2 µmol Fe/g DW), and its baked garlic with DI water showed no difference in its antioxidant activity (9.6 µmol Fe/g DW).

Determination of total phenolics in the garlic crude extract

The total phenolic contents of the garlic extract were determined using Folin-Ciocalteu method. Briefly, the appropriate extract dilution was oxidized with the Folin-Ciocalteu reagent and then neutralized the reaction with sodium carbonate. The absorbance of the resulting blue color was measured at 745 nm after 30 min using UV-Visible spectrophotometer. The calibration curve of gallic acid in the range of 1.0-32.0 µg GA; y = 0.03x + 0.05 with r^2 of 0.9988 was constructed. The total phenolics content was expressed as µg GAE/g DW.

From the results (Table 3), the effect of organic solvents on the extraction can be expected that the total phenolics content of the garlic extract is highly depending on the polarity of solvents. Ethanol and methanol are moderately low polarity with the polarity index of 6.0 and 5.1, respectively. By comparing, the extract of fresh garlic clove with 100% EtOH gave the phenolics (1866 µg GAE/g DW) ($p < 0.05$) significantly higher than other solvents (Figure 5). For garlic capsule, its extract with DI water gave the phenolics (554 µg GAE/g DW) higher than other solvent compositions, and that of the baked garlic extract (536 µg GAE/g DW) of 20% EtOH was also significantly higher than other solvents used.

Determination of the antioxidant activity of the allicin extract

The antioxidant activities of the allicin extract, which were obtained after clean-up the garlic crude extract with C18 SPE, assayed by DPPH using BHT as mentioned earlier were comparatively shown in Table 4 and Figure 6.

From the results, there were not significantly different ($p > 0.05$) in the antioxidant activities of the allicin extract obtained from various methanol

Table 5. The antioxidant activities of the allicin extracts of fresh garlic clove, garlic capsule and baked garlic samples determined by Phen assay (mean ± SD, n = 3)

Solvent (v/v)	Antioxidant activity (μmol Fe^{2+}/g DW)		
	Fresh garlic	Garlic powder	Baked garlic
5% MeOH	1.08±0.0	1.00±0.02	0.85±0.01
10% MeOH	1.16±0.04	1.02±0.01	0.94±0.01
20% MeOH	2.36±0.02	1.11±0.02	1.00±0.01
50% MeOH	2.73±0.02	0.99±0.0	0.85±0.03
75% MeOH	2.91±0.02	0.88±0.02	0.73±0.04
100% MeOH	0.41±0.01	0.49±0.02	0.46±0.02
5% EtOH	0.41±0.01	0.83±0.02	1.00±0.04
10% EtOH	0.96±0.0	0.89±0.03	1.01±0.03
20% EtOH	2.64±0.02	0.90±0.01	0.94±0.01
50% EtOH	2.35±0.04	0.88±0.03	0.95±0.03
75% EtOH	2.04±0.05	0.93±0.0	0.86±0.02
100% EtOH	1.81±0.01	0.65±0.01	0.70±0.02
Deionized water	2.16±0.01	1.00±0.04	1.06±0.04

Table 6. Total phenolic contents of the allicin extracts of fresh garlic clove, garlic capsule and baked garlic samples (mean ± SD, n = 3)

Solvent (v/v)	Total phenolic (μg GAE/g DW)		
	Fresh garlic	Garlic powder	Baked garlic
5% MeOH	61.5±0.06	7.0±0.02	6.4±0.04
10% MeOH	65.2±0.01	25.8±0.02	24.6±0.03
20% MeOH	99.5±0.05	38.5±0.04	26.8±0.01
50% MeOH	107.6±0.06	39.7±0.01	30.6±0.02
75% MeOH	98.9±0.01	32.7±0.02	29.7±0.03
100% MeOH	142.7±0.02	35.6±0.01	35.3±0.04
5% EtOH	67.1±0.05	4.7±0.03	7.0±0.03
10% EtOH	67.0±0.02	8.5±0.03	8.4±0.01
20% EtOH	109.4±0.02	40.9±0.01	29.9±0.03
50% EtOH	120.2±0.01	42.0±0.04	31.7±0.05
75% EtOH	132.5±0.01	35.1±0.02	32.8±0.01
100% EtOH	153.0±0.07	50.6±0.03	35.8±0.03
Deionized water	61.4±0.01	22.9±0.01	22.0±0.03

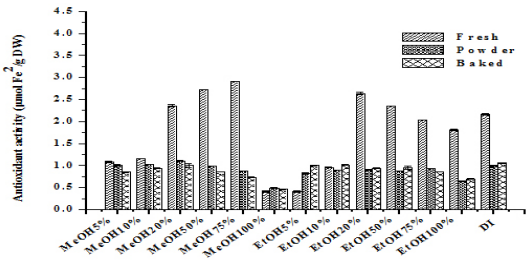

Figure 7. The comparison of the antioxidant capacities of the allicin extracts in various solvent compositions of fresh garlic clove, garlic capsule and baked garlic samples determined by Phen assay

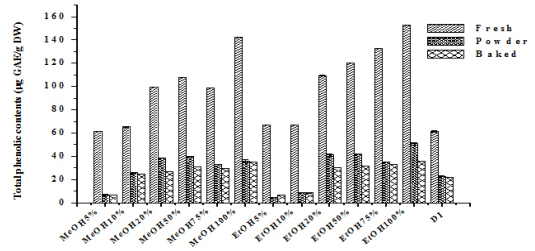

Figure 8. The comparison of total phenolic contents of the allicin extracts found in various solvent compositions of fresh garlic clove, garlic capsule and baked garlic samples

or ethanol compositions in deionized water. It was evident that the allicin extract from garlic capsule exhibited low antioxidant activities in the range of 19.1-27.6 μg BHT/g DW compared with those of baked garlic (22.2-45.1 μg BHT/g DW). While the allicin extract (in 20% ethanol) from fresh garlic clove gave the highest one, 215.9 μg BHT/g DW. However, it still needs deionized water to modify the polarity of the two alcohols, although its extraction efficiency is well suitable for the organosulfurs. Therefore, the antioxidant activities of the allicin extract were found to be 28, 216 and 45 μg BHT/g DW for garlic capsule, fresh garlic clove and baked garlic, respectively.

In the same manner, the antioxidant activities using Phen assay of the allicin extract were also investigated as shown in Table 5 and Figure 7. The antioxidant activities of the allicin extract were found to be 2.9, 1.1 and 1.1 μmol Fe^{2+}/g DW for fresh garlic clove, garlic capsule and baked garlic samples, respectively.

Determination of total phenolics in the allicin extract

Total phenolic contents found in the allicin extract were shown in Table 6 and Figure 8. The total phenolics of the allicin extracts were widely ranged from 4.7 to 153 μg GAE /g DW. For garlic capsule, the phenolic content of the allicin extract was found to be 50.6 μg GAE /g DW. While that of the allicin extract from fresh garlic clove was about three times higher (153 μg GAE/g DW). However, the allicin extract obtained from its baked garlic also contained low contents of total phenolics (35.8 μg GAE/g DW) with no significant difference when compared with other solvent compositions used ($p > 0.05$).

Conclusion

As expected to get partially purified allicin in the garlic extract, the allicin extract obtained from garlic samples was preliminarily identified and characterized by HPLC-PDA and FTIR, since it was somewhat difficult to find an allicin standard. It was noted that their separation behaviors on the reversed phase column of garlic capsule, fresh garlic clove and its baked garlic were similar with respect to the number of components and the maximum absorption wavelength, even though it had not been precisely indicated. Regarding to IR spectra, there are also identity peaks similarly comparing among those of the allicin extracts. The crude extracts of various solvent compositions of both methanol and ethanol in deionized water were evaluated their antioxidant activity using both DPPH and Phen assays compared with that of the allicin extracts. Total phenolic contents between the crude extract and its allicin extract by the Folin-Ciocalteu method were also comparatively determined. When removal of total phenolics at least 10 folds using C18-SPE, its antioxidant activity of the garlic capsule drastically decreased (88%) as the total phenolics decreased about 91%. As usually,

that of the baked garlic powder decreased with the removal of some phenolics, while the antioxidant activity of fresh garlic remained increase of 14% as total phenolics decereased about 92%. Therefore, the total phenolics would make much synergic role on the antioxidant activity of the organosulfurs of the garlic extracts.

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