Research Article

Effect of Extraction Methods on Antibacterial Activity and Chemical Composition of Chinese Chives (*Allium tuberosum* Rottl. ex Spreng) Extract

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Abstract
The Bang-Phae organic vegetable grower community enterprise group, Ratchaburi province is one of the largest Chinese chives (*Allium tuberosum* Rottl. ex Spreng) growers in Thailand. However, not all of its Chinese chives meet the standard of retail markets and were sorted out. Producing Chinese chives extract is an alternative way to add value to the rejected produces. The Chinese chives essential oil contains many new and known bioactive compounds. Therefore, this experiment was aimed to study the antibacterial activity of Chinese chives extracts using 17 difference extraction conditions (Steam distillation for 1.0, 2.0, 2.5, 3.0 h; Ohmic pretreatment followed by steam distillation for 1.0, 2.0, 2.5, 3.0 h; 95% ethanol, hexane, soy bean oil extraction using dried, fresh and frozen Chinese chives) against 6 different pathogenic bacterium (*Escherichia coli* ATCC25822, *Salmonella enterica* Typhimurium U302, *S. enterica* Enteritidis, *S. enterica* 4,5,12:i (human) US clone, *Bacillus cereus* and *Listeria monocytogenes* 10403S). The disc agar diffusion method with 3 different concentrations of extracts (25, 50, 75 mg/mL) was used to evaluate the antibacterial activity. The results showed that different extraction conditions significantly affected the antibacterial activity. The higher extract concentration resulted in the better

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antibacterial activity. This finding indicated that both extraction condition and extract concentration significantly influence antibacterial activity. The extracts obtained from thermal extraction seem to have higher antibacterial activity than the ones obtained from cold extraction. The extract obtained from steam distillation for 2.5 h resulted in the highest antibacterial activity; 75 mg/mL of the extract caused 0.688 ±0.023 cm clear zone against *B. cereus* and *L. monocytogenes* 10403S. The chemical composition profile using GC-MS showed high percentage of organosulfide volatile compounds in thermally extracted Chinese chives. Therefore, the organosulfide volatile compounds might be responsible for effective bacteria inhibition.

Keywords: Antibacterial activity, *Allium tuberosum* Rottl. ex Spreng, Chinese chives, Organosulfide

1 Introduction

Allium tuberosum Rottl. ex Spreng is commonly called garlic chives or Chinese chives. It belongs to the genus Allium and family Liliaceae similar to garlic, white lily and onion. Chinese chives are widely found in South East Asia, South Asia and some countries in Middle East, such as Iran [1]. The Allium species are rich in sulfide compounds which are responsible for their antimicrobial and antioxidant properties [2], [3].

For Chinese chives plantation, fresh leaves were cut several times within the growing season. After 1 year, the crop yield reduced and the plant was discarded as agricultural by-products. To maximize the usefulness of Chines chives by-products, the Bang-Phae organic vegetable grower community enterprise group, Ratchaburi province, which is one of the largest Chinese chives growers in Thailand, is interested in producing the Chinese chives leaf oil from the by-products. The chives leaf oil has a potential to be used as a food preservative and alternative to synthetic antioxidant [2].

Steam distillation is commonly used for extracting volatile organic compounds from biological materials. The basic principle of steam distillation is separation of mixed compound by using water as a solvent. This method is suitable for compound that is easy to volatile, insoluble and does not react with water. However, steam distillation needs long processing time and often results in low extraction yield and degradation of heat sensitive compounds.

Ohmic pretreatment has recently been explored to increase the tissue permeability and, thus, increase extraction efficiency [4]. The principle of ohmic heating is to generate internal heating via passage of electrical current through conductive material. Besides heating effect, during ohmic treatment electroporation may also occur; if the electrical charges build up across the cell wall exceeds the critical limit, pores will be formed on the cell wall causing loss in membrane integrity which can accelerate mass transfer of biological materials inside the cell [5]. However, the effect of ohmic pretreatment on changes in chemical composition is still needed to be studied.

For extraction of heat sensitive compounds from biological materials, solvent extraction is commonly used. This technique generally minimizes the compound deterioration and requires simple equipment [6]. However, toxicity of the solvent residue is a major concern of solvent extraction. Due to the increasing demand for natural ingredients, the use of plant extract appears as a viable alternative product for healthy life style. Therefore, this work aimed to evaluate effect of extraction methods on antibacterial activity and chemical composition of Chinese chives (*A. tuberosum* Rottler. ex Spreng) crude extracts.

2 Materials and Method

2.1 *Preparation of Chinese chives*

Chinese chives (*A. tuberosum* Rottler. ex Spreng) were obtained from Bang-Phae organic vegetable grower community enterprise group, Ratchaburi province, Thailand. The harvesting age of Chinese chives was around 1 year. The Chinese chives leaves were stored at 2–4°C for less than 1 week before use. There were three forms of Chinese chives used in solvent extraction experiment which are fresh, dried and frozen. Only fresh sample was used in hydrodistillation experiment. Fresh Chinese chives was cleaned and chopped into small pieces. For dried Chinese chives, the fresh Chinese chives were dried at 45°C in tray dryer (model UM 500, Memmert) until the weight was constant. Then, the dried Chinese chives were ground into powder and kept in an air-tight container before

use. For frozen Chinese chives, the fresh Chinese chives were cleaned and chopped into small pieces. Then, the sample was frozen in the freezer at –20°C for 24 h.

2.2 *Solvent extractions*

Three forms of Chinese chives were extracted with three different solvents including 95% alcohol, hexane and soy bean oil. The samples were mixed with each solvent by using 1:5 ratio (g/mL) which showed the best yield from our previous study (data not published). The mixtures were macerated at 120 rpm, 25°C for 48 h in an incubator shaker. After 48 h, the mixtures were filtered by using Whatman filter paper No.4. Then, crude extracts were concentrated by using rotary evaporator (BUCHI Rota-vapor R-205) at 45°C until constant weight was obtained [2]. The concentrated crude extracts were diluted with dimethyl sulfoxide (DMSO) and kept at –20°C before use.

2.3 *Steam distillation extraction*

Fresh Chinese chives were washed with tap water and cut into 1 cm length before mixing with distilled water by using 1:3 ratio (by weight). After that, petroleum ether was added to the mixture at the ratio of 10:1 (mL/L) of water. The mixture was distilled at 100°C for 1.0, 2.0, 2.5 and 3.0 h. After distillation, the water fraction was discarded. Then, 10 g of sodium anhydrous was added in order to remove the excess water. The petroleum ether was removed using a rotary evaporator (BUCHI Rota-vapor R-205) at the temperature of 60°C and pressure drop of 838 mPa for 20 min or until petroleum ether was no longer condensed.

2.4 *Ohmic pretreatment*

For ohmic pretreatment, Chinese chives leaves were cut into 1 cm length and mixed with distilled water at the weight ratio of 1:2 before filling into a static ohmic cell. The sample was treated at 75 V/cm and the cut off temperature of 60°C. After that, the sample was subjected to steam distillation as mentioned in section 2.3.

The schematic diagram of a static ohmic treatment system was shown in Figure 1. The detail of ohmic treatment system setup was described in [4].

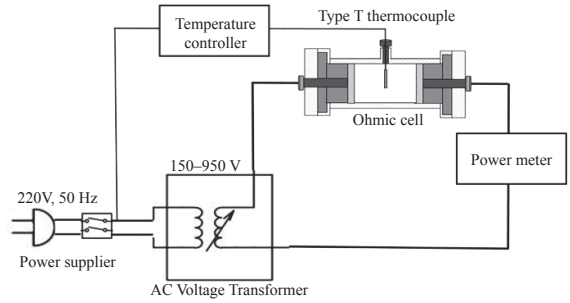

Figure 1: The schematic diagram of static ohmic treatment system.

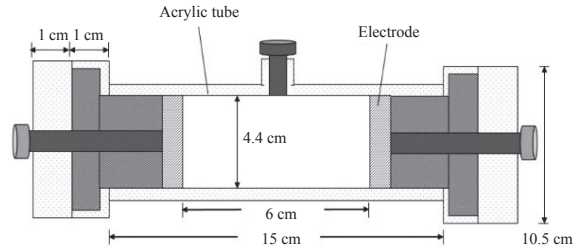

Figure 2: Static ohmic cell for solid food.

The ohmic cell was made from a 5 cm-outer diameter and 15 cm-long acrylic tube and two 316 stainless steel electrodes with the diameter of 4.4 cm. The distance between the electrodes was fixed at 6 cm (Figure 2). During ohmic pretreatment, the sample was placed between the electrodes inside the ohmic cell when electrical field was applied. When the temperature reaches the cut off temperature, a temperature controller (SFN 48 90-250 VAC, Sigma, Patumthani, Thailand) cuts off the electrical current applied to the sample.

2.5 *Antimicrobial activity*

The modified agar well diffusion method [2] was modified and used in this experiment. One hundred microliter of bacteria (approximately 1.5×10^8 CFU/mL) was swab on Mueller-Hinton agar (MHA) plate. Twenty microliter of 25, 50 and 75 mg/mL Chinese chives crude extracts were used to test antibacterial activity against human foodborne pathogen, i.e., *Escherichia coli* ATCC25822, *Salmonella enterica* Typhimurium U302, *S. enterica* Enteritidis, S. enterica 4,5,12;i- human (US clone), *Bacillus cereus* and *Listeria monocytogenes* 10403S using MHA plate. Inhibition zones were measured with a vernier caliper to determine the

effectiveness of the Chinese chives crude extract against each bacterium. The experiment was done in duplicate (or two plates per test condition) and three replications independently.

2.6 *Chemical profile analysis*

Chemical composition of Chinese chives crude extracts was analyzed by using Gas Chromatography-Mass Spectrometry (GC-MS) at King Mongkut's University of Technology Thonburi, Thailand. The analysis was performed using Agilent Technologies 7890A GC System, 5975C inert XL EI/el MSD with triple-axis detector and GC sampler 80 using HP 5 MS Ultra Inert type (30 m × 250 µm × 0.25 µm) column, using He as carrier gas, sample injection volume was 0.1 µl. The oven temperature was 60°C hold for 4 min, 250°C at 20°C/min hold for 1 min. The obtained peaks were matched with NIST database year 2011 by using Benzyl alcohol as a standard.

2.7 *Statistical analysis and experimental design*

All experiments were conducted in three replications and statistical analysis was performed using ANOVA with Duncan's multiple range tests ($p < 0.05$) by SAS software version 9.3.

3 Results and Discussion

3.1 *Effect of extraction method on antibacterial activity of Chinese chives crude extracts*

The *in vitro* antibacterial activity of Chinese chives crude extracts using thermal and solvent extraction against the tested pathogen (Gram-positive and Gram-negative bacteria) was assessed by modified agar well diffusion method [7] by measuring the inhibition zones. All extracts showed ability to inhibit bacteria against *E. coli* ATCC25822, *S. enterica Typhimurium* U302, *S. enterica* Enteritidis. *S. enterica* 4,5,12:i (human), *B. cereus*, and *L. monocytogenes* 10403S. The results in Table 1 indicated that all Chinese chives crude extracts can inhibit the growth of gram positive bacteria better than gram negative bacteria. It was shown that all Chinese chives crude extracts exhibited concentration-dependent against all pathogen inhibition; the higher the crude extract concentration, the better the antibacterial activity against all different pathogenic bacteria. The concentration of 75 mg/mL of Chinese chives crude extract gave the highest antibacterial activity among all extracts. The results in Table 1 also showed that Chinese chives crude extracts using steam distillation for 2.5 h gave the highest antibacterial against all bacteria (0.69±0.02 to 0.28±0.03 cm). Thus, this condition was chosen for analysis of chemical composition profile using GC-MS.

When compare the *in vitro* antibacterial activity of Chinese chives crude extracts using thermal with the one using solvent extractions, it was found that Chinese chives crude extracts using thermal extraction showed higher antibacterial activity than the ones using solvent extraction.

For solvent extraction, the *in vitro* antibacterial activity of the dried, fresh, and frozen Chinese chives crude extracts obtained from three different solvents (i.e. 95% alcohol, hexane and soy bean oil) was assessed. It was found that the form of Chinese chives (i.e. dried, fresh, and frozen) affected the antibacterial activity of crude extracts obtained from solvent extraction as shown in Table 1. The frozen Chinese chives crude extracts gave the highest antibacterial activity against all six different pathogenic bacteria followed by fresh and dried Chinese chives, respectively. The frozen Chinese chives crude hexane extract gave the highest antibacterial activity (0.50±0.03 to 0.29±0.03 cm) among all solvent extraction procedures against all tested pathogens. The different solvent extraction methods also showed significantly difference in antibacterial activity. Considering the antibacterial activity against six different pathogenic bacteria, the crude extract obtained from hexane extraction gave the highest activity among three different solvents used in this study.

3.2 *Chemical composition profile of Chinese chives and its antibacterial mechanism*

Chinese chives crude extracts using steam distillation for 2.5 h, which gave the highest antibacterial against all bacteria, was chosen to study for its chemical composition using GC-MS as the shown in Figure 3.

The GC-MS result was shown in Table 2. It was found that most of the chemical compounds found in Chinese chives crude extracts using thermal extraction were organosulfur compounds (39.07% Dimethyl

trisulfide and 27.20% Methyl 2-propenyl disulfide) and aldehyde. This finding could explain why the Chinese chives extract exhibited antibacterial activity; the main mechanism of bacterial inhibition was bonding between the organosulfur compounds and biomolecules, such as, protein, enzyme and so on. Its reduced forms were able to form superoxide radical anions and further bound with diverse metal ion and interacted readily with protein and cellular membranes. After binding, it generated the sulfide metal binding superoxide signal, strong electrophiles alkylation of DNA and oxidative stress, respectively. All of them caused the death of bacterial cells [8]. Moreover, there was also aldehyde in Chinese chives crude extracts obtained from thermal extraction but at lower percentage than the organosulfur compounds. These aldehyde groups may also contribute to the antibacterial activity of the extracts since aldehyde can bind with amino group on the protein cell wall causing denaturation of proteins on the cell wall. Thus, aldehyde could inhibit the transport of ions across the cell wall and enzyme system by prohibiting substrate enzyme [9].

Table 1: The antibacterial activity in terms of inhibition zone (cm) of Chinese chives (*A. tuberosum Rottler*. ex Spreng) crude extracts under different extraction methods against pathogenic bacteria

Extraction Methods	Concentration (mg/mL)	Inhibition Zone Diameter (cm)					
		***E. coli* ATCC25822**	***S. enterica* Typhimurium U302**	***S. enterica* Enteritidis**	***S. enterica* 4,5,12:i (human)**	***B. cereus***	***L. Monocytogenes* 10403S**
Steam Distillation for 1.0 h	25	$0.00 \pm 0.00^{A,l}$	$0.00 \pm 0.00^{A,n}$	$0.00 \pm 0.00^{A,p}$	$0.00 \pm 0.00^{A,q}$	$0.00 \pm 0.00^{A,r}$	$0.00 \pm 0.00^{A,n}$
	50	$0.00 \pm 0.00^{B,l}$	$0.00 \pm 0.00^{B,n}$	$0.00 \pm 0.00^{B,p}$	$0.07 \pm 0.05^{A,p}$	$0.09 \pm 0.03^{A,p,q}$	$0.07 \pm 0.05^{A,m}$
	75	$0.00 \pm 0.00^{C,l}$	$0.09 \pm 0.03^{B,j,k}$	$0.11 \pm 0.02^{B,l,m}$	$0.19 \pm 0.02^{A,k,l}$	$0.20 \pm 0.03^{A,m}$	$0.19 \pm 0.02^{A,h}$
Steam Distillation for 2.0 h	25	$0.00 \pm 0.00^{D,l}$	$0.06\pm 0.05^{C,k,l,m}$	$0.07 \pm 0.05^{C,n,o}$	$0.16 \pm 0.02^{B,m}$	$0.30 \pm 0.03^{A,i}$	$0.14\pm 0.02^{B,j,k,l}$
	50	$0.10 \pm 0.00^{E,i}$	$0.14 \pm 0.02^{Dg,h,i}$	$0.16 \pm 0.02^{D,I,j,k}$	$0.21 \pm 0.02^{C,i,j,k}$	$0.39 \pm 0.02^{A,g}$	$0.25 \pm 0.03^{B,g}$
	75	$0.14 \pm 0.02^{E,g,h}$	$0.18\pm 0.03^{D,d,e}$	$0.18\pm 0.03^{D,h,i}$	$0.26 \pm 0.02^{C,g}$	$0.45 \pm 0.03^{A,f}$	$0.31 \pm 0.02^{B,e}$
Steam Distillation for 2.5 h	25	$0.11 \pm 0.02^{E,h,i}$	$0.14 \pm 0.02^{D,g,h,i}$	$0.14 \pm 0.02^{D,j,k}$	$0.30 \pm 0.03^{C,f}$	$0.50 \pm 0.03^{A,e}$	$0.34 \pm 0.02^{B,d}$
	50	$0.18 \pm 0.03^{F,f}$	$0.23 \pm 0.03^{E,c}$	$0.32 \pm 0.03^{D,c,d}$	$0.38 \pm 0.03^{C,c}$	$0.64 \pm 0.02^{A,b}$	$0.50 \pm 0.03^{B,b}$
	75	$0.28 \pm 0.03^{C,c}$	$0.29 \pm 0.02^{C,a}$	$0.47 \pm 0.03^{B,a}$	$0.48 \pm 0.03^{B,a}$	$0.69 \pm 0.02^{A,a}$	$0.69 \pm 0.02^{A,a}$
Steam Distillation for 3.0 h	25	$0.00 \pm 0.00^{D,l}$	$0.00 \pm 0.00^{D,n}$	$0.08 \pm 0.04^{C,n,o}$	$0.18 \pm 0.03^{B,l}$	$0.24 \pm 0.02^{A,k,l}$	$0.21 \pm 0.02^{A,h}$
	50	$0.00 \pm 0.00^{E,l}$	$0.09 \pm 0.03^{D,j,k}$	$0.10 \pm 0.00^{D,m,n}$	$0.22 \pm 0.03^{C,h,I,j}$	$0.34 \pm 0.02^{A,h}$	$0.29 \pm 0.02^{B,e,f}$
	75	$0.13 \pm 0.03^{D,g,h}$	$0.13 \pm 0.03^{D,g,h,i}$	$0.18 \pm 0.03^{C,h,i}$	$0.29 \pm 0.02^{B,f}$	$0.39 \pm 0.02^{A,g}$	$0.40 \pm 0.02^{A,c}$
Ohmic Pretreatment before Steam Distillation for 1.0 h	25	$0.00 \pm 0.00^{B,l}$	$0.00 \pm 0.00^{B,n}$	$0.00 \pm 0.00^{B,p}$	$0.00 \pm 0.00^{B,q}$	$0.07 \pm 0.05^{A,q}$	$0.00 \pm 0.00^{B,n}$
	50	$0.00 \pm 0.00^{D,l}$	$0.00 \pm 0.00^{D,n}$	$0.08 \pm 0.04^{C,n,o}$	$0.14 \pm 0.02^{B,m,n}$	$0.21 \pm 0.02^{A,l,m}$	$0.19 \pm 0.02^{A,h,i}$
	75	$0.12 \pm 0.03^{D,g}$	$0.17 \pm 0.03^{C,e,f}$	$0.18 \pm 0.03^{C,h,i}$	$0.19 \pm 0.03^{C,k,l}$	$0.34 \pm 0.02^{A,h}$	$0.30 \pm 0.03^{B,e}$
Ohmic Pretreatment before Steam Distillation for 2.0 h	25	$0.00 \pm 0.00^{C,l}$	$0.00 \pm 0.00^{C,n}$	$0.00 \pm 0.00^{C,p}$	$0.06 \pm 0.05^{B,p}$	$0.19 \pm 0.02^{A,m}$	$0.08 \pm 0.05^{B,m}$
	50	$0.00 \pm 0.00^{E,l}$	$0.06 \pm 0.05^{D,k,l,m}$	$0.13 \pm 0.03^{C,k,l}$	$0.14 \pm 0.02^{C,m}$	$0.29 \pm 0.02^{A,i}$	$0.20 \pm 0.03^{B,h}$
	75	$0.11 \pm 0.02^{E,h,i}$	$0.14 \pm 0.02^{D,f,g,h}$	$0.18 \pm 0.03^{C,h,i}$	$0.24 \pm 0.02^{B,g,h,i}$	$0.39 \pm 0.02^{A,g}$	$0.25 \pm 0.03^{B,g}$
Ohmic Pretreatment before Steam Distillation for 2.5 h	25	$0.00 \pm 0.00^{E,l}$	$0.00 \pm 0.00^{E,n}$	$0.09 \pm 0.03^{Dm,n,o}$	$0.13 \pm 0.03^{C,m,n,o}$	$0.39 \pm 0.02^{A,g}$	$0.19 \pm 0.02^{B,h,i}$
	50	$0.14 \pm 0.02^{E,g}$	$0.18 \pm 0.03^{D,d,e}$	$0.21 \pm 0.02^{C,f,g}$	$0.21 \pm 0.02^{C,h,i,j}$	$0.54 \pm 0.02^{A,d}$	$0.35 \pm 0.03^{B,d}$
	75	$0.24 \pm 0.02^{E,d}$	$0.26 \pm 0.02^{E,b}$	$0.34 \pm 0.02^{D,b,c}$	$0.38 \pm 0.03^{C,c}$	$0.59 \pm 0.02^{A,c}$	$0.50 \pm 0.03^{B,b}$

Table 1: (continuous) The antibacterial activity in terms of inhibition zone (cm) of Chinese chives (*A. tuberosum Rottler*. ex Spreng) crude extracts under different extraction methods against pathogenic bacteria

Extraction Methods	Concentration (mg/mL)	Inhibition Zone Diameter (cm)					
		***E. coli* ATCC25822**	***S. enterica* Typhimurium U302**	***S. enterica* Enteritidis**	***S. enterica* 4,5,12:i (human)**	***B. cereus***	***L. Monocytogenes* 10403S**
Ohmic Pretreatment before Steam Distillation for 3.0 h	25	$0.00 \pm 0.00^{C,l}$	$0.00 \pm 0.00^{C,n}$	$0.00 \pm 0.00^{C,p}$	$0.00 \pm 0.00^{C,q}$	$0.24 \pm 0.02^{A,k}$	$0.06 \pm 0.05^{B,m}$
	50	$0.00 \pm 0.00^{D,l}$	$0.00 \pm 0.00^{D,n}$	$0.09 \pm 0.03^{C,m,n,o}$	$0.11 \pm 0.02^{C,o}$	$0.34 \pm 0.02^{A,h}$	$0.16 \pm 0.02^{B,i,j}$
	75	$0.07 \pm 0.05^{E,j}$	$0.13 \pm 0.03^{D,g,h,i}$	$0.17 \pm 0.03^{C,h,i,j}$	$0.19 \pm 0.02^{C,j,k,l}$	$0.40 \pm 0.03^{A,g}$	$0.29 \pm 0.02^{B,e,f}$
Dried *Allium tuberosum* Rottl. ex Spreng Extracted with 95% Ethanol	25	$0.00 \pm 0.00^{A,l}$	$0.00 \pm 0.00^{A,n}$	$0.00 \pm 0.00^{A,p}$	$0.00 \pm 0.00^{A,q}$	$0.00 \pm 0.00^{A,r}$	$0.00 \pm 0.00^{A,n}$
	50	$0.00 \pm 0.00^{B,l}$	$0.00 \pm 0.00^{B,n}$	$0.00 \pm 0.00^{B,p}$	$0.00 \pm 0.00^{B,q}$	$0.09 \pm 0.04^{A,p,q}$	$0.00 \pm 0.00^{B,n}$
	75	$0.07 \pm 0.05^{D,j}$	$0.09 \pm 0.03^{D,j,k}$	$0.14 \pm 0.02^{C,j,k}$	$0.19 \pm 0.02^{B,k,l}$	$0.24 \pm 0.02^{A,k,l}$	$0.20 \pm 0.03^{B,h}$
Fresh *Allium tuberosum* Rottl. ex Spreng Extracted with 95% Ethanol	25	$0.00 \pm 0.00^{C,l}$	$0.00 \pm 0.00^{C,n}$	$0.00 \pm 0.00^{C,p}$	$0.14 \pm 0.02^{A,m}$	$0.00 \pm 0.00^{C,r}$	$0.25 \pm 0.05^{B,g}$
	50	$0.13 \pm 0.03^{B,g,h}$	$0.06 \pm 0.05^{C,k,l,m}$	$0.09 \pm 0.03^{C,m,n,o}$	$0.21 \pm 0.02^{A,i,j,k}$	$0.14 \pm 0.02^{B,o}$	$0.15 \pm 0.03^{B,j}$
	75	$0.23 \pm 0.03^{C,d}$	$0.12 \pm 0.03^{E,h,i}$	$0.19 \pm 0.02^{D,g,h}$	$0.30 \pm 0.03^{A,f}$	$0.24 \pm 0.02^{C,k,l}$	$0.26 \pm 0.02^{B,f,g}$
Frozen *Allium tuberosum* Rottl. ex Spreng Extracted with 95% Ethanol	25	$0.07 \pm 0.05^{C,j}$	$0.00 \pm 0.00^{D,n}$	$0.08 \pm 0.05^{C,n,o}$	$0.24 \pm 0.02^{A,g,h}$	$0.11 \pm 0.02^{B,p}$	$0.12 \pm 0.03^{B,k,l}$
	50	$0.18 \pm 0.03^{C,f}$	$0.17 \pm 0.03^{C,e,f}$	$0.19 \pm 0.02^{C,g,h}$	$0.34 \pm 0.02^{A,d}$	$0.22 \pm 0.03^{B,k,l,m}$	$0.24 \pm 0.02^{B,g}$
	75	$0.27 \pm 0.03^{D,c}$	$0.20 \pm 0.02^{E,d,e}$	$0.29 \pm 0.02^{C,D,e}$	$0.43 \pm 0.03^{A,b}$	$0.30 \pm 0.03^{C,i}$	$0.34 \pm 0.02^{B,d}$
Dried *Allium tuberosum* Rottl. ex Spreng Extracted with Hexane	25	$0.00 \pm 0.00^{A,l}$	$0.00 \pm 0.00^{A,n}$	$0.00 \pm 0.00^{A,p}$	$0.00 \pm 0.00^{A,q}$	$0.00 \pm 0.00^{A,r}$	$0.00 \pm 0.00^{A,n}$
	50	$0.00 \pm 0.00^{D,l}$	$0.00 \pm 0.00^{D,n}$	$0.11 \pm 0.02^{C,l,m}$	$0.13 \pm 0.03^{B,m,n,o}$	$0.21 \pm 0.02^{A,m}$	$0.14 \pm 0.02^{B,j,k}$
	75	$0.18 \pm 0.03^{D,f}$	$0.13 \pm 0.03^{E,g,h,i}$	$0.19 \pm 0.02^{C,D,g,h}$	$0.21 \pm 0.02^{C,i,j,k}$	$0.34 \pm 0.02^{A,h}$	$0.30 \pm 0.03^{B,e}$
Fresh *Allium tuberosum* Rottl. ex Spreng Extracted with Hexane	25	$0.13 \pm 0.03^{B,g,h}$	$0.00 \pm 0.00^{D,n}$	$0.08 \pm 0.05^{C,n,o}$	$0.08 \pm 0.05^{C,p}$	$0.20 \pm 0.00^{A,m}$	$0.14 \pm 0.02^{B,j,k,l}$
	50	$0.24 \pm 0.02^{B,d}$	$0.13 \pm 0.03^{D,g,h,i}$	$0.19 \pm 0.02^{C,g,h}$	$0.24 \pm 0.02^{B,g,h}$	$0.27 \pm 0.03^{A,j}$	$0.25 \pm 0.03^{A,B,g}$
	75	$0.32 \pm 0.03^{C,b}$	$0.19 \pm 0.02^{E,d,e}$	$0.24 \pm 0.02^{D,f}$	$0.31 \pm 0.02^{C,e,f}$	$0.44 \pm 0.02^{A,f}$	$0.40 \pm 0.03^{B,c}$
Frozen *Allium tuberosum* Rottl. ex Spreng Extracted with Hexane	25	$0.18 \pm 0.03^{B,f}$	$0.11 \pm 0.02^{C,i,j}$	$0.18 \pm 0.03^{B,h,i}$	$0.19 \pm 0.02^{B,j,k,l}$	$0.31 \pm 0.02^{A,i}$	$0.20 \pm 0.03^{B,h}$
	50	$0.32 \pm 0.02^{C,b}$	$0.20 \pm 0.00^{D,d}$	$0.31 \pm 0.02^{C,d,e}$	$0.33 \pm 0.03^{B,C,d,e}$	$0.41 \pm 0.02^{A,g}$	$0.35 \pm 0.03^{B,d}$
	75	$0.43 \pm 0.02^{B,a}$	$0.28 \pm 0.03^{D,a}$	$0.37 \pm 0.03^{C,b}$	$0.49 \pm 0.03^{A,a}$	$0.50 \pm 0.03^{A,e}$	$0.50 \pm 0.02^{A,b}$
Dried *Allium tuberosum* Rottl. ex Spreng Extracted with Soy Bean Oil	25	$0.00 \pm 0.00^{A,l}$	$0.00 \pm 0.00^{A,n}$	$0.00 \pm 0.00^{A,p}$	$0.00 \pm 0.00^{A,q}$	$0.00 \pm 0.00^{A,r}$	$0.00 \pm 0.00^{A,n}$
	50	$0.00 \pm 0.00^{A,l}$	$0.00 \pm 0.00^{A,n}$	$0.00 \pm 0.00^{A,p}$	$0.00 \pm 0.00^{A,q}$	$0.00 \pm 0.00^{A,r}$	$0.00 \pm 0.00^{A,n}$
	75	$0.04 \pm 0.05^{A,k}$	$0.04 \pm 0.05^{A,m}$	$0.06 \pm 0.05^{A,o}$	$0.08 \pm 0.05^{A,p}$	$0.09 \pm 0.04^{A,p,q}$	$0.08 \pm 0.05^{A,m}$
Fresh *Allium tuberosum* Rottl. ex Spreng Extracted with Soy Bean Oil	25	$0.00 \pm 0.00^{B,l}$	$0.00 \pm 0.00^{B,n}$	$0.00 \pm 0.00^{B,p}$	$0.11 \pm 0.02^{A,n,o}$	$0.00 \pm 0.00^{B,r}$	$0.00 \pm 0.00^{B,n}$
	50	$0.06 \pm 0.05^{B,j}$	$0.08 \pm 0.04^{B,k,l}$	$0.06 \pm 0.05^{B,o}$	$0.14 \pm 0.02^{A,m}$	$0.08 \pm 0.05^{B,q}$	$0.00 \pm 0.00^{C,n}$
	75	$0.15 \pm 0.03^{B,C,g}$	$0.15 \pm 0.03^{B,C,f,g}$	$0.13 \pm 0.03^{C,D,k,l}$	$0.24 \pm 0.02^{A,g,h}$	$0.17 \pm 0.03^{B,n}$	$0.11 \pm 0.02^{D,l}$
Frozen *Allium tuberosum* Rottl. ex Spreng Extracted with Soy Bean Oil	25	$0.00 \pm 0.00^{C,l}$	$0.05 \pm 0.05^{B,l,m}$	$0.00 \pm 0.00^{C,p}$	$0.20 \pm 0.03^{A,j,k,l}$	$0.00 \pm 0.00^{C,r}$	$0.00 \pm 0.00^{C,n}$
	50	$0.11 \pm 0.02^{B,h,i}$	$0.12 \pm 0.03^{B,h,i}$	$0.09 \pm 0.03^{C,B,m,n,o}$	$0.29 \pm 0.02^{A,f}$	$0.11 \pm 0.02^{B,p}$	$0.08 \pm 0.05^{C,m}$
	75	$0.21 \pm 0.02^{B,e}$	$0.20 \pm 0.03^{B,d}$	$0.14 \pm 0.02^{C,j,k}$	$0.39 \pm 0.02^{A,c}$	$0.20 \pm 0.03^{B,m}$	$0.14 \pm 0.02^{C,j,k}$

Note: Capital letter superscript showed significant different at $p < 0.05$ within a row
Small letter superscript showed significant different at $p < 0.05$ within a column

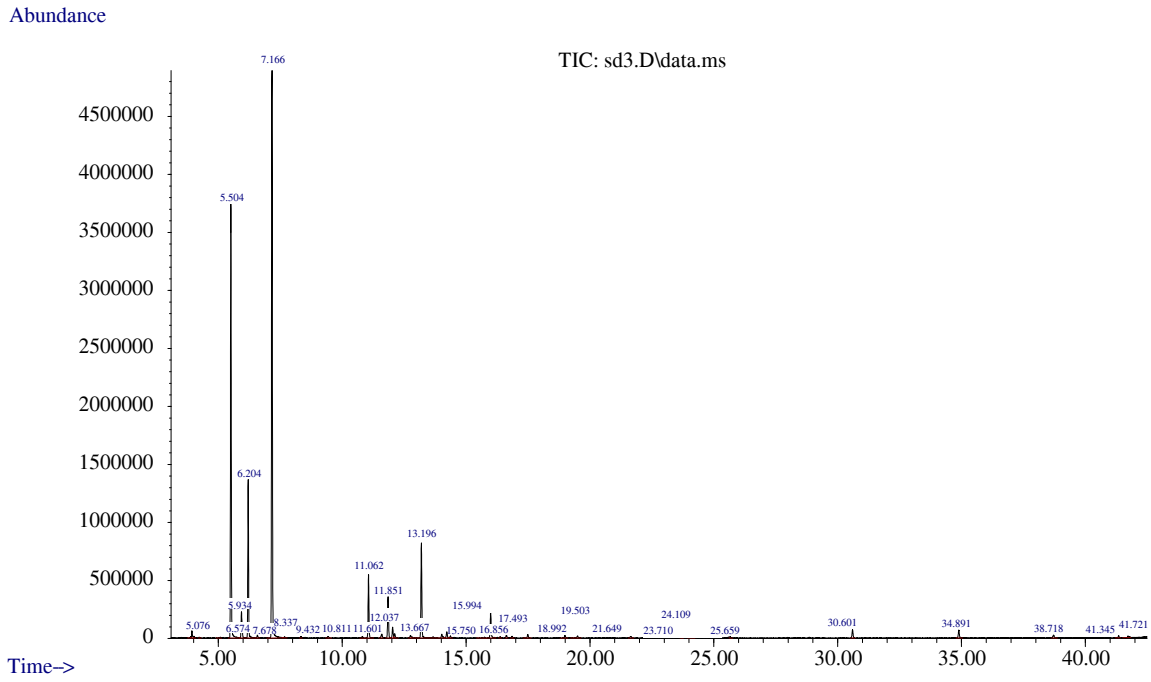

Figure 3: Peak of chemical composition profile of Chinese chives (*A. tuberosum* Rottler. Ex) extract using stream distillation for 2.5 h analyzed by GC-MS.

Table 2: The chemical compound profile of Chinese chives (*A. tuberosum* Rottler. Ex) extract using stream distillation for 2.5 h analyzed by GC-MS

RT (min)	Compound Name	Peak Area	%Area
3.933	(Z)-3-Hexen-1-ol	1260242	0.42
5.505	Methyl 2-propenyl disulfide	81363375	27.20
5.935	1,3-Dithiane	36824660	12.31
6.204			
7.165	Dimethyl trisulfide	116886353	39.08
11.062	Diallyl disulphide	13834446	4.62
11.601	2-Vinyl-1,3-dithiane	1081858	0.36
11.851	1-Oxa-4,6-diazacyclooctane-5-thione	9241203	3.09
12.036	Nonanal	2508019	0.84
12.758	Methyl (methylthio)methyl Disulfide	666512	0.22
13.197	(Methylthio)-acetonitrile	21280145	7.11
14.022	(Z)-1-(methylthio)-1-Propene	1096536	0.37
14.225	Allyl methyl Sulfide	1609967	0.54
15.993	Dimethyl Tetrasulfide	6404305	2.14
17.493	N,N-dimethyl-Methanethioamide	896989	0.30
18.993	Di-2-propenyl Trisulfide	746585	0.25
21.65	tert-Butly methyl sulfoxide	682512	0.23
24.108	S-Methyl methanethiosulfinate	562156	0.19
34.892	2,2,7,7-tetramethyl- 3-Oxa-6-thia-2,7-disilaoctane	2174708	0.73

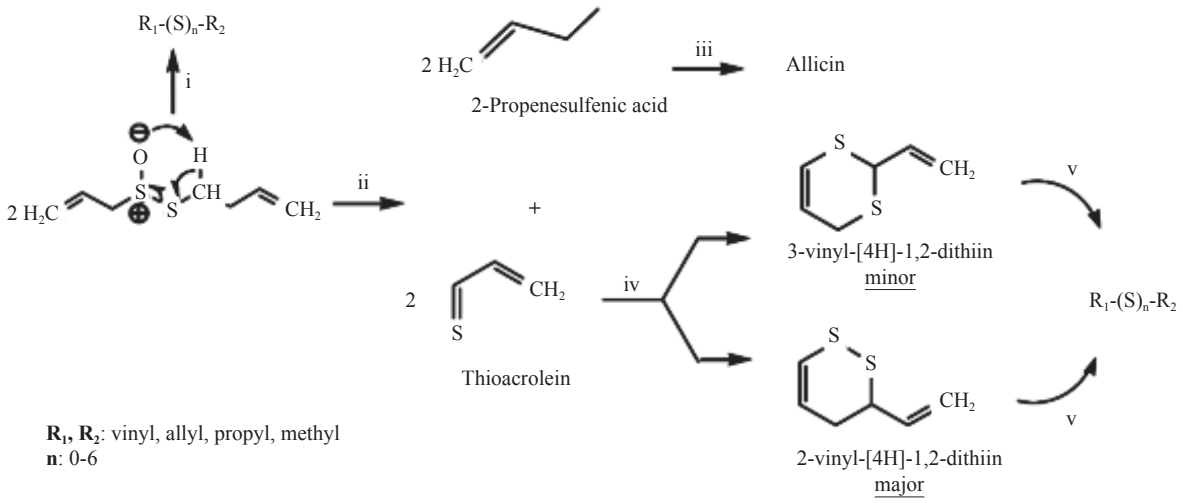

Figure 4: Allicin's decomposition as postulated [18]: (i) allicin's degradation to sulfur acyclic components found in garlic distilled oil, (ii) allicin's self-decompose to two molecules of 2-propenesulfenic acid and two molecules of thioacrolein, (iii) 2-propenesulfenic acids self-condensation to allicin, (iv) thioacrolein's self-condensation to two isomer cyclic dithiins and (v) cyclic dithiins thermal decomposition to variousacyclic molecules similar to those found in garlic distilled oil.

The chemical composition of Chinese chives extract obtained from steam distillation for 2.5 h was analysed by GC-MS and the result was shown in Table 2. It was found that organosulfide volatile compounds (i.e., Dimethyl trisulfide and Methyl 2-propenyl disulphide) were the major chemical compounds. These organosulfide volatile compounds might be the one responsible for antibacterial activity. From the previously proposed degradation mechanism [10], allicin (dially thiosulfinate) was highly unstable and could decompose to dithine compounds (cyclic molecules) which could further decompose by heat to organosulfide (acyclic molecules), such as mono- to tetrasulfide compounds as showed in Figure 4. Our results could also be explained by this phenomenon as showed in Figure 4 and, thus, high concentration of organosulfide volatile compounds (Dimethyl trisulfide and Methyl 2-propenyl disulfide) was observed in the extract obtained from 2.5 h stream distillation treatment.

The GC-MS results of Chinese chives crude extracts using solvent extracts (data not published) showed high percentage of phenol. The phenol can act as both antimicrobial and antioxidant agents. Phenol has long been used for its antiseptic, disinfectant, or preservative properties. It has been known for many years [10] that, although phenol has often been referred to as "general protoplasmic poison", it has membrane-active properties which also contribute to its overall activity [11]. Phenol induces progressive leakage of intracellular constituents, including the release of K+, the first index of membrane damage [12], and of radioactivity from 14C-labeled *E. coli* [13], [14]. Pulvertaft and Lumb [15] demonstrated that low concentrations of phenols (0.032%, 320 mg/mL) and other (nonphenolic) agents lysed rapidly growing cultures of *E. coli*, *Staphylococci*, *Streptococci* and concluded that autolytic enzymes were not involved. Phenol antimicrobial mechanism involves physical interactions by penetration or partition into phospholipid bilayer; possible displacement of phospholipid molecules; intramembrane molecular cycling resulting in leakage, disruption of transport, respiratory and energy coupling processes [16].

The result of this study indicated that the major player in antibacterial activity of Chinese chives crude extracts was organosulfur compounds because organosulfides was detected in the extracts at the higher concentration than aldehyde and phenol. In addition, a greater number of sulfide atoms also resulted in a higher antimicrobial activity [17].

4 Conclusion

Chinese chives crude extracts exhibited very promising antibacterial activity. The extraction method significantly affected the bioavailability of the extracts. This study indicated that different extraction methods gave different profiles of bioactive compound.

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