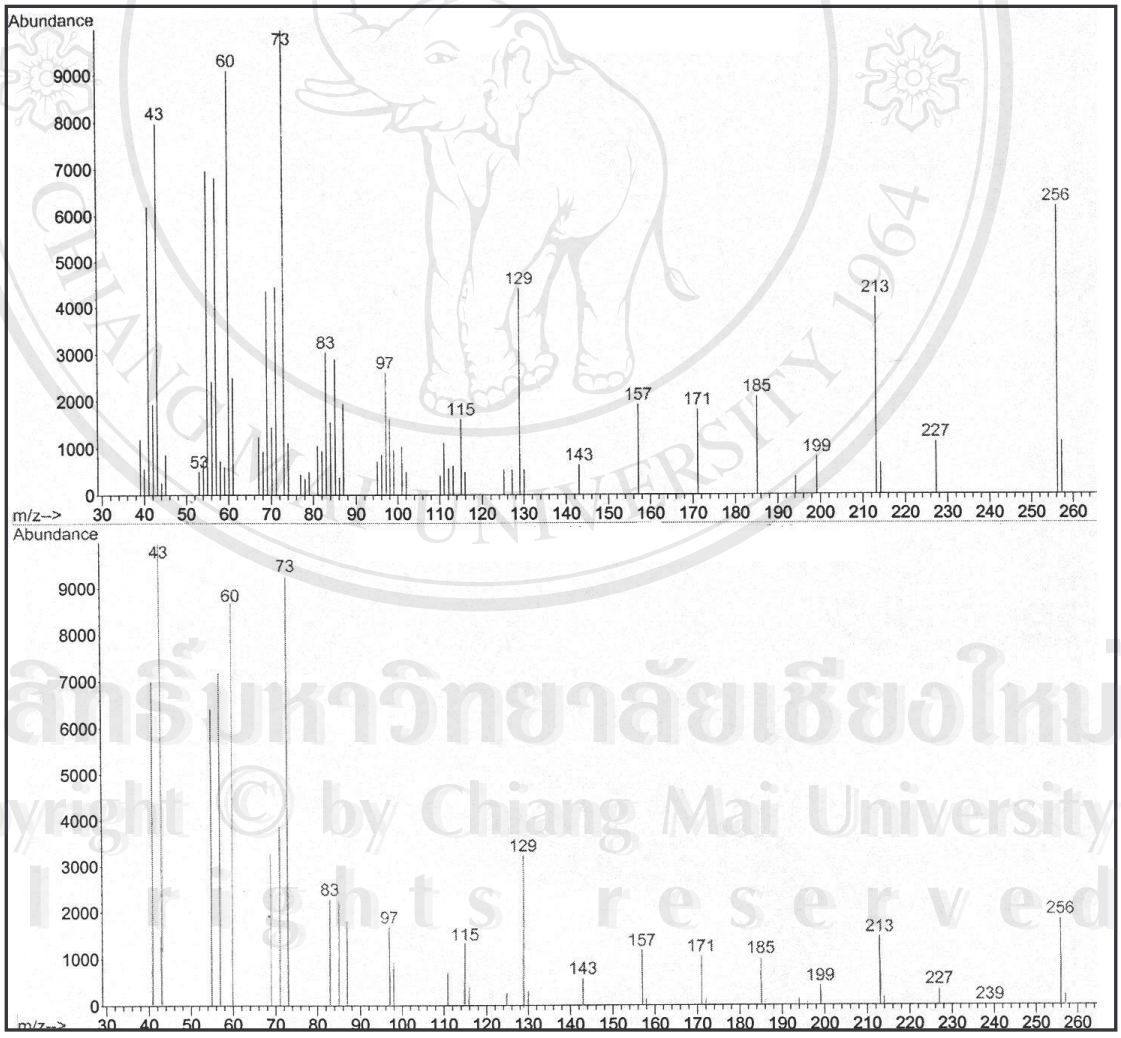
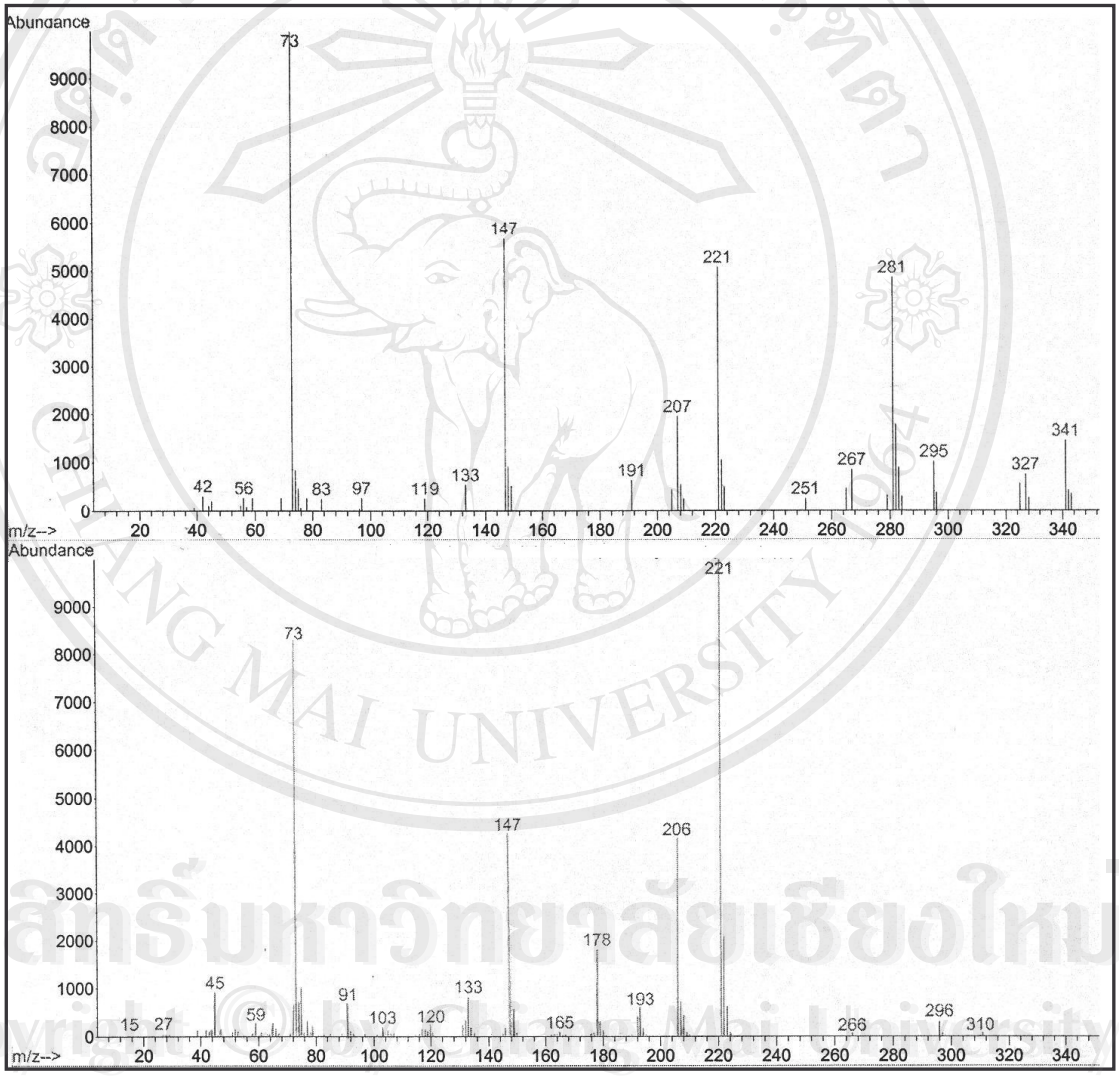
APPENDIX

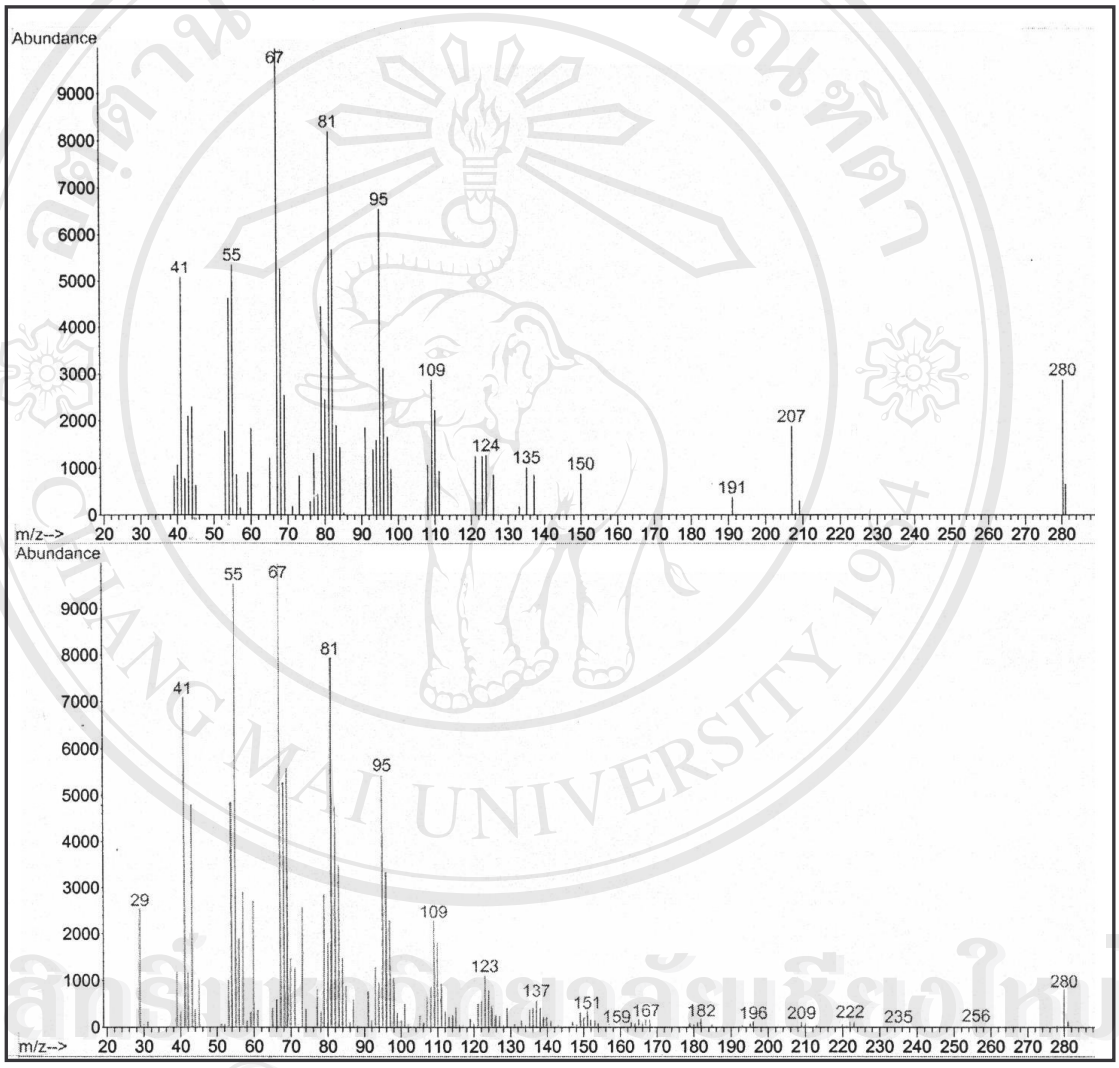
A-1 Mass spectrum of antimicrobial compound from *Pimpinella anisum* (spot 1) at retention time 19.31 min (above), and reference spectrum of hexadecanoic acid (bottom)



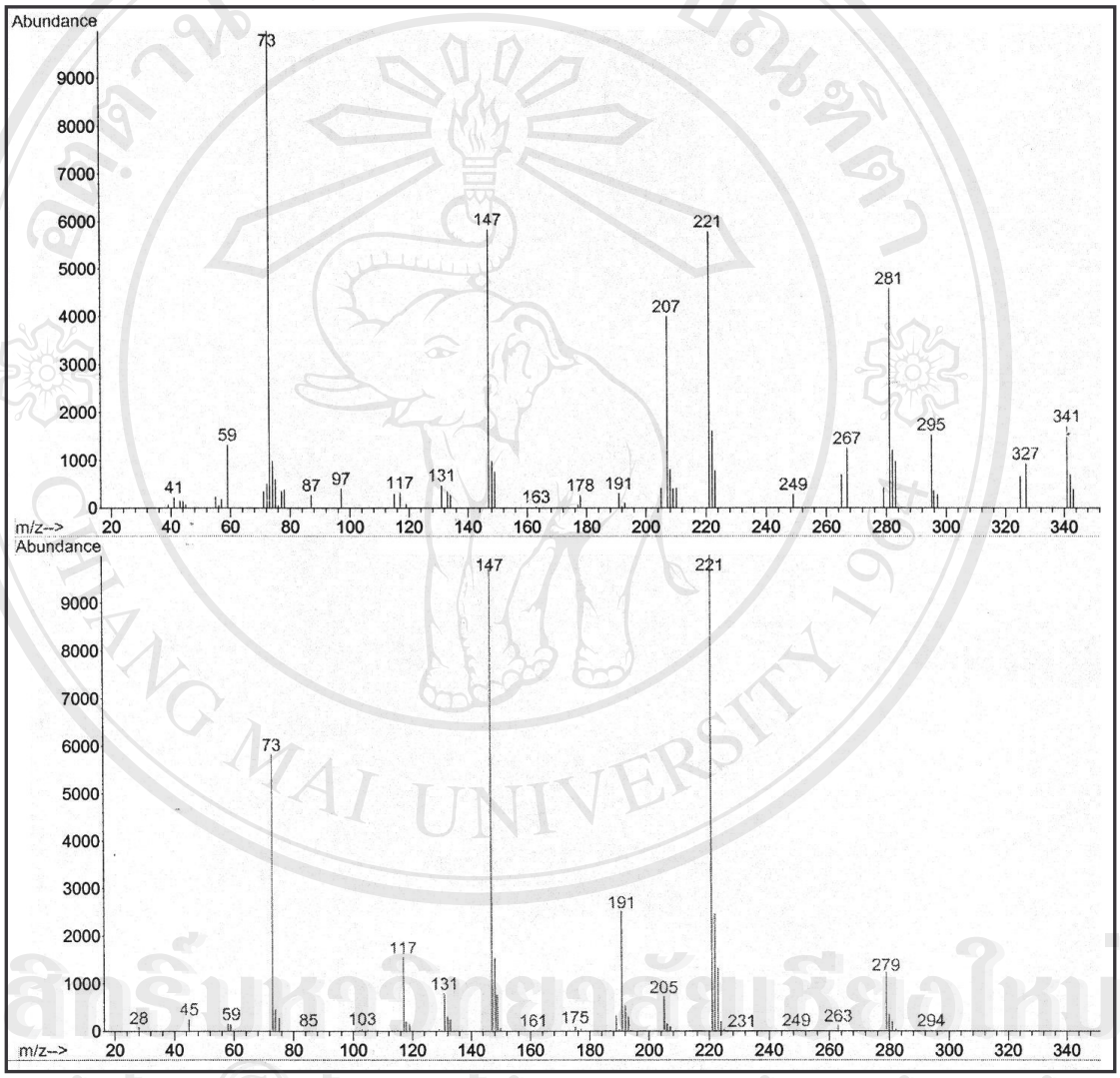
A-2 Mass spectrum of antimicrobial compound from *Pimpinella anisum* (spot 1) at retention time 21.08 min (above), and reference spectrum of 2,6-dimethyl-3,4-bis (trimethylsilyloxymethyl) pyridine (bottom)



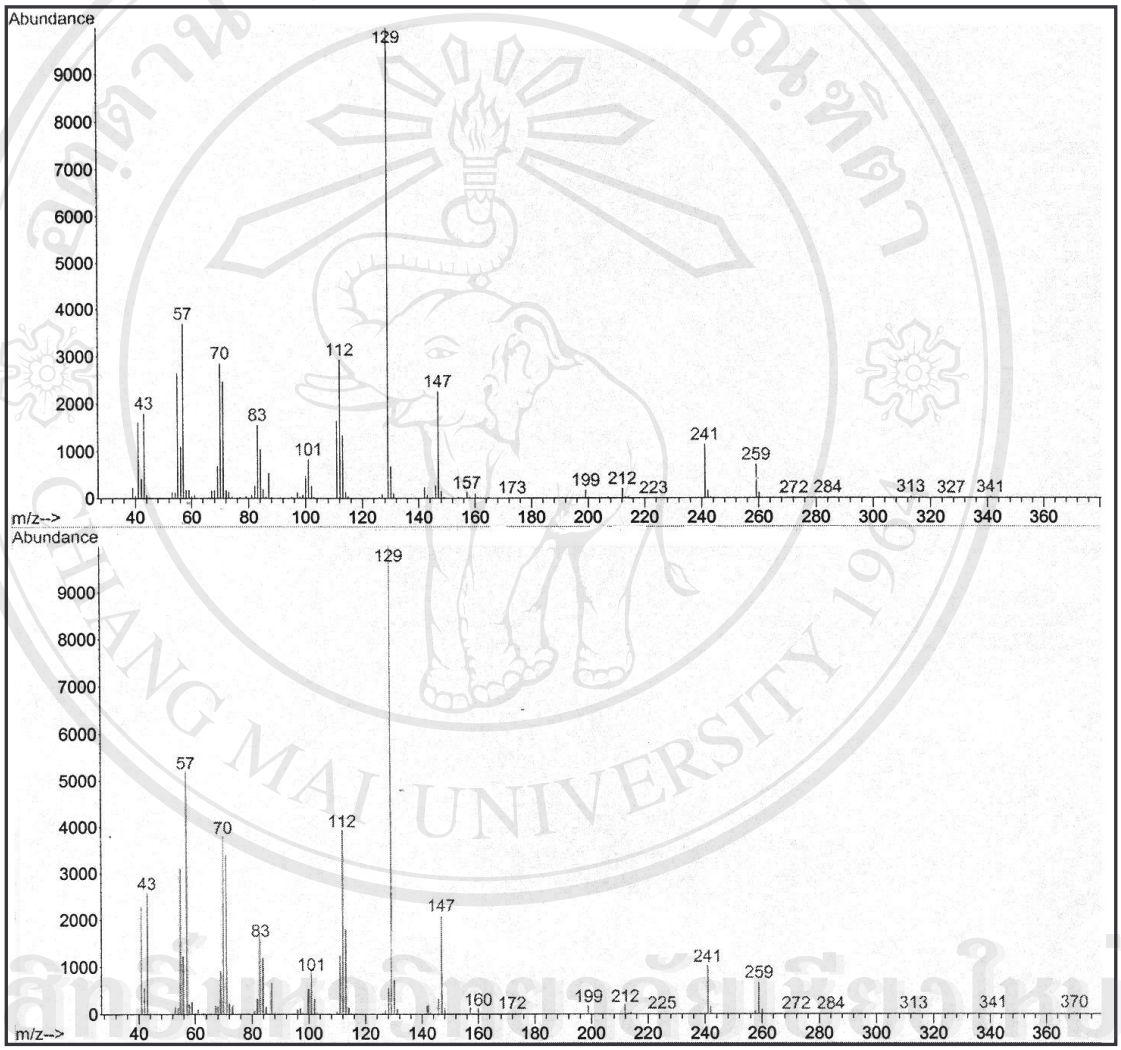
A-3 Mass spectrum of antimicrobial compound from *Pimpinella anisum* (spot 1) at retention time 21.25 min (above), and reference spectrum of 9,12-octadecadienoic acid (bottom)



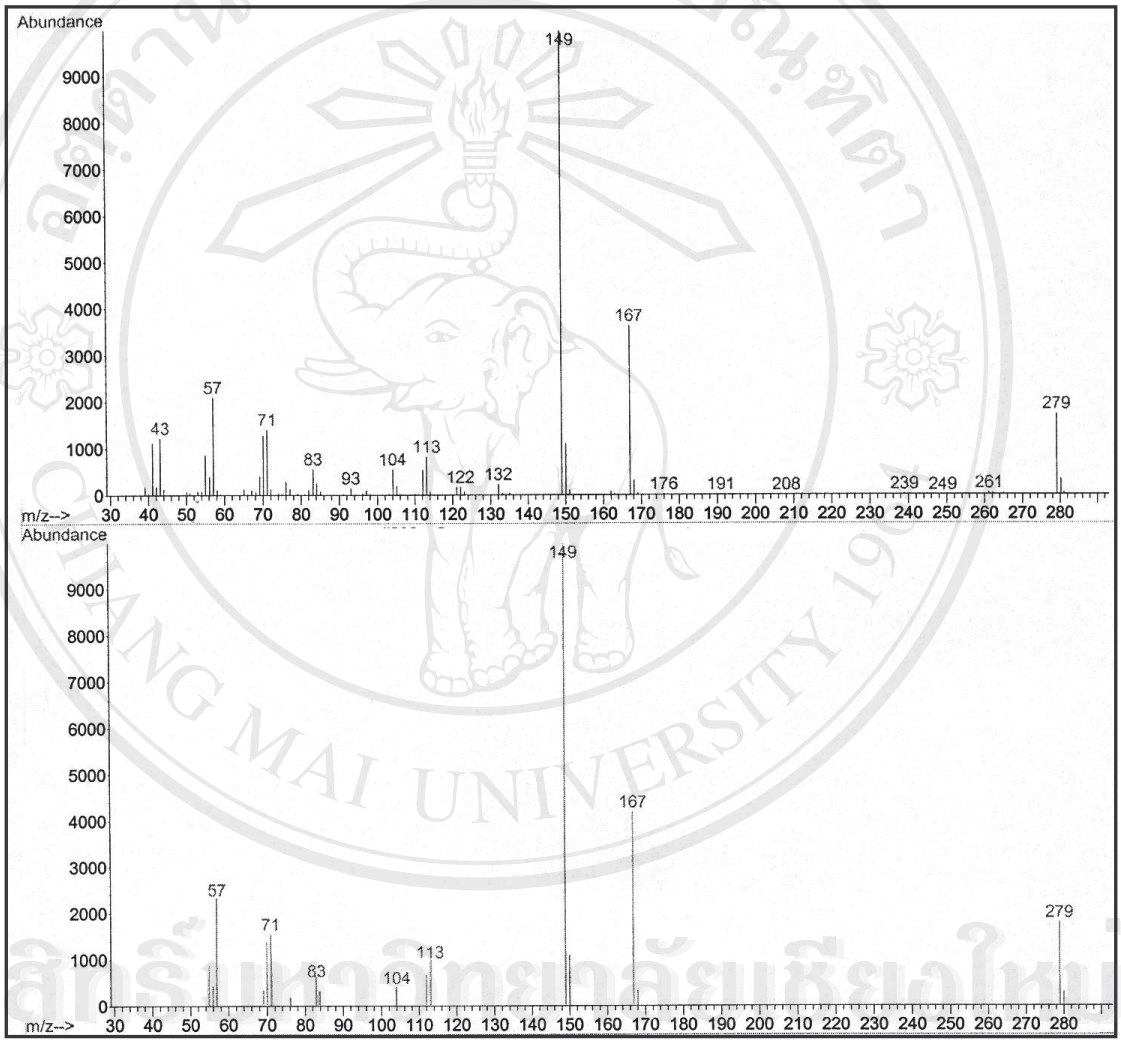
A-4 Mass spectrum of antimicrobial compound from *Pimpinella anisum* (spot 1) at retention time 23.45 min (above), and reference spectrum of 3,6-dioxa-2,4,5,7-tetrasilaoctane (bottom)



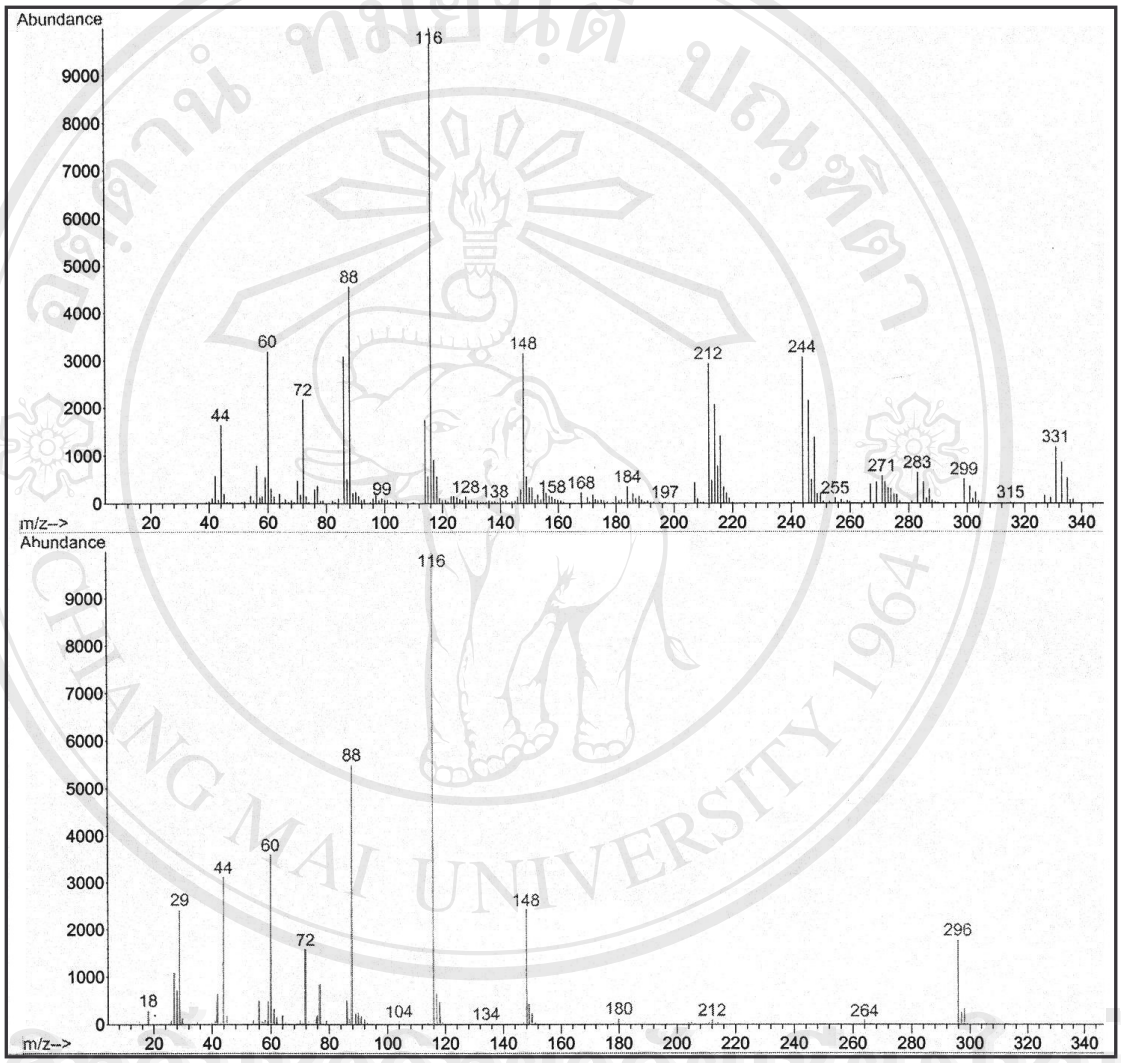
A-5 Mass spectrum of antimicrobial compound from *Pimpinella anisum* (spot 1) at retention time 23.93 min (above), and reference spectrum of dioctyl hexanedioate (bottom)



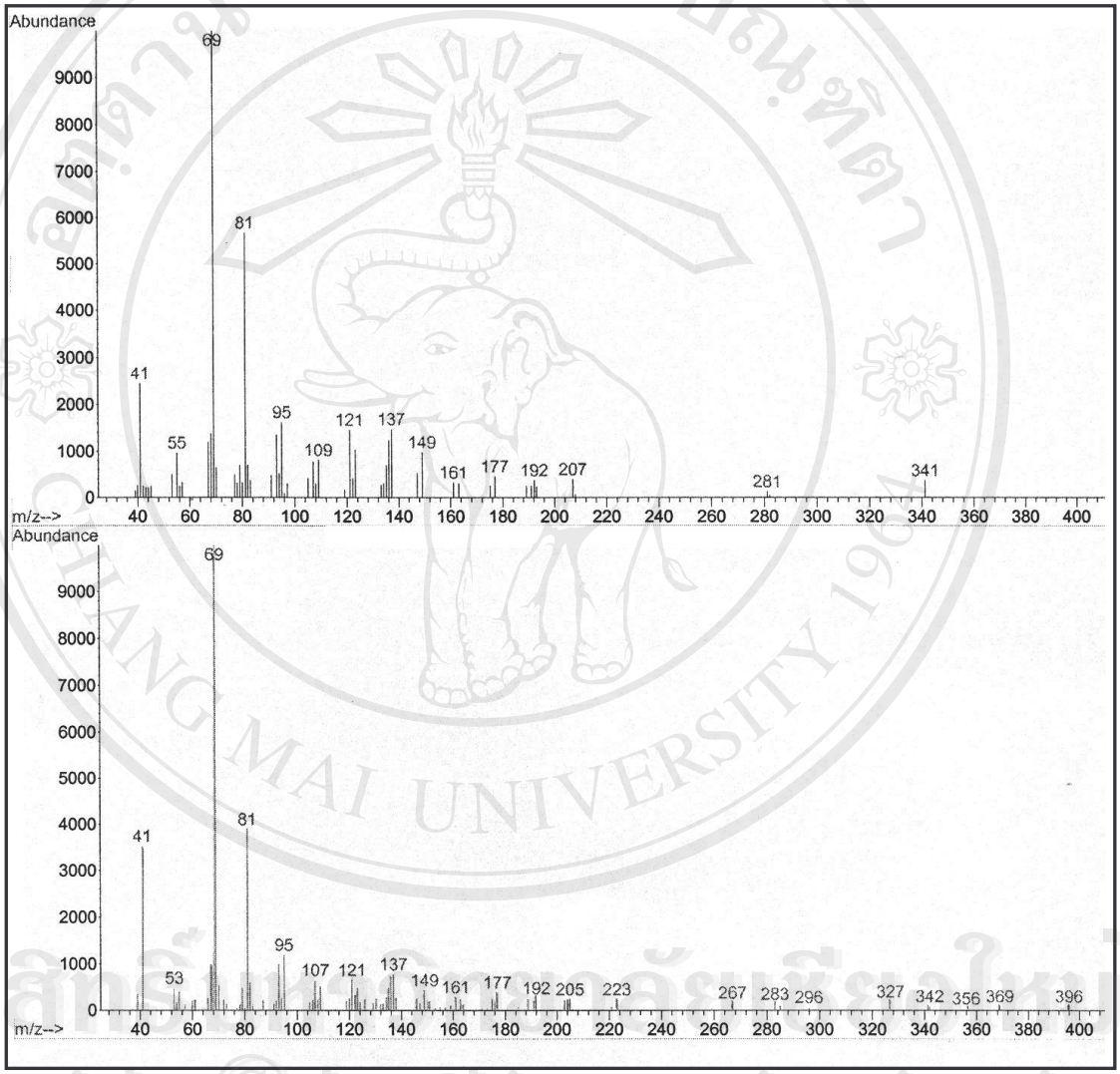
A-6 Mass spectrum of antimicrobial compound from *Pimpinella anisum* (spot 1) at retention time 26.38 min (above), and reference spectrum of bis (2-ethylhexyl) 1,2-benzenedicarboxylate (bottom)



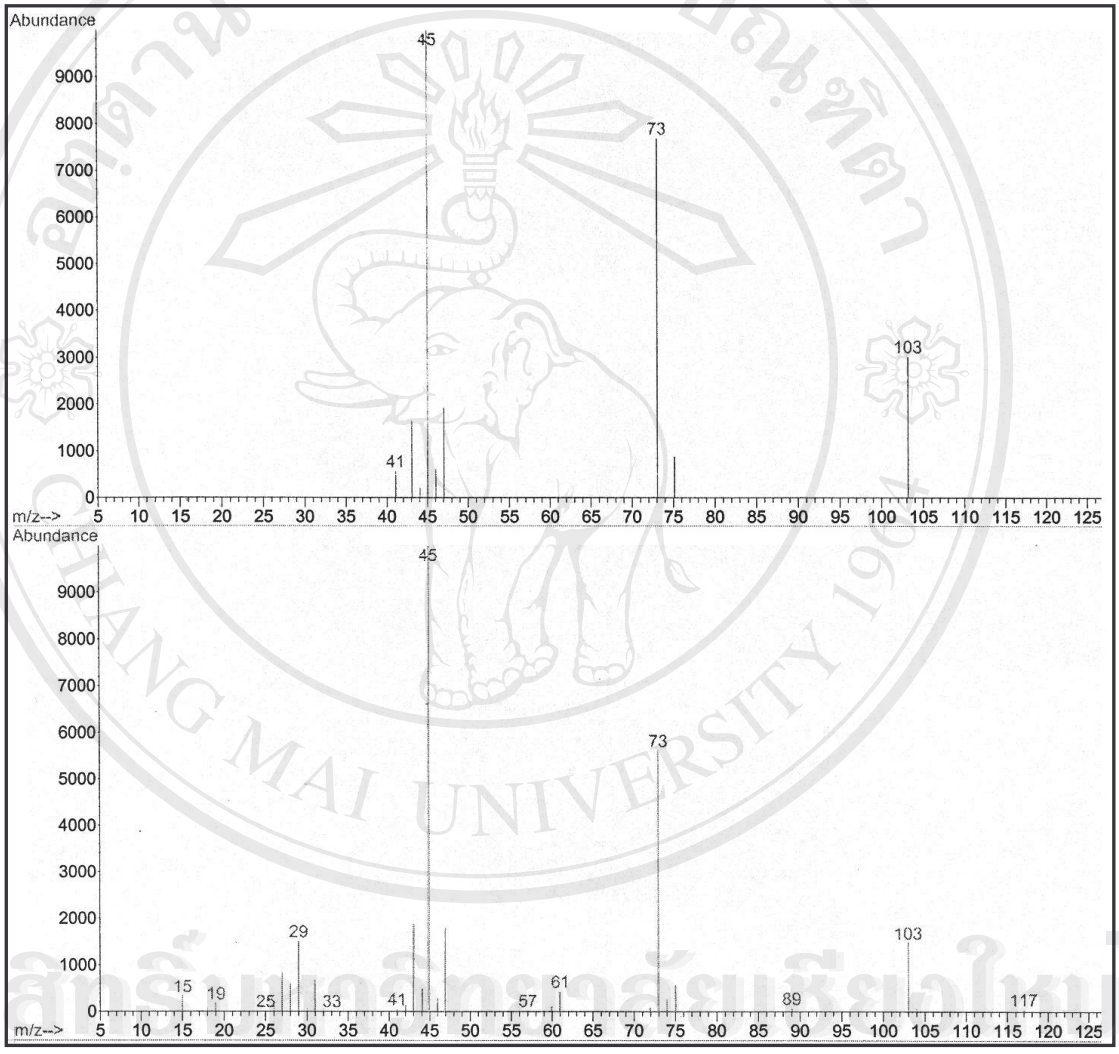
A-7 Mass spectrum of antimicrobial compound from *Pimpinella anisum* (spot 1) at retention time 32.07 min (above), and reference spectrum of disulfiram (bottom)



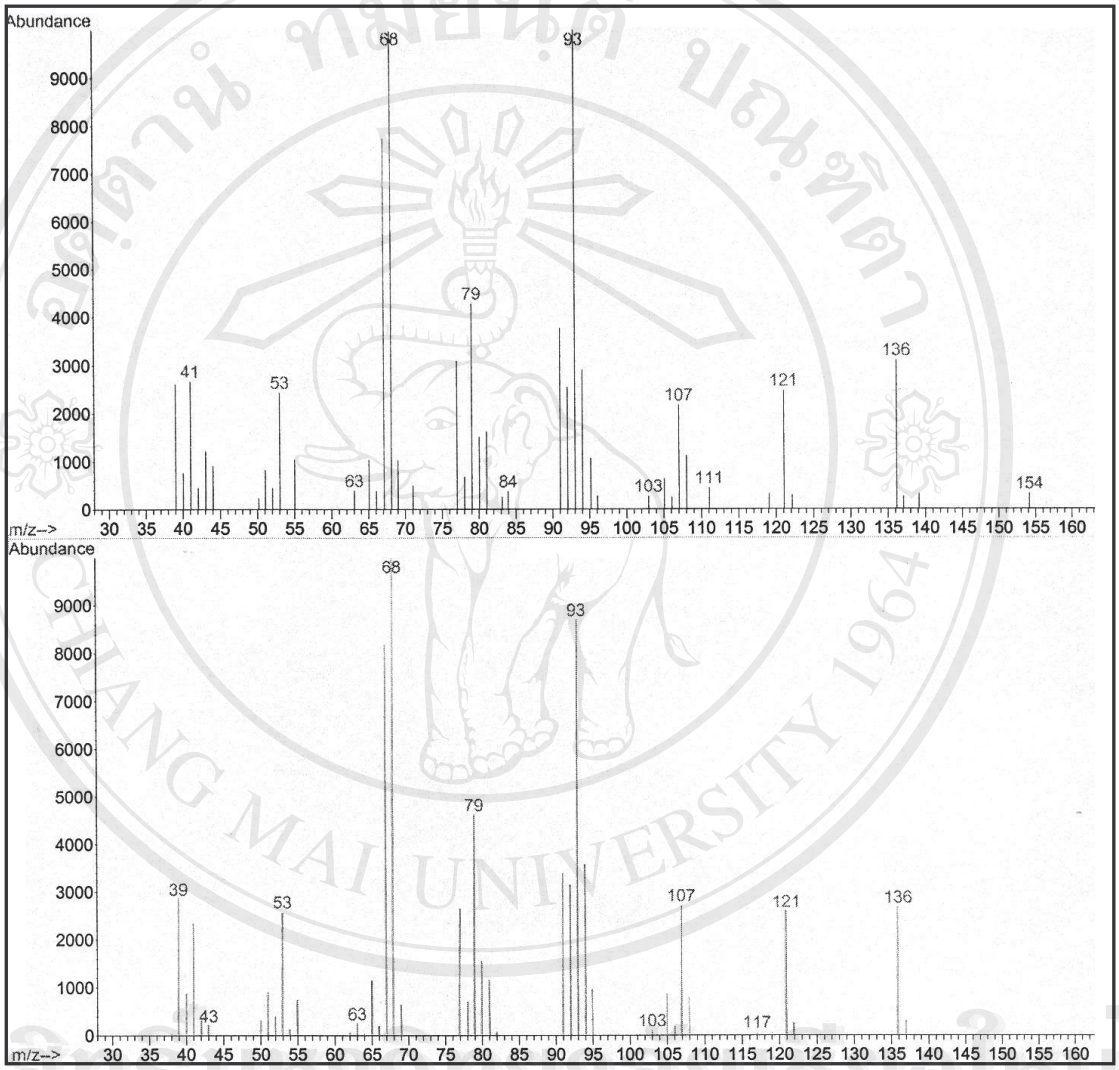
A-8 Mass spectrum of antimicrobial compound from *Pimpinella anisum* (spot 1) at retention time 35.65 min (above), and reference spectrum of 10-demethylsqualene (bottom)



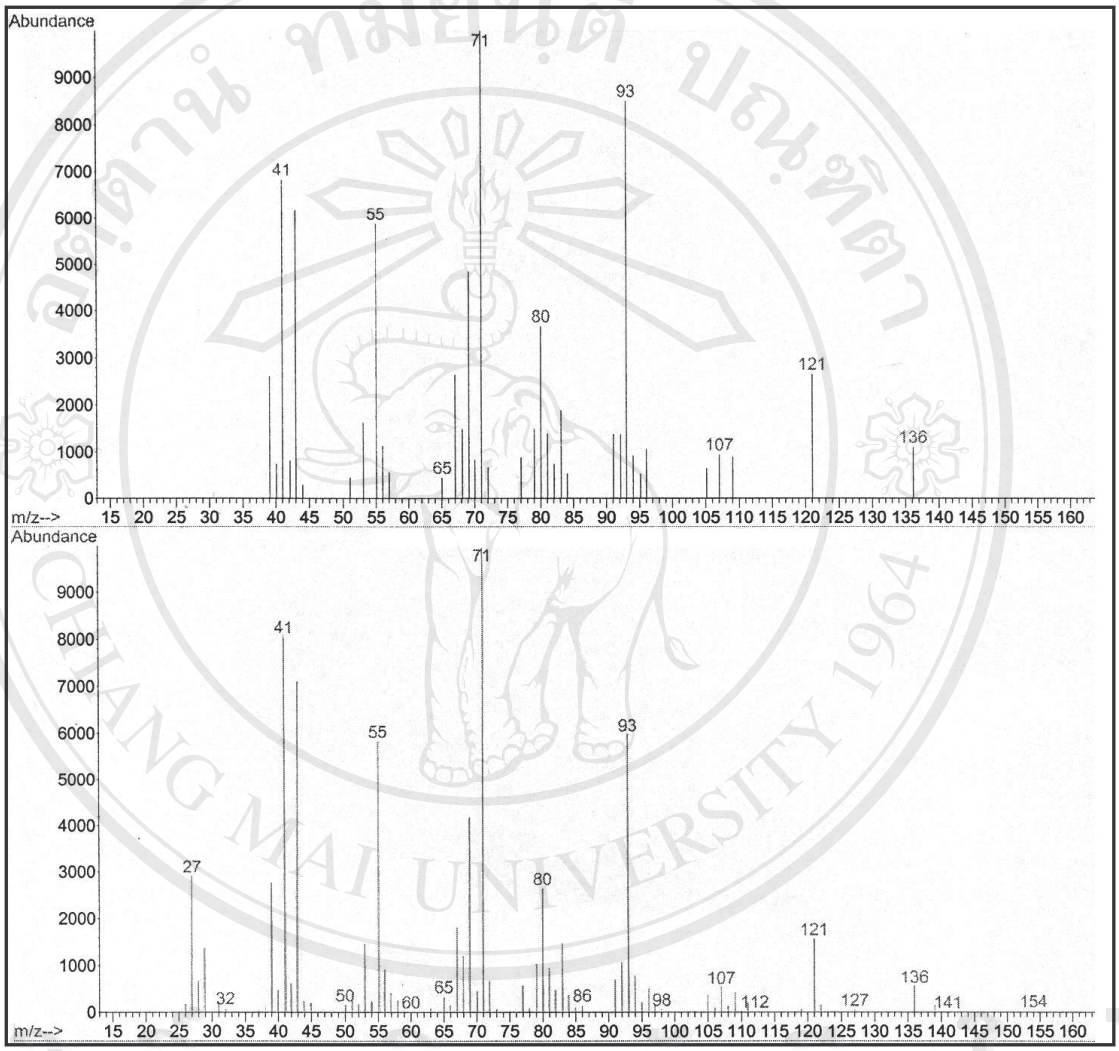
A-9 Mass spectrum of antimicrobial compound from *Pimpinella anisum* (spot 3) at retention time 2.25 min (above), and reference spectrum of 1,1-diethoxy ethane (bottom)



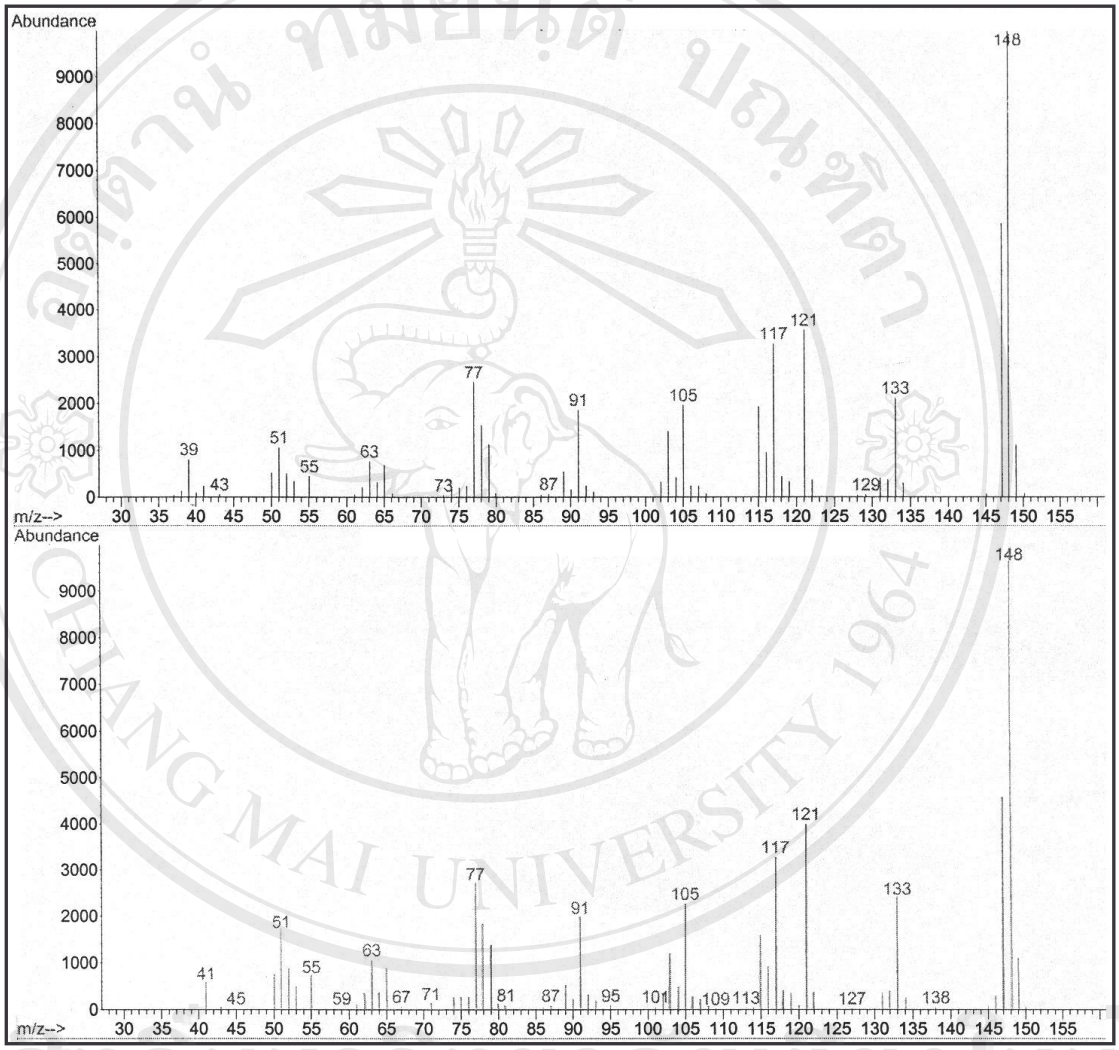
A-10 Mass spectrum of the essential oil of star anise at retention time 5.57 min (above), and reference spectrum of limonene (bottom)



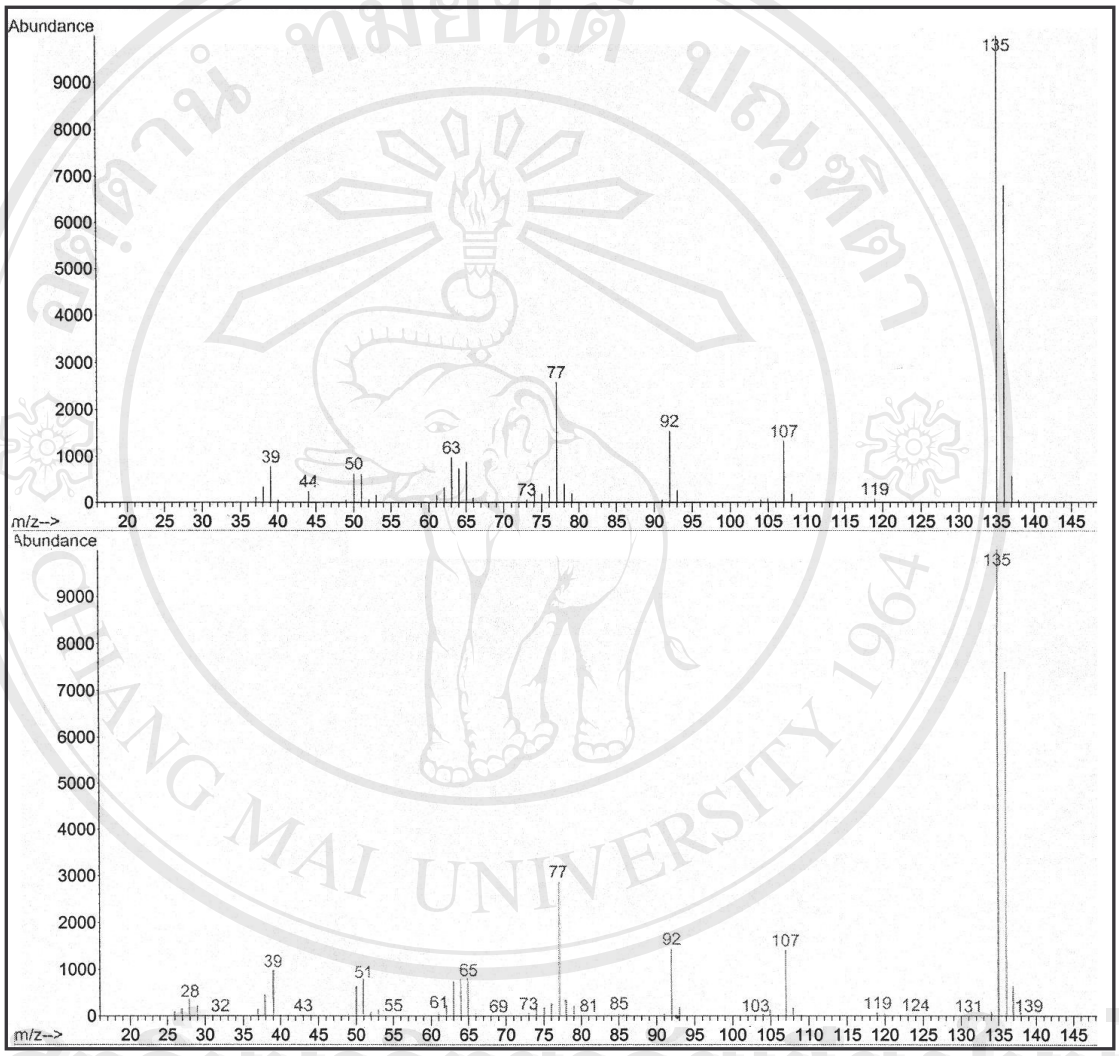
A-11 Mass spectrum of the essential oil of star anise at retention time 6.57 min (above), and reference spectrum of linalool (bottom)



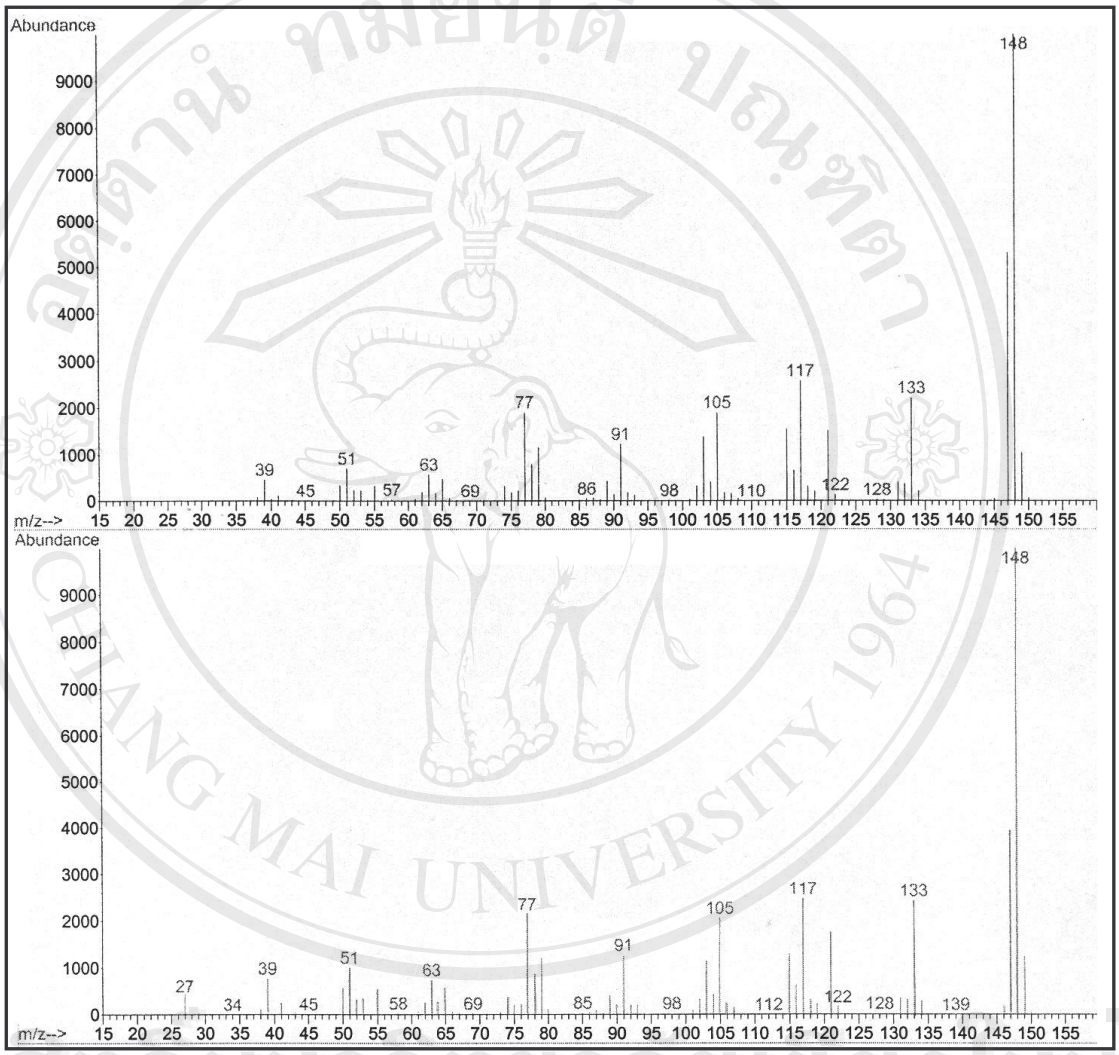
A-12 Mass spectrum of the essential oil of star anise at retention time 8.12 min (above), and reference spectrum of methyl chavicol (bottom)



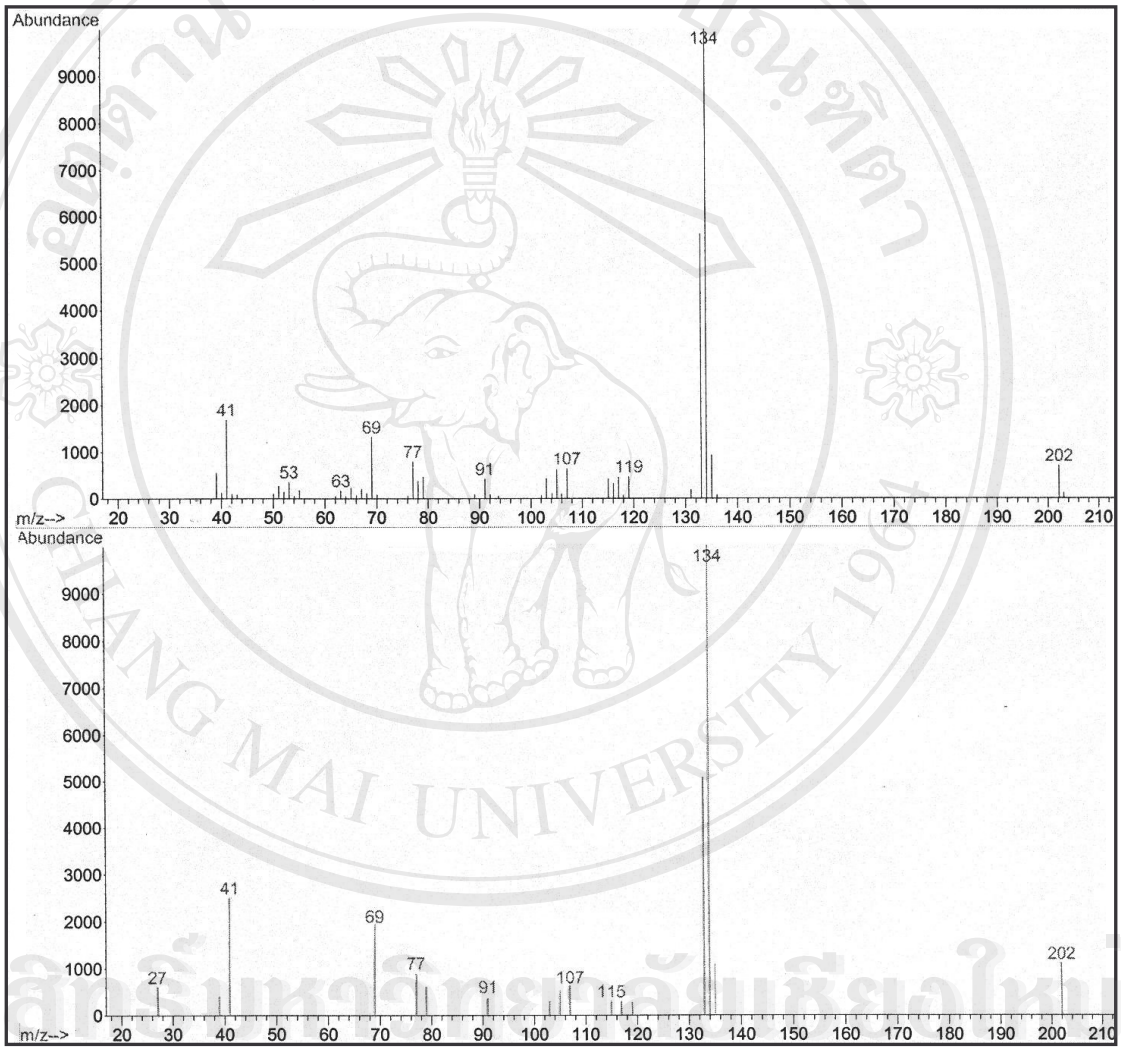
A-13 Mass spectrum of the essential oil of star anise at retention time 8.78 min (above), and reference spectrum of anisaldehyde (bottom)



A-14 Mass spectrum of the essential oil of star anise at retention time 9.58 min (above), and reference spectrum of anethole (bottom)



A-15 Mass spectrum of the essential oil of star anise at retention time 15.56 min (above), and reference spectrum of 1-(3-methyl-2-butenoxy)-4-(1-propenyl) benzene (bottom)



A-16 Supporting papers

1. **Chanwitheesuk, A.**, Teerawutgulrag, A., and Rakariyatham, N. 2005. Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand. *Food Chemistry*. 92(3), 491-497.
2. **Chanwitheesuk, A.**, Teerawutgulrag, A., Wongchanapiboon, T., Kilburn, J. D., and Rakariyatham, N. 2005. Antimicrobial activity of anise (*Pimpinella anisum* L.) seed extracts. *Chiang Mai Journal of Science*. 32(1), 53-59.

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 92 (2005) 491–497
Food Chemistry
www.elsevier.com/locate/foodchem

Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand

Anchana Chanwitheesuk, Aphiwat Teerawutgulrag, Nuansri Rakariyatham *

Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand

Received 28 June 2004; accepted 28 July 2004

Abstract

A large number of plants, which have been used as food and herbs in Thailand, were investigated for their antioxidant activity by using a β-carotene bleaching method. The contents of plant chemicals, such as vitamin C, vitamin E, carotenoids, tannin, and total phenolics, were also determined. The results showed that the highest antioxidant activity was found in the plant *Gymnema inodorum*, followed by *Piper sarmentosum* and *Mentha arvensis*, respectively. *G. inodorum* also contained the highest amount of vitamin E, and *M. arvensis* contained the highest amount of total xanthophylls. Correlations between the chemical content of each plant and the antioxidant index were observed. The results suggest that chemicals such as vitamin C, vitamin E, carotenoids, and phenolic compounds are the contributors to the antioxidant activity in the plants.

Keywords: Antioxidant activity; Antioxidant compounds; Edible plants

1. Introduction

The oxidative deterioration of lipid-containing food is responsible for the rancid odours and flavours during processing and storage, consequently decreasing the nutritional quality and safety of foods due to the formation of secondary, potentially toxic compounds (Zainol, Abd-Hamid, Yusof, & Muse, 2003). Moreover, in humans, lipid oxidation is also thought to induce physiological obstruction, causing aging of the cells and carcinogenesis (Lampart-Szczapa, Korczak, Nogala-Kalucka, & Zawirska-Wojtasiak, 2003). A large number of experimental studies indicate that lipid oxidation products, called free radicals, can harm healthy cells, create harmful molecules, and contribute to the degenerative processes related to aging and diseases, e.g. cancer, cardiovascular disease, and neurodegenerative disorders, such as Alzheimer's disease (Croft, 1999; Lemberkovics, Czinner, Szentmihályi, Balázs, & Szöke, 2002; Sami, 1995; Shon, Kim, & Sung, 2003). The antioxidants are now known to play an important role in protection against disorders caused by oxidant damage. The term "antioxidants" refers to compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reaction (Velioglu, Mazza, Gao, & Oomah, 1998), and which can thus prevent or repair damage done to the body's cells by oxygen. They act in one or more of the following ways: reducing agents, free radical scavengers, potential complexers of pro-oxidant metals, and quenchers of singlet oxygen (Hudson, 1990).

Recently, there has been a considerable interest in finding natural antioxidants from plant materials to replace synthetic ones. Natural antioxidant substances are presumed to be safe since they occur in plant foods, and

* Corresponding author. Tel.: +66 53 943341-45x342; fax: +66 53 892277.
E-mail address: nuansril@yahoo.com (N. Rakariyatham).

0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.07.035

are seen as more desirable than their synthetic counterparts. Data from both scientific reports and laboratory studies show that plants contain a large variety of substances called "plant chemicals" or "phytochemicals" that possess antioxidant activity (Pratt, 1992). Natural antioxidants occur in all higher plants, and in all parts of the plant (wood, bark, stems, pods, leaves, fruit, roots, flowers, pollen, and seeds). Typical compounds that exhibit antioxidant activity include vitamins, carotenoids, and phenolic compounds. Therefore, recommendations have been made to increase the daily intake of fruit and vegetables, which are rich in these nutrients that lower the risk of chronic health problems associated with the diseases mentioned above (Klipstein-Grobusch et al., 2000; Moeller, Jacques, & Blumberg, 2000; Morris et al., 1998; Slattery et al., 2000).

The antioxidant compounds of higher plants have been demonstrated, in vitro experiments, to protect against oxidation damage by inhibiting or quenching free radicals and reactive oxygen species. The roles of these compounds as potential antioxidants can be inferred by their similarity to synthetic antioxidants, of related structures (Larson, 1988). Vitamin C has been proposed, for a long time, as a biological antioxidant. It was found to act as a chain-breaking scavenger for peroxy radicals and also to act as a synergist with vitamin E, since vitamin C can donate a hydrogen atom to the vitamin E-derived phenolate radical, thus regenerating its activity. Vitamin E is one of the best quenchers for singlet oxygen, and can act as a chain-breaking antioxidant. Furthermore, singlet oxygen is very powerfully quenched by carotenoids, especially β-carotene. In the case of phenolic compounds, the ability of the phenolics to act as antioxidants depends on the redox properties of their phenolic hydroxyl groups, that allow them to act as reducing agents, hydrogen-donating antioxidants, oxygen quenchers (Rice-Evans & Miller, 1996).

In the present study, we collected 43 edible plant species from eight families that are widely consumed in Thailand, and analyzed antioxidant activities of methanolic extracts prepared from these plants. The contents of six antioxidant compounds, including vitamin C, vitamin E, total carotenes, total xanthophylls, tannins, and total phenolics, were also investigated in the plants.

2. Materials and methods

2.1. Plant material

The plant species used were purchased from a local market in Chiang Mai, Thailand, during June–October 2000. They were identified botanically at the Biology Department of Chiang Mai University. The scientific names of the plants which were used in the study are given in Table 1. The plants were cleaned and cut into small pieces before being dried in a hot air-blowing oven at 50 °C. All samples, after drying, had water contents below 10%. They were ground to a fine powder in a mechanical blender and kept at room temperature prior to extraction. The dried plants were used for the analysis of antioxidant activity, vitamin C, vitamin E, carotenoids, tannins and phenolic compounds.

2.2. Determination of antioxidant activity

A dried plant was soaked overnight in methanol at room temperature in a flask. Each flask contained 0.5 g sample in 10 ml of methanol. The extract was filtered through Whatman No. 42 filter paper, and the residue was washed with hot methanol. The insoluble residue was discarded. The filtrate was evaporated in a water bath at 40 °C to a final volume 1 ml. Antioxidant activity was determined by measuring the coupled oxidation of carotene and linoleic acid, as described by Hammerschmidt and Pratt (1978). One ml of β-carotene solution in chloroform (1 mg/10 ml) was pipetted into a flask, which contained linoleic acid and Tween 40. After removal of the chloroform on a water bath at 50 °C, 50 ml of distilled water (which was bubbled by an air pump for 1 h) were added to the flask with vigorous swirling. Five ml aliquots of this emulsion were placed in test tubes which contained 0.2 ml of the extracts. Samples were read against a blank containing the emulsion minus the carotene. A reading at 470 nm was taken immediately ($t = 0$) and then at 15-min intervals for 105 min. The bleaching rate of β-carotene was determined by the difference in the spectral absorbance reading between the initial and last reading of bleaching that remained essentially linear divided by time. The antioxidant index was the ratio of the bleaching rate of control (system with no added test compound) to the bleaching rate when a test compound was in the system.

2.3. Determination of vitamin C content

The content of vitamin C in the plants was determined by the spectrophotometric procedure of Bajaj and Kaur (1981). A known mass of dried plant material (0.5 g) was extracted overnight with 10 ml of oxalic acid–EDTA solution at room temperature. The extract was filtered through a filter paper. A 2.5 ml aliquot was then transferred into a 25-ml volumetric flask. Then, the other reagents (2.5 ml of oxalic acid-EDTA solution, 0.5 ml of metaphosphoric acid-acetic acid solution, 0.1 ml of sulfuric acid solution, and 2 ml of ammonium molybdate reagent) were added. After that, the solvent was adjusted to a volume of 25 ml with distilled water. The molybdenum blue complex, which was formed by the reduction of ammonium molybdate with ascorbic acid (vitamin C), was

Table 1
Edible plants used in this study

Scientific name	Common name	Part used	Uses
(Family Araliaceae)			
Acanthopanax trifoliatum	–	Leaves	–
Macropanax dispermus	–	Leaves	Digestive tonic
Polycia fruticosa	–	Leaves	Poultice for swelling, antipyretic
(Family Asclepiadaceae)			
Dregea volubilis	–	Leaves	Poultice for swelling
Gymnema inodorum	–	Leaves	Antihyperglycemia
Marsdenia glabra	–	Leaves	Digestive tonic, restorative, antipyretic
Telosma minor	Tonkin jasmine	Flowers	Restorative, detoxicant
(Family Cucurbitaceae)			
Coccinia grandis	Ivy gourd	Leaves	Antihyperglycemia
Cucurbita moschata	Pumpkin	Leaves	Carminative, expectorant
Momordica charantia	Bitter gourd	Fruit	Antihyperglycemia
Sechium edule	Chayote	Leaves	–
(Family Labiatae)			
Coleus amboinicus	Country borage	Leaves	Cure otorrhea, otitis
Mentha arvensis	Japanese mint	Leaves	Relieve headache, antipyretic
M. cordifolia	Kitchen mint	Leaves	Relieve headache, carminative
Ocimum americanum	Hairy basil	Leaves	Carminative, relieve cold
O. basilicum	Sweet basil	Leaves	Digestive tonic, carminative, expectorant, relieve headache
O. gratissimum	–	Leaves	Expectorant, carminative, relieve stomach ache and flatulence
O. sanctum	Holy basil	Leaves	Expectorant, carminative, relieve stomach ache and flatulence
Orthosiphon grandiflorus	Cat's whisker	Leaves	Uretic
(Family Leguminosae)			
Acacia pennata	Acacia leaf	Shoot tips	Carminative, relieve flatulence
Bauhinia purpurea	–	Leaves	Antitussive, uretic
Caesalpinia mimosoides	–	Shoot tips	Digestive tonic
Cassia siamea	Thai copper pod	Leaves	Laxative, haemagogic, uretic
Leucaena leucocephala	Lead tree	Shoot tips	Relieve diarrhea
Neptunia oleracea	Water cress	Leaves	Detoxicant
Pueraria mirifica	–	Stem	Restorative
Tamarindus indica	Tamarind	Shoot tips	Antitussive, expectorant
(Family Piperaceae)			
Piper retrofractum	Long pepper	Flower	Antidiarrheic, carminative
P. sarmentosum	–	Leaves	Digestive tonic, carminative, expectorant, antihyperglycemia, antimalaria
(Family Umbelliferae)			
Anethum graveolens	Dill	Seed	Restorative, carminative, expectorant, relieve nausea
Apium graveolens	–	Leaves	Restorative, carminative, uretic
Centella asiatica	Pennywort	Leaves	Poultice for wound, scar or ulcer
Coriandrum sativum	Coriander	Leaves	Carminative, relieve flatulence, antipyretic, antitussive
Eryngium foetidum	False coriander	Leaves	Digestive tonic, carminative, relieve flatulence
Oenanthe stolonifera	Chinese celery	Leaves	Digestive tonic, expectorant, relieve flatulence,
(Family Zingiberaceae)			
Amomum krervanh	Siam cardamom	Fruit	Carminative, relieve flatulence
Boesenbergia pandurata	Wild ginger	Rootstock	Restorative, carminative, relieve flatulence, uretic, haemagogic
Curcuma xanthorrhiza	–	Rootstock	Relieve stomachache
Kaempferia galanga	–	Rootstock	Carminative, relieve flatulence, haemagogic
Languas galanga	Greater galanga	Rootstock	Carminative, relieve flatulence
Zingiber cassumunar	–	Rootstock	Carminative, haemagogic, relieve muscle pain and beriberi
Z. officinale	Ginger	Rootstock	Restorative, carminative, relieve flatulence
Z. zerumbet	Zerumbet ginger	Rootstock	Restorative, carminative, uretic, expectorant

measured for absorbance after 15 min at 760 nm. The content of vitamin C was then determined by referring to the calibration graph, using ascorbic acid solution as a standard solution.

2.4. Determination of vitamin E content

Dried plant material (0.5 g) was immersed in 20 ml of ethanol for 30 min in a water bath at 85 °C. The solution

was allowed to cool and then filtered into a separating funnel. Heptane (10 ml) was added, and the solution was shaken for 5 min. Then, 20 ml of 1.25% sodium sulfate was added and the solution was shaken again for 2 min, and allowed to separate into layers. Total tocopherols were determined by a reaction with cupric ions and complexation with 2,2′-biquinoline (cuproine) according to Contreras-Guzmán and Strong (1982). A volume of 0.5 ml of α-tocopherols in ethanol was processed in the same way as a sample, and used as a standard.

2.5. Determination of carotenoids content

Dried plant material (0.5 g) was extracted with 30 ml of hexane at room temperature, with hot saponification, as described by Helrich (1990). The isolation of carotenes and xanthophylls in the extract of dried samples was performed by column chromatography. For this condition, Hyflo Super-Cel was used as a stationary phase, and solutions, of hexane–acetone (9:1 v/v) and hexane–acetone–methanol (8:1:1 v/v/v) were used as a mobile phase for the separation of carotenes and xanthophylls, respectively. The content of carotenes and xanthophylls was determined by measuring absorbance at 436 and 474 nm, respectively.

2.6. Determination of tannins content

Dried plant material (0.5 g) was extracted with 300 ml of diethyl ether for 20 h at room temperature. The residue was boiled for 2 h with 100 ml of distilled water, and then allowed to cool, and was filtered. The extract was adjusted to a volume of 100 ml in a volumetric flask. The content of tannins in the extract was determined colorimetrically using Folin–Denis reagent, and by measuring absorbance of the blue complex at 760 nm, using tannic acid solution as a standard solution, as described by Helrich (1990).

2.7. Determination of total phenolics content

The extraction of total phenolics was performed according to Naczk, Wanasundara, and Shahidi (1992) with a slight modification. One gramme of dried plant material was extracted three times with 20 ml of acetone–methanol–water (7:7:6 v/v/v) at room temperature. The extract was centrifuged at 6000 rpm for 10 min. After centrifugation, the combined supernatant was analyzed for total phenolics according to Hammerschmidt and Pratt (1978) with some modifications. The procedure consisted of combining 1 ml of test solution with 10 ml of deionized water and 2 ml of Folin–Denis reagent. After 5 min, 2 ml of saturated sodium carbonate solution were added, and the mixture was incubated for 1 h at room temperature. Absorbance was then measured at 640 nm. Pyrocatechol solution was used as a standard for the determination of total phenolics content.

2.8. Statistical analysis

Mean values and standard deviations (S.D.) were calculated from the results. One way analysis of variance (ANOVA) was applied for comparison of the mean values. P value < 0.05 was regarded as significant. Correlation coefficients (R), to determine the relationship between two variables (between antioxidant index and content of antioxidant compounds in the plants), were also calculated by using the Statistical Package for Social Science (SPSS) programme.

3. Results and discussion

The antioxidant activity of plants is mainly contributed by the active compounds present in them. In this study, the antioxidant activity and the content of active compounds of 43 edible plants belonging to eight families were determined. The results are summarized in Table 2. It was found that the methanolic extracts of all plants showed antioxidant activity. Stronger activity is indicated by a higher antioxidant index. Significant differences ($P < 0.05$) were found in the antioxidant indices of the tested plants. The methanolic extract of the leaves of *Gymnema inodorum* exhibited the highest level of antioxidant activity with the index of 14.8, followed by *Piper sarmentosum* (13) *and Mentha arvensis* (10.9), respectively. The plants *G. inodorum* and *P. sarmentosum* are native herbs in Southeast Asia. They are used as food and traditional medicine in Thailand. The leaves of *G. inodorum* have been known to be effective for some diseases, including diabetes mellitus, rheumatic arthritis, and gout. A few studies on pharmacological activities of the *G. inodorum* extract have been scientifically investigated. It has been reported that the extract of *G. inodorum* inhibits the increase in blood sugar level by interfering with the intestinal glucose absorption process (Shimizu et al., 2001; Shimizu et al., 1997). The plant *P. sarmentosum* is one of the medicinal plants which possess antimalarial activity (Rahman, Furuta, Kojima, Takane, & Mohd, 1999), and neuromuscular blocking activity (Ridtitid, Rattanaprom, Thaina, Chittrakarn, & Sunbhanich, 1998). The water decoction of the whole plant of this species has also been traditionally used to treat diabetic patients. It has been reported that the aqueous extract of *P. sarmentosum* has a hypoglycemic effect in rats (Peungvicha et al., 1998). Antibacterial and antifungal activities have been investigated in the plant *M. arvensis* (Imai et al., 2001; Kishore, Mishra, & Chansouria, 1993). The result of this study suggests that the three plants can be used as a potential source of natural antioxidants, with their pharmaceutical applications.

Table 2
Antioxidant index and content of antioxidant compounds of the tested plants (±standard deviation)

Plants	Antioxidant index	Antioxidant compound content (mg%)					
		Vitamin C	Vitamin E	Total carotenes	Total xanthophylls	Tannins	Total phenolics
Araliaceae							
A. trifoliatum	4.37 ± 0.17	15.3 ± 0.07	0.0055 ± 0.0002	2.54 ± 0.03	3.17 ± 0.04	57.3 ± 0.10	275 ± 0.11
M. dispermus	1.27 ± 0.10	31.0 ± 0.07	0.0064 ± 0.0004	3.89 ± 0.06	1.06 ± 0.04	37.4 ± 0.11	651 ± 0.10
P. fruticosa	2.92 ± 0.12	9.11 ± 0.08	0.0201 ± 0.0015	2.52 ± 0.07	2.13 ± 0.03	24.3 ± 0.12	46.3 ± 0.09
Asclepiadaceae							
D. volubilis	7.20 ± 0.85	20.0 ± 0.08	0.0015 ± 0.0002	6.14 ± 0.07	1.07 ± 0.03	17.7 ± 0.08	100 ± 0.09
G. inodorum	14.8 ± 0.98	19.3 ± 0.08	0.0301 ± 0.0011	1.31 ± 0.03	1.07 ± 0.03	11.1 ± 0.11	188 ± 0.12
M. glabra	2.06 ± 0.63	22.7 ± 0.06	0.0018 ± 0.0005	8.92 ± 0.04	7.42 ± 0.04	4.47 ± 0.07	51.5 ± 0.10
T. minor	2.85 ± 0.39	23.6 ± 0.05	0.0070 ± 0.0011	1.29 ± 0.04	4.24 ± 0.03	17.7 ± 0.09	98.4 ± 0.11
Cucurbitaceae							
C. grandis	2.87 ± 0.05	16.6 ± 0.07	0.0070 ± 0.0013	1.94 ± 0.03	2.65 ± 0.04	17.7 ± 0.10	74.7 ± 0.10
C. moschata	0.91 ± 0.03	27.8 ± 0.06	0.0045 ± 0.0007	1.92 ± 0.04	1.59 ± 0.05	4.48 ± 0.06	87.8 ± 0.12
M. charantia	1.49 ± 0.18	13.8 ± 0.06	0.0024 ± 0.0005	1.31 ± 0.07	0.54 ± 0.04	4.48 ± 0.05	37.0 ± 0.13
S. edule	4.89 ± 0.68	17.9 ± 0.06	0.0197 ± 0.0010	3.83 ± 0.04	2.13 ± 0.03	4.48 ± 0.08	66.1 ± 0.14
Labiatae							
C. amboinicus	4.22 ± 0.43	10.2 ± 0.05	0.0054 ± 0.0011	2.54 ± 0.05	4.24 ± 0.03	24.3 ± 0.09	54.8 ± 0.11
M. arvensis	10.9 ± 0.55	12.8 ± 0.08	0.0294 ± 0.0015	4.48 ± 0.06	26.5 ± 0.03	21.0 ± 0.9	70.0 ± 0.13
M. cordifolia	7.45 ± 0.53	11.3 ± 0.05	0.0016 ± 0.0007	2.58 ± 0.06	4.24 ± 0.03	73.7 ± 0.10	280 ± 0.14
O. americanum	3.67 ± 0.24	5.48 ± 0.05	0.0040 ± 0.0010	5.12 ± 0.04	9.52 ± 0.03	11.1 ± 0.06	43.6 ± 0.04
O. basilicum	5.12 ± 0.45	6.79 ± 0.05	0.0101 ± 0.0012	10.8 ± 0.06	13.3 ± 0.05	30.9 ± 0.10	83.3 ± 0.09
O. gratissimum	10.8 ± 0.79	5.09 ± 0.06	0.0206 ± 0.0018	2.56 ± 0.09	7.94 ± 0.03	24.3 ± 0.08	125 ± 0.11
O. sanctum	4.23 ± 0.21	9.23 ± 0.07	0.0202 ± 0.0014	5.13 ± 0.04	3.18 ± 0.04	40.8 ± 0.10	91.8 ± 0.12
O. grandiflorus	1.99 ± 0.32	7.69 ± 0.07	0.0011 ± 0.0004	3.20 ± 0.06	25.4 ± 0.02	30.9 ± 0.11	145 ± 0.12
Leguminosae							
A. pennata	1.46 ± 0.27	22.3 ± 0.08	0.0015 ± 0.0005	1.27 ± 0.05	1.59 ± 0.04	11.1 ± 0.09	121 ± 0.09
B. purpurea	3.73 ± 0.52	13.9 ± 0.05	0.0103 ± 0.0011	2.55 ± 0.07	3.72 ± 0.04	24.3 ± 0.07	59.5 ± 0.13
C. mimosoides	5.43 ± 0.61	20.1 ± 0.07	0.0105 ± 0.0009	1.92 ± 0.05	5.28 ± 0.04	484 ± 0.13	1924 ± 0.31
C. siamea	1.92 ± 0.50	48.4 ± 0.16	0.0204 ± 0.0014	1.92 ± 0.05	1.59 ± 0.06	110 ± 0.10	384 ± 0.11
L. leucocephala	9.37 ± 0.50	48.5 ± 0.13	0.0202 ± 0.0006	0.64 ± 0.05	3.18 ± 0.03	60.6 ± 0.11	405 ± 0.18
N. oleracea	1.16 ± 0.07	12.9 ± 0.07	0.0066 ± 0.0005	3.18 ± 0.08	1.06 ± 0.02	21.0 ± 0.10	104 ± 0.14
P. mirifica	6.54 ± 0.72	5.63 ± 0.08	0.0011 ± 0.0002	0.63 ± 0.03	0.52 ± 0.04	1.18 ± 0.05	15.8 ± 0.10
T. indica	1.10 ± 0.12	4.96 ± 0.04	0.0015 ± 0.0005	0.64 ± 0.04	1.05 ± 0.04	77.0 ± 0.08	121 ± 0.04
Piperaceae							
P. retrofractum	3.36 ± 0.45	9.37 ± 0.04	0.0053 ± 0.0009	1.28 ± 0.05	5.31 ± 0.03	7.78 ± 0.06	57.5 ± 0.12
P. sarmentosum	13.0 ± 0.84	16.6 ± 0.06	0.0100 ± 0.0006	3.82 ± 0.06	5.81 ± 0.04	17.7 ± 0.06	123 ± 0.12
Umbelliferae							
An. graveolens	1.09 ± 0.21	11.7 ± 0.07	0.0039 ± 0.0002	0.64 ± 0.03	0.53 ± 0.02	7.78 ± 0.06	66.1 ± 0.15
Ap. graveolens	6.39 ± 0.67	6.65 ± 0.07	0.0070 ± 0.0012	1.28 ± 0.05	3.72 ± 0.05	4.49 ± 0.07	31.1 ± 0.14
C. asiatica	7.10 ± 0.62	3.28 ± 0.05	0.0031 ± 0.0005	12.8 ± 0.04	10.6 ± 0.04	24.3 ± 0.06	98.5 ± 0.10
C. sativum	1.41 ± 0.11	11.0 ± 0.06	0.0038 ± 0.0006	2.52 ± 0.03	1.05 ± 0.05	24.3 ± 0.08	33.0 ± 0.14
E. foetidum	5.65 ± 0.46	11.4 ± 0.07	0.0069 ± 0.0007	1.92 ± 0.03	1.60 ± 0.05	17.7 ± 0.08	98.4 ± 0.08
O. stolonifera	7.92 ± 0.93	9.11 ± 0.07	0.0051 ± 0.0009	3.83 ± 0.05	14.8 ± 0.06	34.2 ± 0.09	329 ± 0.19
Zingiberaceae							
A. krervanh	4.00 ± 0.72	2.52 ± 0.05	0.0043 ± 0.0006	1.29 ± 0.04	1.07 ± 0.03	7.77 ± 0.09	46.3 ± 0.13
B. pandurata	0.91 ± 0.16	0.98 ± 0.03	0.0032 ± 0.0004	0.64 ± 0.03	0.52 ± 0.03	4.48 ± 0.07	20.5 ± 0.08
C. xanthorrhiza	0.69 ± 0.08	7.69 ± 0.05	0.0085 ± 0.0006	1.28 ± 0.04	0.65 ± 0.03	14.4 ± 0.08	112 ± 0.13
K. galanga	3.15 ± 0.22	5.37 ± 0.04	0.0035 ± 0.0005	1.91 ± 0.02	1.59 ± 0.02	4.48 ± 0.09	26.4 ± 0.11
L. galanga	3.24 ± 0.53	7.87 ± 0.05	0.0042 ± 0.0011	0.64 ± 0.02	1.05 ± 0.04	17.7 ± 0.05	63.4 ± 0.10
Z. cassumunar	10.9 ± 0.84	13.4 ± 0.04	0.0065 ± 0.0009	1.27 ± 0.04	0.54 ± 0.04	1.18 ± 0.07	83.9 ± 0.13
Z. officinale	2.00 ± 0.75	9.38 ± 0.03	0.0039 ± 0.0006	0.64 ± 0.04	1.06 ± 0.03	11.1 ± 0.10	60.1 ± 0.14
Z. zerumbet	3.00 ± 0.61	5.87 ± 0.05	0.0209 ± 0.0012	1.29 ± 0.07	4.23 ± 0.04	17.7 ± 0.08	79.3 ± 0.13

Typical compounds that possess antioxidant activity have been characterized as vitamin C, vitamin E, carotenoids and phenolic compounds. It was reasonable to investigate their total level in the tested plants. Significant differences ($P < 0.05$) were found in all of these amounts. The content of vitamin C of the plants varied from 0.98 to 48.5 mg%. The highest amount was found in the extract of *Leucaena leucocephala*. *A* high content

of vitamin C was also present in *Cassia siamea* and *Macropanax dispermus*. Correlation between the vitamin C content and the antioxidant index was only found in plants of the families Piperaceae ($R = 0.99$) and Zingiberaceae ($R = 0.63$). The content of vitamin E (mg%) ranged from 0.0011 to 0.0301. The highest amounts were found in the extracts of *G. inodorum* and *M. arvensis*. A correlation between the content of vitamin E and the antioxidant index was observed in several families (Asclepiadaceae, $R = 0.87$; Cucurbitaceae, $R = 0.90$; Labiatae, $R = 0.69$; Leguminosae, $R = 0.39$; and Piperaceae, $R = 0.96$), indicating that vitamin E was likely to contribute to the antioxiant activity of these plants.

Regarding the content of carotenoids (mg%), total carotenes varied from 0.63 to 12.8, and total xanthophylls varied from 0.52 to 26.5. The lowest amount of both carotenoids was found in *Pueraria mirifica*, while *Centella asiatica* and *M. arvensis* contained the highest amounts of total carotenes and total xanthophylls, respectively. In this study, a correlation between the content of total carotenes and the antioxidant index was found in Cucurbitaceae ($R = 0.87$), Piperaceae ($R = 0.99$) and Umbelliferae ($R = 0.46$). However, it can be observed that the amount of total xanthophylls in the plants showed a correlation with the antioxidant index in many families (Araliaceae, $R = 0.98$; Cucurbitaceae, $R = 0.56$; Leguminosae, $R = 0.41$; Piperaceae, $R = 0.98$; Umbelliferae, $R = 0.77$).

Among phenolic compounds (mg%), tannins ranged from 1.18 to 484,and total phenolics from 15.8 to 1924. The plant *Caesalpinia mimosoides* exhibited the highest amount of both tannins and total phenolics, while the lowest amounts of both compounds were observed in the extract of *P. mirifica*. A correlation between the content of tannins and the antioxidant index was only found in the Araliaceae and Piperaceae families ($R = 0.72$ and 0.99, respectively). A correlation between the content of total phenolics and the antioxidant index was found in some families (Asclepiadaceae, $R = 0.94$; Piperaceae, $R = 0.99$; Umbelliferae, $R = 0.57$).

From Table 2, by employing correlation coefficients, it was revealed that vitamin E and total xanthophylls had a relationship with the antioxidant index in five of eight families tested in this study. However, vitamin C, total carotenes, tannins, and total phenolics also showed a relationship with antioxidant index in some families.

Several comprehensive reviews of the literature on natural antioxidants have recently appeared. Many published data highlight the potential role of the phenolic components of fruit, vegetables, beverages, and grain, which may act as antioxidants (Ancos, Gonzáles, & Pilar Cano, 2000; Miliauskas, Venskutonis, & van Beek, 2004; Miranda et al., 2000; Zainol et al., 2003; Zielinski & Kozlowska, 2000). However, the results in this study demonstrated that the plants which contained a high amount of vitamins or carotenoids, also possessed a high level of antioxidant activity. These results are in agreement with those in previously published literature. Larson (1988) reported the roles of many other compounds as potential antioxidants of higher plants (e.g. vitamins C and E, carotenoids, chlorophyll derivatives, alkaloids, flavonoids, phenolic acids and other phenol). A similar finding was reported by Rice-Evans and Miller (1996), who determined the relative antioxidant potentials of vitamins, carotenoids, and phenolics in many kinds of fruit and vegetables. Javanmardi, Stushnoff, Locke, and Vivanco (2003) also found that the antioxidant activity of plant extracts is not limited to phenolics. The activity may also come from the other antioxidant secondary metabolites, such as volatile oils, carotenoids and vitamins.

4. Conclusions

The study shows that there are differences in the contents of antioxidant compounds of the plants commonly consumed in Thailand. Some of the plants can be considered as good sources of natural antioxidants since their extracts were found to possess high antioxitant activity. The highest activity was detected in *G. inodorum*, followed by *P. sarmentosum* and *M. arvensis*, respectively. Inspection reveals that contents of vitamin C, vitamin E, total carotenes, total xanthophylls, tannins and total phenolics in the test plants correlated with the antioxidant index. The results suggest that the antioxidant activities of these plants may be attributed to the chemical components present, especially vitamin E and xanthophylls.

Acknowledgements

The authors thank Asst. Prof. Paritat Trisonthi, Department of Biology, Faculty of Science, Chiang Mai University, for the plant identification. This research was financially supported by the Thailand Research Fund (TRF) through the Royal Golden Jubilee Ph.D. Program.

References

Ancos, B., Gonzáles, E. M., & Pilar Cano, M. (2000). Ellagic acid, vitamin C, and total phenolic contents and radical scavenging capacity affected by freezing and frozen storage in raspberry fruit. *Journal of Agricultural and Food Chemistry, 48*(10), 4565–4570.

Bajaj, K. L., & Kaur, G. (1981). Spectrophotometric determination of L-ascorbic acid in vegetables and fruits. *The Analyst, 106*(1), 117–120.

Contreras-Guzmán, E., & Strong, F. C. III, (1982). Determination of total tocopherols in grains, grain products, and commercial oils, with slight saponification, and by a new reaction with cupric ion. *Journal of Agricultural and Food Chemistry, 30*(6), 1109–1112.

Croft, K. D. (1999). Antioxidant effects of plant phenolic compounds. In T. K. Basu, N. J. Temple, & M. L. Garg (Eds.), *Antioxidants in human health and disease* (pp. 109–112). New York: CABI Publishing.

Hammerschmidt, P. A., & Pratt, D. E. (1978). Phenolic antioxidants of dried soybeans. *Journal of Food Science, 43*(2), 556–559.

Helrich, K. (1990) *Official methods of analysis of the association of official analytical chemist* (15th ed., Vol. 2, pp. 703, 1048–1049). Virginia: Association of Official Analytical Chemists.

Hudson, B. J. F. (1990). *Food antioxidants*. London: Elsevier Applied Science.

Imai, H., Osawa, K., Yasuda, H., Hamashima, H., Arai, T., & Sasatsu, M. (2001). Inhibition by the essential oils of peppermint and spearmint of the growth of pathogenic bacteria. *Microbios, 106*(1), 31–39.

Javanmardi, J., Stushnoff, C., Locke, E., & Vivanco, J. M. (2003). Antioxidant activity and total phenolic content of Iranian *Ocimum* accessions. *Food Chemistry, 83*(4), 547–550.

Kishore, N., Mishra, A. K., & Chansouria, J. P. (1993). Fungitoxicity of essential oils against dermatophytes. *Mycoses, 36*(5–6), 211–215.

Klipstein-Grobusch, K., Launer, L. J., Geleijnse, J. M., Boeing, H., Hofman, A., & Witterman, J. C. (2000). Serum carotenoids and atherosclerosis: the Rotterdam study. *Atherosclerosis, 148*(1), 49–56.

Lampart-Szczapa, E., Korczak, J., Nogala-Kalucka, M., & Zawirska-Wojtasiak, R. (2003). Antioxidant properties of lupin seed products. *Food Chemistry, 83*(2), 279–285.

Larson, R. A. (1988). The antioxidant of higher plants. *Phytochemistry, 27*(4), 969–978.

Lemberkovics, É., Czinner, E., Szentmihályi, K., Balázs, A., & Szöke, É. (2002). Comparative evaluation of *Helichrysi flos* herbal extracts as dietary sources of plant polyphenols, and macro- and microelements. *Food Chemistry, 78*(1), 119–127.

Miliauskas, G., Venskutonis, P. R., & van Beek, T. A. (2004). Screening of radial scavenging activity of some medicinal and aromatic plant extracts. *Food Chemistry, 85*(2), 231–237.

Miranda, C. L., Steven, J. F., Ivanov, V., McCall, M., Frei, B., Deinzer, M. L., & Buhler, D. R. (2000). Antioxidant and prooxidant actions of prenylated and nonprenylated chalcones and flavanones in vitro. *Journal of Agricultural and Food Chemistry, 48*(9), 3876–3884.

Moeller, S. M., Jacques, P. F., & Blumberg, J. B. (2000). The potential role of dietary xanthophylls in cataract and age-related macular degeneration. *Journal of the American College Nutrition, 19*(5), 522s–527s.

Morris, M. C., Bekett, L. A., Scherr, P. A., Hebert, L. E., Bennett, D. A., Field, T. S., & Evans, D. A. (1998). Vitamin E and vitamin C supplement use and risk of incident Alzheimer disease. *Alzheimer Disease and Associated Disorders, 12*(3), 121–126.

Naczk, M., Wanasundara, P. K. J. P. D., & Shahidi, F. (1992). Facile spectrophotometric quantification method of sinapic acid in hexane-extracted and methanol–ammonia–water-treated mustard and rapeseed meals. *Journal of Agricultural and Food Chemistry, 40*(3), 444–448.

Peungvicha, P., Thirawarapan, S., Temsiririrkkul, R., Watanabe, H., Prasain, J. K., & Kadota, S. (1998). Hypoglycemic effect of the water extract of *Piper sarmentosum* in rats. *Journal of Ethnopharmacology, 60*(1), 21–32.

Pratt, D. E. (1992). Natural antioxidants from plant material. In I. M. T. Huang, C. T. Ho, & C. Y. Lee (Eds.), *Phenolic compounds in food and their effects on health* (pp. 54–72). New York: American Chemical Society.

Rahman, N. N. N. A., Furuta, T., Kojima, S., Takane, K., & Mohd, M. A. (1999). Antimalarial activity of extracts of Malaysian medicinal plants. *Journal of Ethnopharmacology, 64*(3), 249–254.

Rice-Evans, C. A., & Miller, N. J. (1996). Antioxidant activities of flavonoids as bioactive components of food. *Transactions and Biochemical Society, 24*(3), 790–795.

Ridtitid, W., Rattanaprom, W., Thaina, P., Chittrakarn, S., & Sunbhanich, M. (1998). Neuromuscular blocking activity of methanolic extract of *Piper sarmentosum* leaves in the rat phrenic nerve–hemidiaphragm preparation. *Journal of Ethnopharmacology, 61*(2), 135–142.

Sami, A. (1995). *Oxidative stress and antioxidant defenses in biology*. New York: Chapman & Hall.

Shimizu, K., Ozeki, M., Iino, A., Nakajyo, S., Urakawa, N., & Atsuchi, M. (2001). Structure–activity relationships of triterpenoid derivatives extracted from *Gymnema inodorum* leaves on glucose absorption. *The Japanese Journal of Pharmacology, 86*(2), 223–229.

Shimizu, K., Ozeki, M., Tanaka, K., Itoh, K., Nakajyo, S., Urakawa, N., & Atsuchi, M. (1997). Suppression of glucose absorption by extracts from the leaves of *Gymnema inodorum*. *The Journal of Veterinary Medical Science, 59*(9), 753–757.

Shon, M. Y., Kim, T. H., & Sung, N. J. (2003). Antioxidants and free radical scavenging activity of *Phellinus baumii* (*Phellinus* of Hymenochaetaceae) extracts. *Food Chemistry, 82*(4), 593–597.

Slattery, M. L., Benson, J., Curtin, K., Ma, K. N., Schaeffer, D., & Potter, J. D. (2000). Carotenoids and colon cancer. *American Journal of Clinical Nutrition, 71*(2), 575–582.

Velioglu, Y. S., Mazza, G., Gao, L., & Oomah, B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural and Food Chemistry, 46*(9), 4113–4117.

Zainol, M. K., Abd-Hamid, A., Yusof, S., & Muse, R. (2003). Antioxidant activity and total phenolic compounds of leaf, root and petiole of four accessions of *Centella asiatica* (L.) urban. *Food Chemistry, 81*(4), 575–591.

Zielinski, H., & Kozlowska, H. (2000). Antioxidant activity and total phenolics in selected cereal grains and their different morphological fractions. *Journal of Agricultural and Food Chemistry, 48*(6), 2008–2016.

Chiang Mai J. Sci. 2005; 32(1) : 53-59
www.science.cmu.ac.th/journal-science/josci.html
Contributed Paper

Antimicrobial Activity of Anise (*Pimpinella anisum* L.) Seed Extracts

Anchana Chanwitheesuk [a], Aphiwat Teerawutgulrag [a], Teerapol Wongchanapiboon [a], Jeremy D. Kilburn [b] and Nuansri Rakariyatham* [a]
[a] Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand.
[b] School of Chemistry, University of Southampton, Southampton, U.K.
*Author for correspondence, e-mail : nuansri1@yahoo.com

Received : 24 May 2004
Accepted : 3 November 2004

ABSTRACT

Different extracts of anise (*Pimpinella anisum* L.) seed were investigated for their antimicrobial activity on several microorganisms including bacteria, yeast, dermatophytic fungi, and plant pathogenic fungi by using disc diffusion method. All extracts (acetone, aqueous, chloroform, and ethanolic extracts) showed strong antibacterial activity against the bacterium *Vibrio cholerae*, while *Pseudomonas aeruginosa* showed resistance to all types of the extracts. Among various extracts, ethanolic extract was found to show the strongest and broadest spectrum of antimicrobial activity as compared to the other extracts. The most sensitive microbial strain to the ethanolic extract of anise seed was the yeast *Candida albicans*, with MIC of 7.81 mg/ml. Bioautographic assay of the ethanolic extract demonstrated the presence of two compounds which are active constituents responsible for antimicrobial activity against the test strains.

Keywords : anise, antimicrobial activity, *Pimpinella anisum*.

1. INTRODUCTION

Anise, *Pimpinella anisum* L. (Umbelliferae), Thai name : Tean-Sut-Ta-Boot, is an annual herb that reaches a height of about 0.5 meters. This plant is a native of the Levant and was consumed by the ancient Egyptians, Greeks, and Romans. It spreads to the rest of southern and central Europe in the Middle Ages, and was sometimes grown in Britain. Today, it is grown commercially in suitably warm climates all over the world, particularly in Southeast Europe, North Africa, and India [1].

Like coriander, the leaves at the base are lobed, but further up the stem they become more finely divided. The flowers are yellowish or white [1]. Anise fruits, commonly called anise seed or aniseed, are tiny, brown, and oval, with an unmistakable flavor of licorice [2]. Chemical studies have demonstrated that the anise seed is composed of essential oil, mainly trans-anethole (80-90%) [3-6], methylchavicol and anisaldehyde [6-7], pseudoisougenol [6], and terpene hydrocarbons [8].

Anise seed has a sweet licorice-like taste, and is warm, fruity and camphoraccous. It is widely used for flavoring curries, savory dishes, soups, breads, cakes, candies, cookies, desserts, such liqueurs as anisette and arrack, and even juice drinks and tea [1, 2, 5, 9]. The volatile or essential oil of the anise seed is valuable in perfumery and soaps, and has been used in toothpaste, mouthwashes, and skin creams [9]. As a medicinal plant, it has been reported that anise has several therapeutic effects. It has been used as a carminative, expectorant, stimulant, and stomachic [3, 4, 9]. It has also been used as a medicine in coughs and pectoral affections. It is greatly used in the form of lozenges, and

the seed has also been used for smoking, to promote expectoration [3]. In addition, it has been used to promote lactation in nursing mothers [9]. Traditionally, Europeans used anise to treat epilepsy and to ward off evil, the Aztecs drank tea made from its flowers and leaves to relieve coughing, while in India, anise seeds are served after meals to aid digestion and sweeten breath [5]. However, there is no information about the antimicrobial activity of this plant extract. The purpose of the present study was to examine antibacterial and antifungal activities of various extracts of the anise seed on several pathogenic microorganisms, which can cause diseases in human and plants.

2. Materials and Methods

2.1 Plant Material and Extraction

Dried seeds of *P. anisum* were obtained from a local market. The plant was identified by Asst. Prof. Paritat Trisonthi, Department of Biology, Faculty of Science, Chiang Mai University, Thailand. The dried seeds (50 g) were extracted with 500 ml of various solvents (distilled water, 95% ethanol, chloroform, and acetone) by soaking at room temperature for 72 h. The crude extracts were filtered, and the filtrates were then evaporated under reduced pressure. The extracts containing 250 mg/ml were prepared in the same solvent of extraction and kept at 4 °C until used.

2.2 Microorganisms

The microorganisms used in the study (Table 1) consisted of 8 strains of human pathogenic bacteria, 1 yeast, and 5 filamentous fungi (including 2 dermatophytic fungi). All the bacterial strains, yeast, and dermatophytic fungi were obtained from the Department of Clinical Microbiology, Faculty of Associated Medical Sciences, Chiang Mai University. The plant pathogenic fungi were obtained from the Department of Plant Pathology, Faculty of Agriculture, Chiang Mai University. The bacteria were grown and maintained on nutrient agar slants, while yeast and fungi on potato dextrose agar slants. The inoculated agar slants were incubated at 37 °C for bacteria and yeast, and at 30 °C for fungi.

2.3 Antimicrobial Activity Test

The antimicrobial activity of the crude extracts was determined by disc diffusion method [10], using bacterial cell suspension which equilibrated their concentration to a 0.5 McFarland standard, or 10^5-10^6/ml of yeast cell or fungal spore suspension. Each bacterial suspension (100 μl) was spread on Mueller-Hinton agar plate, while yeast or fungal suspension on Sabouraud dextrose agar plate. Sterile paper discs, 6 mm diameter, were impregnated with 20 μl of each crude plant extracts. The discs were allowed to dry and then placed on the agar surface of each Petri dish which had previously been inoculated

Table 1. Microorganisms used in this study.

Bacteria	Yeast	Filamentous fungi
Gram negative bacteria	*Candida albicans*	*Aspergillus* sp.
Escherichia coli		*Fusarium* sp.
Klebsiella pneumoniae		*Microsporum gypseum**
Pseudomonas aeruginosa		*Penicillium* sp.
Salmonella typhi		*Trichophyton rubrum**
Vibrio cholerae		
Gram positive bacteria		
Enterococcus faecalis		
Staphylococcus aureus		
S. epidermidis		

* Dermatophytes

Chiang Mai J. Sci. 2005; 32(1)

with the above microorganisms. Discs with the solvents used for extraction were used as negative controls, while two standard antibiotics (10 μg streptomycin discs and 100 units nystatin discs) were used as positive controls. The plates were incubated at 37 °C overnight for bacteria and yeast, or at 30 °C for 3 days for fungi. After incubation, zones of inhibition appearing around the discs were measured and recorded. The values were the average of three measurements per disc, taken at three different directions in order to mistake minimization. The inhibition zones were expressed as the mean of four separation experiments.

2.4 Determination of the Minimum Inhibitory Concentration

Minimum inhibitory concentrations (MICs) were determined by two-fold serial dilution of the extracts which showed antimicrobial activity beyond the level where no inhibition of growth of the test microorganisms was observed.

2.5 Bioautographic Assay

Separation of compounds from the extract of which showed the strongest antimicrobial activity was carried out by two-dimensional paper chromatography [11]. The chromatograms were developed with n-butanol-glacial acetic acid-water (6:1:2 v/v/v) in one direction, and 2% glacial acetic acid in the other direction. The air-dried chromatograms were examined under ultra-violet (UV) light (254 and 365 nm) to indicate the compounds. For bioautography, the compounds on the chromatograms were tested for antimicrobial activity by using the diffusion technique on solid media [12]. The bacteria *Escherichia coli* and *Staphylococcus aureus*, and the dermatophytic fungi *Microsporum gypseum* and *Trichophyton rubrum* were used as test microbial strains. Individual compounds on the chromatograms mentioned above were cut out, and placed on plates of Mueller-Hinton agar or Sabouraud dextrose agar which had been surface spread with 100 μl of microbial suspension. The plates were incubated at 37 °C overnight for bacteria, or 30 °C for 3 days for fungi. Zone of inhibition of the microbial growth could be seen around the active compounds.

3. Results and Discussion

The antimicrobial activity of the anise seed extracts and their potency were assessed quantitatively by the presence or absence of inhibition zone, and zone diameter, respectively as given in Table 2. A total of 14 microorganisms which consisted of 8 bacteria, 1 yeast, and 5 filamentous fungi were tested. Standard antibiotics were used and are mentioned in Table 2. The solvents used for extraction were also used for dissolving the extracts, and all the solvent controls did not show any activity (Table 2). As shown in Table 2, aqueous extract has activity against the bacterium *Vibrio cholerae* only, which is one of the most common of the bacteria causing food poisoning. All of the extracts also showed strong antibacterial activity against *V. cholerae*. However, the anise seed extracts did not inhibit the growth of *Pseudomonas aeruginosa*. Acetone and chloroform extracts also showed activity against some bacteria such as *E. coli*, *Salmonella typhi*, *V. cholerae*, *S. aureus*, *S. epidermidis*, and *T. rubrum*. The growth of *Candida albicans*, which is the microbe responsible for most clinical yeast infections, was inhibited only by ethanolic extract. Moreover, the ethanolic extract showed a broad spectrum of activity against the test microorganisms except the bacteria *P. aeruginosa* and the fungi *T. rubrum*. Based on these results, it is possible to conclude that the ethanolic extract has the strongest and broadest spectrum of antimicrobial activity as compared to the other test extracts. This observation confirmed the evidence in a previous study which reported that alcohol was found to be a better solvent for extraction of antimicrobially active substances [13].

To compare the sensitivity of the microorganisms to the different extracts, MIC values were determined for the extracts which showed antimicrobial activity (Table 3). There

Table 2. Antimicrobial activity of the anise seed extracts (5 mg/disc) against microorganisms tested based on disc diffusion method.

Microorganisms	Inhibition zones in diameter (mm)									
	Anise seed extracts				Negative controls			Positive controls		
	AcE	AqE	CE	EE	Acetone	Water	Chloroform	Ethanol	Streptomycin	Nystatin
Gram negative bacteria										
E. coli	7.25±0.25	-	-	19.75±0.43	-	-	-	-	16.00±0.71	NT
K. pneumoniae	-	-	-	10.00±0.71	-	-	-	-	16.50±0.50	NT
P. aeruginosa	-	-	-	-	-	-	-	-	13.75±0.43	NT
S. typhi	7.00±0.71	-	7.50±0.87	16.50±0.50	-	-	-	-	17.00±0.71	NT
V. cholerae	11.25± 0.43	15.50±0.50	12.25±0.43	25.50±0.50	-	-	-	-	20.75±0.43	NT
Gram positive bacteria										
E. faecalis	-	-	-	11.75±0.83	-	-	-	-	19.00±0.71	NT
S. aureus	9.25±0.43	-	14.00±0.71	23.25±0.43	-	-	-	-	16.25±0.43	NT
S. epidermidis	8.50±0.50	-	13.25±0.43	13.50±0.50	-	-	-	-	20.50±0.50	NT
Yeast										
C. albicans	-	-	-	13.75±0.43	-	-	-	-	NT	26.25±0.43
Filamentous fungi										
Aspergillus sp.	-	-	-	8.50±0.87	-	-	-	-	NT	21.50±0.50
Fusarium sp.	-	-	-	14.00±0.71	-	-	-	-	NT	16.00±0.71
M. gypseum	-	-	-	40.25±0.43	-	-	-	-	NT	22.75±0.43
Penicillium sp.	-	-	-	7.50±0.50	-	-	-	-	NT	18.25±0.83
T. rubrum	-	-	9.50±0.50	-	-	-	-	-	NT	18.50±0.50

Values are mean±SD (mm) of four separate experiments; AcE : acetone extract; AqE : aqueous extract; CE : chloroform extract; EE : ethanolic extract; - : no inhibition zone; NT : not tested.

Chiang Mai J. Sci. 2005; 32(1)

Table 3. MIC values in mg/ml of the anise seed extracts against test microorganisms.

Microorganisms	MICs (mg/ml)			
	AcE	AqE	CE	EE
Gram negative bacteria				
E. coli	250.00	-	-	31.25
K. pneumoniae	-	-	-	31.25
P. aeruginosa	-	-	-	-
S. typhi	250.00	-	250.00	15.63
V. cholerae	62.50	125.00	250.00	15.63
Gram positive bacteria				
E. faecalis	-	-	-	250.00
S. aureus	250.00	-	250.00	31.25
S. epidermidis	250.00	-	125.00	31.25
Yeast				
C. albicans	-	-	-	7.81
Filamentous fungi				
Aspergillus sp.	-	-	-	125.00
Fusarium sp.	-	-	-	31.25
M. gypseum	-	-	-	15.63
Penicillium sp.	-	-	-	125.00
T. rubrum	-	-	15.63	-

AcE : acetone extract; AqE : aqueous extract; CE : chloroform extract; EE : ethanolic extract; - : no inhibition zone

were substantial differences between the MICs of the different extracts. The activity of the ethanolic extract against the yeast was higher than the other microorganisms tested. This extract displayed the lowest MIC (7.81 mg/ml) against the yeast *C. albicans*. The Gram-positive bacteria *Enterococcus faecalis* was the most resistant to the extract since the highest MIC (250 mg/ml) was observed. The ethanolic extract was also active against the filamentous fungi except *T. rubrum*.

The results in Tables 2 and 3 indicate that the ethanolic extract of anise seed has considerable *in vitro* activity against most of the test microbial strains. Thus, this extract was analyzed for active compounds by means of bioautography with two bacterial strains (*E. coli* and *S. aureus*), and two dermatophytic fungi (*M. gypseum* and *T. rubrum*). Two-dimensional paper chromatograms presented 3 spots of compounds as shown in Table 4. Bioautography of these compounds worked well with *S. aureus*, but did not affect the other test microbes. Two of three spots on the

Table 4. R_f values, color under UV light, and antimicrobial activity of the compounds from the ethanolic extract of anise seed against the bacterium *S. aureus*.

Spot No.	R_f values in solvent system*		Color under UV light		Activity against
	a	b	254 nm	365 nm	*S. aureus*
1.	0.65	0.08	Bright blue	Bright blue	+
2.	0.58	0.48	Bright blue	Bright blue	-
3.	0.70	0.80	Bright blue	Bright blue	+

* Solvent systems : a = n-butanol-glacial acetic acid-water (6:1:2 v/v/v); b = 2% glacial acetic acid

chromatogram showed inhibition zone against *S. aureus* indicating the presence of active compounds (Figure 1). Thus, the compounds spot 1 and 3 seem to be active components for antimicrobial activity. Preliminary identification of the constituents in the compounds was carried out by gas chromatography and gas chromatography-mass spectrometry compared with standard anethole, methylchavical, and anisaldehyde (data not shown). The result showed that both compounds were not the chemicals mentioned above.

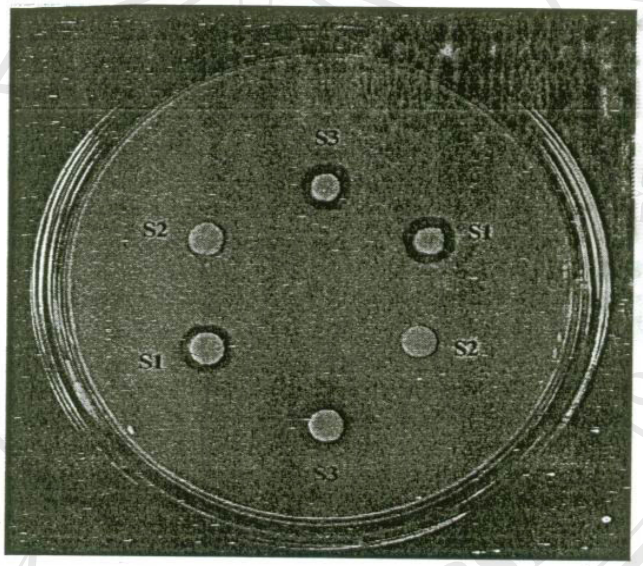


Figure 1. Antimicrobial activity of the compounds from the ethanolic extract of anise seed against the bacterium *S. aureus* (S1: spot 1; S2: spot 2; and S3: spot 3 on the chromatogram).

4. Conclusion

The present investigation confirms that there are antimicrobial properties in the crude extracts of anise seed against several different bacterial and fungal strains. The ethanolic extract showed the strongest and broadest spectrum of antimicrobial activity as compared to the other extracts tested in this study. However, the identity of compounds in the extract is still unclear. Therefore, our future aim is to purify and identify the active compounds from anise seed, and explore the potential for their use as an antimicrobial drug.

Acknowledgements

The authors wish to thank the Department of Clinical Microbiology, Faculty of Associated Medical Sciences, Chiang Mai University, and the Department of Plant Pathology, Faculty of Agriculture, Chiang Mai University for providing some of the microorganisms used in this study. This research was financially supported by the Thailand Research Fund (TRF) through the Royal Golden Jubilee Ph.D. Program.

References

[1] Stobart T., *Herbs, Spices and Flavorings*, Harmondsworth: Peguin, 1977.

[2] Mawson M., *Herb and Spice Cookery*, London: Hamlyn, 1972.

[3] Grieve M., *A Modern Herbal: The Medical, Culinary, Cosmetic, and Economic Properties, Cultivation, and Folklore of Herbs, Grasses, Fungi, Shrubs, and Trees With All Their Modern Scientific Uses*, New York: Hafner Press, 1974.

[4] Flück H., *Medicinal Plants and Their Uses*, London: W. Foulsham, 1988.

[5] Uhl S.R., *Handbook of Spices, Seasonings, and Flavorings*, Lancaster, Pa.: Technomic Pub. Co., 2000.

[6] Rodrigues V.M., Rosa P.T., Marques M. O., Petenate A.J. and Meireles M.A., Supercritical Extraction of Essential Oil from Aniseed (*Pimpinella anisum* L.) Using CO_2: Solubility, Kinetics, and Composition Data, *J. Agric. Food Chem.*, 2003; **51**: 1518-1523.

[7] Wagner H., Baldt S. and Zgainski E.M., *Plant Drug Analysis*, New York: Springer Verlay, 1984.

[8] Brukhardt G., Reichling I., Martin R. and Becker H., Terpene Hydrocarbons in *Pimpinella anisum*, *Pharm. Weekly Sci.*, 1986; **8**: 190-193.

[9] Simon J.E., Chadwick A.F. and Craker L.E., *Herbs : An Indexed Bibliography, 1971-1980, The Scientific Literature on Selected Herbs, and Aromatic and Medicinal Plants of the Temperate Zone*, Hamden, CT.: Archon Book, 1984.

[10] Bauer A.W., Kirby W.M.M., Sherris J.C. and Truck M., Antibiotic Susceptibility Testing by a Standardized Single Disc Method, *Am. J. Clin. Pathol.*, 1966; **45**: 493-496.

[11] Raymond J., Rakariyatham N. and Azanza J.L., Polyphenol-protein Interactions in Sunflower Seed (*Helianthus annuus*), *Sci. Des. Ali.*, 1984; **4**: 95-108.

[12] Betina V., Bioautography in Paper and Thin-layer Chromatography and Its Scope in the Antibiotic Field, *J. Chromatogr.*, 1973; **78**: 41-51.

[13] Ahmad I., Mehmood Z. and Mohammad F., Screening of Some Indian Medicinal Plants for Their Antimicrobial Properties, *J. Ethnopharmacol.*, 1988; **62**: 183-193.

VITA

Name Miss Anchana Chanwitheesuk

Date of birth September 5, 1974

Place of birth Ratchaburi, Thailand

Education 1996: B. S. (Microbiology) Chiang Mai University

2001: M. S. (Biotechnology) Chiang Mai University

2005: Ph. D. (Biotechnology) Chiang Mai University

Poster presentation

1. **Chanwitheesuk, A.**, Rakariyatham, N., and Teerawutgulrag, A. 2002. Phenolic compounds from ivy gourd (*Coccinia grandis* Voigt.). RGJ-Ph.D. Congress III, 25-27 April 2002, Jomthien Palm Beach and Resort, Pattaya, Chonburi, Thailand.
2. **Chanwitheesuk, A.**, and Rakariyatham, N. 2002. Phenolic compounds from *Dregea volubilis* Stapf. (Pak-Huan-Mu). 28th Congress on Science and Technology of Thailand, 24-26 October 2002, Queen Sirikit National Convention Center, Bangkok, Thailand.
3. **Chanwitheesuk, A.**, and Rakariyatham, N. 2002. Preliminary study of antibacterial activity of *Coccinia grandis* Voigt. (ivy gourd). The 14th Annual Meeting of the Thai Society for Biotechnology: Biotechnology for Better Living in the New Economy, 12-15 November 2002, Hotel Sofitel Raja Orchid, Khon Kaen, Thailand.

4. **Chanwitheesuk, A.**, Teerawutgulrag, A., Wongchanapiboon, T., and Rakariyatham, N. 2003. Antioxidant: anti-cancer agent in Thai vegetables and herbs. The 3rd World Congress on Medicinal and Aromatic Plants for Human Welfare (WOCMAP III): From Biodiversity through Science and Technology, Trade and Industry to Sustainable Use, 3-7 February 2003, the Lotus Hotel Pang Suan Kaew, Chiang Mai, Thailand.

Publication

1. **Chanwitheesuk, A.**, Teerawutgulrag, A., and Rakariyatham, N. 2005. Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand. *Food Chemistry*. 92(3), 491-497.
2. **Chanwitheesuk, A.**, Teerawutgulrag, A., Wongchanapiboon, T., Kilburn, J. D., and Rakariyatham, N. 2005. Antimicrobial activity of anise (*Pimpinella anisum* L.) seed extracts. *Chiang Mai Journal of Science*. 32(1), 53-59.

Scholarship

2001-2005 The Royal Golden Jubilee Ph. D. project, Thailand Research Fund, Bangkok, Thailand.