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Research article

Phytochemicals, Antioxidant, and Antibacterial Activities of Fresh and Dried Chinese Chive (*Allium tuberosum* Rottler) Leaf Extracts

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Abstract: Chinese chive (*Allium tuberosum* Rottler) is a nutrient-rich vegetable widely cultivated in Southeast Asia, including Thailand. The objectives of this study were to assess the phytochemical compounds present in fresh and dried Chinese chives obtained through aqueous extraction and to investigate their biological activities, particularly their antioxidant and antibacterial properties. In this study, fresh and dried Chinese chive leaves were extracted using water to obtain fresh Chinese chive extract (FCCE) and dried chive extract (DCCE). The extracts were characterized for their phytochemical compounds and evaluated for their bioactive properties. Based on GC-MS analyses, 5 major bioactive compounds found in both FCCE and DCCE were 2-methoxy-4-vinyl phenol, dimethyl sulfone, n-hexadecanoic acid, 2-hydroxy-gamma-butyrolactone, and furaneol. The FCCE and DCCE extracts exhibited antioxidant properties with the IC50 values of 7.25 ± 0.14/8.62 ± 0.02 mg/ml and 4.91 ± 0.29/6.66 ± 0.03 mg/ml for FCCE and DCCE determined by DPPH/ABTS assays, respectively. Antibacterial activities of FCCE and DCCE against food pathogenic bacteria demonstrated that both extracts could inhibit *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella* sp., *Listeria monocytogenes*, *Escherichia coli*, *Vibrio cholerae*, and *V. parahaemolyticus* with the same MIC value (8 mg/ml). Therefore, this study provided a basic knowledge of Chinese chive as a potentially promising source of natural bioactive ingredients for various applications in food technology.

Keywords: Antibacterial activity; antioxidants; Chinese chives; food pathogens

1. Introduction

Chinese chives, also known as garlic chives, belong to the Liliaceae family and the *Allium* genus, which includes garlic, leek, and onion. Chinese chives are popular in Southeast Asia, South Asia, and some Middle Eastern countries [1]. In Thailand, the province with the largest cultivation area is Ratchaburi. There are also reports that Klong U-Tapao Sub-district, Hat Yai District, Songkhla Province, popularly cultivated large quantities of Chinese chives with a total plantation area of about 96,000 square meters, and most are exported to Malaysia [2]. Thai people usually consume fresh Chinese chives with Thai stir-fried rice noodles (Pad Thai) or cooked as dumplings (Gui Chai). In other countries, some of the most popular dishes from the Chinese chives are Korean chive salad (Kimchi) and Japanese pan-fried dumplings (Gyoza).

and dietary fiber content [3]. Additionally, Chinese chives have health advantages and have been utilized as herbal medicine to treat asthma and conditions including diarrhea, hematemesis, and snakebite [4]. The crushed leaves contained volatile sulfur-containing substances, which could reduce the risk of cardiovascular and inflammatory disorders [5].

Chinese chives are rich in various phytochemicals that offer potential health benefits. Notably, Sulphur compounds, including diallyl disulfide and diallyl trisulfide, are responsible for the distinct aroma and taste of Chinese chives and have been studied for their possible anticancer and anti-inflammatory properties [6]. The presence of phenolic compounds like ferulic acid and caffeic acid provides antioxidant and anti-inflammatory benefits, helping to combat oxidative stress and promote overall health [7]. Additionally, Chinese chives contain flavonoids, a group of polyphenolic compounds known for their strong antioxidant properties. These can counteract harmful free radicals and potentially reduce the risk of chronic diseases, supporting overall well-being [8].

The majority of people around the world are struggling with the problems of foodborne illnesses caused by foodborne pathogens. The food market has identified the pathogenic bacteria, mainly *Bacillus cereus, Listeria* spp., *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp., and *Vibrio* spp. [9]. To reduce cases, antimicrobial substances from edible sources have been studied. Various assays have previously screened the antimicrobial activities of Chinese chives extracted by different methods. For example, antibacterial activities of 25, 50, and 75 mg/ml of Chinese chive crude extracts have been reported by the modified agar well diffusion method and the highest antibacterial activity was obtained from 75 mg/ml of the steam distillation extract against *B. cereus* and *L. monocytogenes* [10]. In addition, the antibacterial activity of a water extract of Chinese chive against 21 *Helicobacter pylori* was shown by the disk diffusion method and the minimum inhibitory concentration (MIC) value obtained by the agar dilution method was 2.45 mg DW/ml [11]. Hence, there is a great interest in examining the possibility of using Chinese chive as natural ingredients in food or food products. Therefore, this study aimed to evaluate the phytochemical compounds of fresh and dried Chinese chives obtained by the aqueous extraction method and determine their biological activity, including antioxidant and antibacterial properties.

2. Materials and Methods

2.1 Bacterial Strains

Food pathogenic bacteria used in this study were *B. cereus* PSU3874, *L. monocytogenes* PSU6205, *S. aureus* ATCC25923, *E. coli* ATCC25922, *Salmonella* sp. PSU411, *V. cholerae* PSU6072, and *V. parahaemolyticus* ATCC17802. All isolates were obtained from the microbial culture collection at the Division of Biological Science, Prince of Songkla University, Thailand, and were stored in 20% glycerol at -80 °C.

2.2 Preparation of fresh and dried Chinese chives

Chinese chives were purchased from a local grower at Khlong-u Tapao in Songkhla province of southern Thailand. Fresh Chinese chives were chopped into small pieces and rinsed under running water. Fresh leaves were dried in a hot air oven at 40 °C for 2 days or until dried for dried Chinese chives. All samples were blended into a fine powder [12].

2.3 Extraction of Chinese chives extract

Chinese chive powders obtained from fresh and dried leaves were extracted with hot water following the method previously described by Hernandez *et al*., 2017 with some modifications [12]. In a heating oven, 100 g of fresh and dried Chinese chives powders were soaked in 500 ml hot distilled water at 60 °C for 30 minutes. After filtration through double layers of muslin, the solutions were concentrated by freeze dryer (Martin Christ, Osterode am Harz, Germany) by freezing at -80 °C for 24 hours before taking to freeze drier at -45 °C for 3 days. The freeze-dried extracts were kept in dark storage at 4 °C until further use.

2.4 GC-MS analysis

Gas chromatography-electron ionization/mass spectrometry (GC-MS) analysis of phytochemical compounds present in fresh (FCCE) and dried (DCCE) Chinese chive leaves was carried out using Perkin

and DCCE powders were dissolved in dimethyl sulfoxide (DMSO) and centrifuged at 10,000 rpm for 10 min at 10 °C before the supernatant was subjected to GC-MS analysis. The Elite-5 capillary column ((5% biphenyl) 95% dimethylpolysiloxane), length 30 m 0.25 mm ID, and film thickness 250 m df were utilized for the GC-MS analysis. A 1 ml/min of helium was used as the carrier gas. The temperature at the injector and interface was 260 °C. The column temperature was programmed from 60 to 300 °C at an increasing rate of 10 min, where it was held for 6 min. The spectrums of the compounds were compared with standard spectra available in the Perkin Elmer GC-MS NIST library [13].

2.5 Antioxidant Activity Assays

The antioxidant activity assays were determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assays of CCE in 96-well microtiter plates using the methods described below.

2.5.1. DPPH Assay

The antioxidant activity of the CCE was evaluated by DPPH radical scavenging. In brief, 180 µl of 0.1 mM DPPH solution in methanol were combined with 20 µl of FCCE, DCCE, or standard solution. A spectrophotometer was used to measure the absorbance at 517 nm after 30 minutes [14].

2.5.2 ABTS Assay

The ABTS stock solution was prepared by mixing 7.75 mM of potassium persulfate with 7.25 mM of ABTS and incubated at room temperature for 16 hours in the dark. The ABTS stock solution was diluted to a concentration of 107.14 µM ABTS solution until use. Then, 280 µl of 107.14 µM ABTS solution was combined with 20 µl of FCCE, DCCE, or standard solution. The plate was mixed and incubated in the dark at room temperature for 6 minutes, and then the absorbance at 734 nm was measured using a spectrophotometer (LUMIstar, BMGLABTECH, Ortenberg, Germany) [15].

2.6 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays

The MICs of FCCE and DCCE against all pathogens were determined by following the Clinical and Laboratory Standard Institute (CLSI) guidelines [16]. The tested isolates were cultured in Mueller–Hinton broth (MHB) supplemented with (for *Vibrio* spp.) or without 1% NaCl for 4 hours. Then, cells were adjusted with fresh media to 10^6 CFU/ml, and 100 µl of cell suspensions were added to 100 µl of FCCE or DCCE solution at final concentrations ranging from 1 to 16 mg/ml. After incubation for 18–20 hours at 30 °C (for *Vibrio* spp.) or for 16 hours at 37 °C (for other species), the MIC values were determined by adding 20 µl of a 0.1% resazurin solution to each well and incubating for 2 h at 30 or 37 °C. For MBC values, a liquid portion from each well that showed no growth was taken and cultivated on Tryptic Soy agar (TSA) with or without 1% NaCl and incubated at 30 °C or 37 °C for 18–24 h.

2.7 Statistical analysis

A completely randomized experimental design was used with three replicates each, and the means and standard deviations (S.D.) were used to present the results. A one-way analysis of variance (ANOVA) was performed to compare more than two means. Duncan's new multiple-range tests were employed to determine the significant differences between the means. Correlations between the DPPH and ABTS assays of antioxidant activity were calculated at a significance level of $p < 0.05$.

3. Results and Discussion

This study investigated the phytochemical components from aqueous extracts of fresh and dried Chinese chives by GC-MS analyses and examined their biological activities, including antioxidants and antibacterial properties.

3.1 The visual appearance and extraction yield of Chinese chive extracts

The visual appearance of Chinese chive extracts from fresh and dried leaves were dry greenish and brown powder, respectively (Figure 1). The extraction yield obtained by water extraction was 290.40 mg/g of dried matter and 37.70 mg/g of fresh weight, respectively (Table 1). As a result, the maximum yield was obtained

water content in dry leaves, which leads to a higher concentration of the desired compounds. Consequently, the extraction process becomes more efficient, resulting in an improved yield. Moreover, the use of dry leaves offers additional advantages. Dry leaves are less susceptible to enzymatic degradation and microbial growth, ensuring the preservation of the plant substances' integrity during extraction. Additionally, their lighter weight and manageable nature make them more convenient to handle during extraction [17, 18].

Figure 1. The visual appearance of Chinese chive extracts. (A) Extract obtained from fresh sample (FCCE) and (B) Extract obtained from dried sample (DCCE)

Table 1. The extraction yield of Chinese chive extracts obtained by water extraction.

Sample	Yield (mg/g)	Characteristics
Fresh leaves	37.70	Dark green powder
Dried leaves	290.4	Brown powder

3.2 GC-MS Analysis

The GC-MS analysis identified 108 and 156 compounds in FCCE and DCCE, respectively. Ten major compounds identified in FCCE (a total of 18.70 mg/g or 49.60% of the extraction yield) were 2-methoxy-4-vinyl phenol, dimethyl sulfone, n-hexadecanoic acid, butanoic acid, 1,2,3-propanetriyl ester2-propanone, 1,3-dihydroxy-, 2-hydroxy-gamma-butyrolactone, furaneol, 1-heptadecanecarboxylic acid, 2-furanmethanol, and pentanoic acid (Table 2). Among these, 8 of 10 were also identified as major compounds in DCCE. The 5 additional compounds among top 10 hits found in DCCE were 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, glycerin, benzofuran, 2,3-dihydro-, 4H-pyran-4-one, 3,5-dihydroxy-2-methyl-, and 2,4(1H,3H)-pyrimidinedione, 5-methyl- (Table 2).

In this study, both FCCE and DCCE exhibited a high content of 2-methoxy-4-vinylphenol, a phenolic compound with antibacterial, antioxidant, anti-inflammatory, analgesic, and anti-germination properties [19-21]. From previous research, 2-methoxy-4-vinylphenol, a major compound in red cabbage, has strong antibacterial activity and possesses higher degrees of interaction with bacteria DNA gyrase, leading to high antimicrobial efficacy [19]. Dimethyl sulfone is an organosulfur compound that is a metabolite of DMSO and certain sulfur-containing amino acids. It is present in some natural green plants, fruits, and vegetables, such as tomatoes, corn, and apples [22]. Several reports provide *in vitro* evidence of the antioxidant, anti-inflammatory, antibacterial, antifungal, and antiviral activity of dimethyl sulfone [22-25]. Moreover, it was also found to inhibit *E. coli* and *S. enterica* serovar Kinshasa [26]. Previous studies showed that one of the organosulfur compounds, dimethyl trisulfide, in Chinese chives could involve its antibacterial activity [10]. The fatty acid n-hexadecanoic acid isolated from the *Canthium parviflorum* leaves exhibited antimicrobial activity against *S. aureus*, *E. coli*, *B. subtilis*, and *Candida albicans* [27]. Antibacterial activity of furaneol has been reported against human pathogens, including *S. aurues*, *S. epidermidis*, *E. coli*, and *Proteus vulgaris* [28]. Thus, these major compounds in FCCE and DCCE might significantly contribute to their antimicrobial activities.

In DFFC, several compounds can be found as major constituents. However, they are not seen as major constituents in FCCE. For example, 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- is the second top component found in the DCCE, which was not found in the extract of fresh Chinese chives (Table 2). The formation of this compound in heat-treated food or dried fruit has been reported due to the Maillard reaction, which lead to changes in the color of the extract from dark green to brown [29, 30].

The DPPH and ABTS assays were used to evaluate the antioxidant activity of Chinese chive extracts. At the highest concentration (16 mg/ml), extracts had a more significant scavenging effect against ABTS than DPPH radicals. FCCE and DCCE possessed free radical scavenging properties but varying degrees, with an IC50 ranging from 7.25 to 8.62 and 4.91 to 6.66 mg/ml for DPPH and ABTS methods, respectively (Table 3). The antioxidant activity of Chinese chive extract may be due to several phytochemical compounds. For example, sulfur compounds have been reported to contribute to various vegetables' antioxidant properties in the *Allium* species [31]. Lachman *et al*., 2003 also reported that polyphenolic compounds in different onions (*Allium cepa* L.) are effective antioxidants due to their potential to scavenge free radicals of fatty acids and oxygen [32]. Additionally, antioxidant activity was found in extracts from all organs of *Allium schoenoprasum* L., which leaves showing the highest antioxidant activity [33].

In a previous study, the IC50 of Chinese chives essential oil extract by turbo hydrodistillation showed an IC50 of 12.16 mg/ml, which showed a slightly higher IC50 value (indicating less potency) in the DPPH assay compared to the current study's extracts. This discrepancy could be due to differences in extraction methods or the presence of varying antioxidant compounds in the different extracts [31]. According to Parvu *et al*., 2010 p-coumaric acid, ferulic acid, isoquercitrin, and rutin are the main polyphenolic compounds found in chives [34]. In addition, the IC50 values for the extract of Chinese chives in water and ethanol were 822.92 g/ml, 735.96 g/ml, 757.0 g/ml, and 650.6 g/ml, respectively, according to the DPPH and ABTS assays. As a result, Chinese chive extract has strong antioxidant activity against the DPPH and ABTS radicals [35]. In this study, it was found that DCCE had a slightly lower antioxidant activity than FCCE. This suggested that treating the Chinese chive leaves at 40 ^{o}C may result in the loss of some antioxidant components. Therefore, the study demonstrated that Chinese chive extracts possess significant antioxidant activity against both DPPH and ABTS radicals, likely due to the presence of various phytochemical compounds with antioxidant properties. However, the specific antioxidant components and their concentration might vary depending on extraction methods and processing conditions.

3.4 Antibacterial activities

The antibacterial activity of Chinese chives extracts against 7 bacterial strains causing foodborne illnesses, including *B. cereus* PSU3874, *L. monocytogenes* PSU6205, *S. aureus* ATCC25923, *E. coli* ATCC25922, *Salmonella* sp. PSU411, *V. cholerae* PSU6072, and *V. parahaemolyticus* ATCC17802 were evaluated using MIC and MBC assays. The result demonstrates that both FCCE and DCCE could inhibit all tested bacteria at the same MIC values (8 mg/ml) (Table 4). All bacterial strains in this investigation had MBC values greater than 16 mg/ml. This was consistent with previous studies showing that Chinese chives have inhibitory effects on Gram-positive and Gram-negative bacteria [11, 19]. In this study, one of the major components present in FCCE and DCCE is 2-methoxy-4-vinylphenol. This compound has been found to interact with DNA gyrase required for DNA synthesis and lipoprotein of the bacterial cell wall [19]. Thus, the inhibition of these targets could contribute to the antibacterial efficacy of the extracts. Both FCCE and DCCE extracts contain very similar active compounds responsible for antibacterial activity. Consequently, if the active ingredients are consistently present in both extracts at similar concentrations, they would exert comparable inhibitory effects on the bacterial strains. The bacterial strains tested might share a similar sensitivity profile to the compounds in both FCCE and DCCE, resulting in similar MIC values. This suggests that Chinese chives may contain a relatively narrow range of compounds with antibacterial properties. The MIC values could be consistently the same if the extracts mainly consist of a few key active compounds effective against the tested bacterial strains [36, 37].

In a previous study, Chinese chives extracted from water could inhibit *E. coli*, *L. monocytogenes,* and *V. parahaemolyticus* with an inhibitory zone of 15, 10, and 14 mm, respectively. However, it could not inhibit *S. aureus* by a disc diffusion assay [38]. In addition, Chinese chives crude extract obtained by steam distillation for 2.5 hours at a concentration of 75 mg/ml could inhibit both Gram-positive and Gram-negative bacteria with the inhibitory zone of 0.688 ± 0.023 cm against *B. cereus* and *L. monocytogenes* [10]. Moreover, the antibacterial study of Chinese chives extract based on the serial double dilution method has indicated that both water and ethanolic extracts exhibited good efficacy against *E. coli* at a MIC value of 64 μg/ml [35]. Consequently, this study demonstrated the potential of Chinese chive extracts as an antibacterial agent against important food pathogenic bacteria.

Table 2. Major compounds identified in Chinese chives extracts.

			FCCE			DCCE		
No.	**Compound Name**	**Formula**	**Amount (mg/g of fresh matter)**	**Retention time**	**Match Factor**	**Amount (mg/g of dried matter)**	**Retention time**	**Match Factor**
1	2-Methoxy-4-vinyl phenol	$C_9H_{10}O_2$	2.88	34.81	95.82	32.20	34.82	96.20
2	Dimethyl sulfone	$C_2H_6O_2S$	2.50	28.55	98.84	7.83	28.38	98.84
3	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	2.40	49.27	97.34	9.57	49.27	96.60
4	Butanoic acid, 1,2,3-propanetriyl ester	$C_{15}H_{26}O_6$	2.32	40.42	92.50			
5	2-Propanone, 1,3-dihydroxy-	$C_3H_6O_3$	1.84	32.32	86.76	3.51	32.26	78.70
6	2-Hydroxy-gamma-butyrolactone	$C_4H_6O_3$	1.60	34.24	93.42	8.46	34.21	85.70
7	Furaneol	$C_6H_8O_3$	1.55	31.23	96.61	17.19	31.22	97.70
8	1-Heptadecanecarboxylic acid	$C_{18}H_{36}O_2$	1.28	52.48	95.16	2.26	52.46	84.00
9	2-Furanmethanol	$C_5H_6O_2$	1.24	24.95	84.53	0.52	26.51	73.70
10	Pentanoic acid	$C_5H_{10}O_2$	1.09	25.00	67.27			
11	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$				23.91	36.34	95.60
12	Glycerin	$C_3H_8O_3$				16.29	37.42	95.20
13	Benzofuran, 2,3-dihydro-	C_8H_8O				9.30	39.09	93.40
14	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-	$C_6H_6O_4$				4.32	36.84	90.50
15	2,4(1H,3H)-Pyrimidinedione, 5-methyl-	$C_5H_6N_2O_2$				4.12	55.26	79.80
	Total			18.70			133.19	

Note: For FCCE, only the top 10 major compounds are listed in this table.

Table 3. Antioxidant activity of Chinese chive Extracts.

Sample	**Antioxidant Activity**[1]	
	DPPH IC_{50} (mg/ml)	**ABTS IC_{50} (mg/ml)**
FCCE	7.25 ± 0.14*	4.91 ± 0.29*
DCCE	8.62 ± 0.02*	6.66 ± 0.03*
Trolox	0.0281 ± 0.78	0.2795 ± 0.69

[1] IC_{50} = half-maximal inhibitory concentration. The value indicates the mean ± SD for three independent experiments performed in triplicates;
* $p < 0.05$ compared between DPPH and ABTS.

Table 4. The MIC and MBC values of Chinese chive extracts against various food pathogenic bacteria.

Bacteria	MIC (mg/ml)		MBC (mg/ml)	
	FCCE	DCCE	FCCE	DCCE
Bacillus cereus PSU3874	8	8	>16	>16
Listeria monocytogenes PSU6205	8	8	>16	>16
Staphylococcus aureus ATCC25923	8	8	>16	>16
Escherichia coli ATCC25922	8	8	>16	>16
Salmonella sp. PSU411	8	8	>16	>16
Vibrio cholerae PSU6072	8	8	>16	>16
Vibrio parahaemolyticus ATCC17802	8	8	>16	>16

4. Conclusions

The study results indicate the good antibacterial and antioxidant properties of Chinese chive extracts, mainly due to phytochemical compounds. Therefore, the extracts from fresh or dried leaves could be applied as natural ingredients in functional food products. The study highlights that the promising activities of Chinese chive extracts could attributed to the presence of phytochemical compounds in the plant. These bioactive compounds, commonly found in plants, offer various health benefits. Furthermore, the study emphasizes the distinct culinary uses of fresh and dried Chinese chive leaves. Fresh leaves add vibrant color, crisp texture, and a strong flavor to dishes, while dried leaves offer a more concentrated and potent taste and an extended shelf life. Although the drying process may lead to a slight reduction in heat-sensitive vitamins, dried chives retain essential nutrients, making them a practical choice for enhancing the nutritional content of meals. The valuable properties of Chinese chive extracts, whether derived from fresh or dried leaves, hold potential as natural ingredients for functional food products. Utilizing these extracts in such applications can offer health benefits and enhance the overall flavor of foods.

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