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Chemical Profiling of Essential Oil Composition and Biological Evaluation of *Anethum graveolens* L. (Seed) Grown in Thailand

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Abstract
In this study, the essential oil of *Anethum graveolens* L. seed (AEo) was studied for their chemical composition, antioxidant potential, antimicrobial and anticancer activities. AEo was obtained from hydro-distillation and their composition was analyzed by GC-MS. The major compositions of AEo are *D*-carvone, carvone, dill apiol and limonene. AEo was investigated for antioxidant activity by DPPH, ABTS, FRAP assay and measured total phenolic content by Folin-Ciocalteu colorimetric method. Agar-well diffusion assay was used to study the anti-microbial activity and also agar-dilution and broth-micro dilution techniques were employed for minimum inhibitory concentration (MIC) determination. In addition, the cytotoxicity and anticancer activity were evaluated on Green fluorescent protein (GFP) and Resazurin micro plate assay (REMA). The results showed that AEo exhibited high total phenolic content (GAE= 4.5746 mg/mL) and antioxidant activities on DPPH (TEAC= 52.5391 mg/mL), ABTS (TEAC= 1.5936 mg/mL) and FRAP assay (TEAC= 0.5469 mg/mL) and also showed potent activity against *Staphylococcus aureus* and *Escherichia coli* at the MIC= 5.99 µg/mL. Furthermore, AEo presented non-cytoxicity in normal cell whereas it exhibited greatly anti-cancer activity on KB-Oral cavity and MCF7-Breast cancer cells.
Keywords: *Anethum graveolens* L., antioxidant activity, antimicrobial activity, anticancer activity, chemical compositions

1. Introduction
Anethum graveolens L. is known in Thai as Pakk Chi Lao which is a member of the Umbelliferae family. This medicinal plant has been extensively cultivated in the northern and northeastern regions of Thailand for household use. The fresh aerial part of plant is used as an edible vegetable and also used as flavor in local cuisine. Moreover, the plant seeds are widely used as spice and an ingredient in Thai traditional medicines. In addition, local wisdom has taught that the seeds can be used for the fermented food to prevent food spoilage. Previous research proved the potential biological activities, e.g., antimicrobial and antioxidant, of *A. graveolens* L. seeds. The essential oil from *A. graveolens* L. seeds inhibited gram-positive and gram-negative bacteria including yeast and mold as well (Abed, 2007; Badar *et al.,* 2008; Delaquis *et al.,* 2002; Lopez *et al.,* 2005). *D*-limonene and *D*-carvone which are chemical compositions exhibited strong activity against *Aspergillus niger*, *Saccharomyces cerevisiae* and *Candida albicans* (Delaquis *et al.,* 2002; Jirovetz *et al.,* 2003; Stavri and Gibbons, 2005). Moreover, some parts of the plant such as the leaf and seeds, including their essential oils, showed good antioxidant activity (Kmiecik *et al.,* 2001; Mohammad and Aburijai, 2004; Singh *et al.,* 2005). The flower extract of *A. graveolens* L. exhibited higher antioxidant potential than leaf and seed extracts (Shyu *et al.,* 2009). Furthermore, methanolic extract of *A. graveolens* L. exhibited activities against tumor cell lines MK-1, HeLa as well as B16F10 (Yazdanparast and Alavi, 2001). The chemical constituents in *A. graveolens* L., e.g., alpha-terpineol, alpha-tocopherol, caffeic acid, hyperoside, iso-quercetin, kaempferol, limonene and rutin showed anti-cancer activity while chlorogenic acid and furulic acid exhibited anti-liver cancer cells as well (Sathya and Gopalakrishnan, 2012). However, the differences in biological activity and chemical composition of the plant may be dependent on many factors, i.e., plant part, harvest time, type of cultivar, geographic origin, storage conditions, extraction method, etc. (Charles *et al.,* 1995; Delaquis *et al.,* 2002; Faber *et al.,* 1997). Therefore, the aim of this research was to investigate chemical constituents and antioxidant, antimicrobial including anticancer activities of AEo that are grown in Thailand.

2. Material and Methods:
2.1 Plant materials
The seeds plant material of *A. graveolens* L. was collected from Khon Kaen Province during November 2011 to January 2012. The identification of plant material was verified by J.F. Maxwell, a taxonomist. The voucher

specimens were deposited in CMU Herbarium at the Department of Biology, Faculty of Science, Chiang Mai University (N. Phoowiang No.7).

2.2 Essential oil extraction

The essential oil from *A. graveolens* L. seeds (AEo) was obtained by hydro-distillation. Then, anhydrous sodium sulfate was used to dry the essential oil, and was placed in a brown bottle that protected it from light. After that, it was stored in the refrigerator for further analysis

2.3 Determination of chemical composition

The concentration of AEo (0.5%) was prepared in ethanol analytical reagent grade. SHIMADZU® GC-2010 instrument with AOC-500 auto injector as well as the flame ionization detector (FID) and GCMS-QP2010 Plus equipment were used for analysis. The injector was set at 180 °C of temperature. The sample solution was separated by DB-5MS capillary column (Agilent Technology), length 30 m, i.d. 0.25 mm, film thickness 0.25 µm of 5% phenylmethypolysiloxane. One microliter of AEo solution was auto injected into the injector port with split ratios of 50:1 and using helium as a carrier gas at the flow rate 1.00 mL/min. The temperature program of reparation was initially created from 60-200 °C at the rate differential as follows: the temperature was started from 60-90 °C at the rate 5 °C/min, next ramped to 95 °C (1°C/min) continuously to 180 °C (5°C/min) after that the temperature was increased to 180 °C at 1°C/min and also successively to 200 °C (10°C/min) with 5 min hold. The identification of volatile components was based on computer matching with WILLEY 7 library as well as by comparison of the mass spectra and Kovat retention indices (KI) with a series of *n*-alkanes and previous information literature (Adam, 1995; NIST web book).

2.4 Determination of total phenolic content

The Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965) was used for examination of the total phenolic content. The sample solution of AEo (250 µL) was mixed with the diluted Folin-Ciocalteau reagent and distilled water in the ratio of 1:10 (2.5 mL). Then, 7.5% of sodium carbonate was added and incubated in the dark at room temperature. After that, measurement was done at 765 nm by spectrophotometer (SHIMADZU® UV-2450) and gallic acid equivalent value (GAE mg/mL) was calculated and compared with the dilution curve of gallic acid standard.

2.5 Determination of antioxidant activities

2.5.1 The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay:

The antioxidant activity by DPPH radical scavenging assay was investigated following the method describe by Wu *et al.,* 2005. The solution of DPPH radical was prepared in ethanol and measured at the wavelength 517 nm (absorbance ≤1.00±0.02). Next, triplicate of the different sample concentrations (20 µL) were transferred into 96-well micro titer plate. Then, the DPPH radical solution was added into each well except blank and shaken, and then left in the dark at room temperature (30 min). After that, measurement of the absorbance (517 nm) and calculation of the percentage of inhibition compared with trolox standard as the formula was undertaken:

$$\%\ \text{Inhibition} = \frac{((A_{test}-A_{Blank})-(A_{s\text{-}test}-A_{s\text{-}Blank}))}{(A_{test}-A_{Blank})} \times 100$$

Where A_{test} is the absorbance of only free-radical solution, A_{Blank} is the absorbance of ethanol which replaces free-radical solution, $A_{s\text{-}test}$ is the absorbance of sample mixed with free-radical solution and $A_{s\text{-}Blank}$ is the absorbance of sample mixed with ethanol. The result was compared with trolox standard and interpreted in terms of Trolox equivalence antioxidant capacity value (TEAC mg/mL).

2.5.2 The 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radial scavenging assay:

The ABTS radical scavenging assay was tested by the modified method from Re *et al.,* 1999. The ABTS radical solution was prepared in the ratio of 2:1 by using ABTS radical solution in water (7 mM) and potassium persulfate solution (2.45 mM). The mixture was stored in the dark at room temperature for 12 hours. Next, the solution was diluted with ethanol and the absorbance was measured at the wavelength 734 nm (absorbance = 0.70-0.90±0.05) before use. Then, 20 µL of the different sample concentrations were added into test tubes and mixed with 80 µL of ethanol including 2 mL of ABTS radical solution. The mixture was left at room temperature for 5 minutes and the absorbance was detected (734 nm). The percentage of inhibition was calculated and compared with trolox standard using the same formula as above.

2.5.3 Ferric reducing antioxidant power (FRAP) assay:

The FRAP assay was monitored with some method modifications of Benzie and Strain, 1996. A freshly-prepared FRAP reagent was done by using a mixture of 200 mL of acetate buffer (300 mM, pH 3.6), 20 mL of TPTZ (2,4,6-tripyridyl-s-triazine) solution (10 mM TPTZ in 40 mM of HCl) and 20 mL of ferric chloride solution (20 mM). The mixture was incubated at 37° C before use. Ten micro liter of sample solution was mixed with 190 µL of FRAP reagent in 96-well micro titer plate, then it was set aside in the dark at room temperature for 30 minutes. Finally, the absorbance was measured at wavelength 593 nm (Beckman® Coulter/DTX880). The linear equation of trolox standard (50-1000 µM) was compared and interpreted in terms of trolox equivalence

antioxidant capacity value (TEAC mg/mL).

2.6 Determination of antimicrobial activity

2.6.1 Microbial strains:

Five species of microorganisms were used in this research, namely, bacteria *Staphylococcus aureus* ATCC25923 (*S*), *Pseudomonas aeruginosa* ATCC27853 (*P*), *Escherichia coli* ATCC25922 (*E*), yeast *Candida albicans* ATCC90028 (*C*) and mold *Aspergillus flavus* (*A*) isolated strain. These microbial strains were obtained from the culture collection of the Department of Medical Technology, Faculty of Associated Medical Sciences, Chiang Mai University. The turbidity of each microorganism was adjusted to match with the turbidity of McFarland No. 0.5 standard. The microorganism concentration was approximately 10^8 CFU/mL. This concentration was used for inhibition zone investigation and suspended in trypticase soy broth (TSB) for bacteria and sabouraud dextrose *broth (SDB)* for fungus. In addition, the diluted microbial strains at 10^6 CFU/mL (bacteria) and 10^4 spore/mL (fungus) were used to determine minimum inhibitory concentration of both dilution techniques.

2.6.2 Agar-well diffusion test:

Agar-well diffusion method was tested for microbial sensitivity compare to antibiotics. The method was described by Bouhdid *et al.,* 2008 with some modification as follows: the first layer of solid medium was prepared from the mixture of TSA (10 mL) and 10^8 CFU/mL of bacteria strains (1 mL). Next, the steriled 12 mm diameter cylinders were deposited, then the same fresh preparation mixture medium was transferred and left until it became medium solidified, then the cylinders were removed. On the other hand, fungus was used for SDA which replaced TSA using the same preparation method as above. After that, 100 µL of pure AEo were poured into the well and incubated at 37° C for 24 hours for bacteria and 25° C for 72 hours for fungus. Finally, the zones of inhibition were measured in millimeters. Gentamicin (75 µg/mL) and Ketoconazole (250 µg/mL) were used as standards.

2.6.3 Agar-dilution test:

Agar-dilution technique was used for determination of minimum inhibitory concentration (MIC) of bacteria strains. The procedures followed NCCLS, 1997b with some modifications. The method of all tests was arranged in TSA mixed with AEo at serial dilution ranging from 0.01-0.20 mg/mL which were transferred into a 24-well micro titer plate. Then, 2 µL of each bacteria strain (10^4CFU) was dropped and evenly spread on the surface mixture solidification medium. After that, the micro plates were inoculated at 37° C for 24 hr and the growth of microorganism was observed.

2.6.4 Broth-micro dilution test:

Broth-micro dilution method was used for the MIC investigation of fungus. The method was described according to the NCCLS, 1997c with some modifications. The essential oil (0.50 mg/mL) was prepared in dimethylsulfoxide (DMSO). Fifty micro liter of medium (SDB) was transferred into 96-well micro titer plate. Next, the highest concentration of AEo (50 µL) was dispensed in the first column wells. Then, the mixture (50 µL) was diluted two-fold serially from the first column wells onto the second column wells and so on, and continued until the final concentration was achieved and removed (50 µL). After that, 10^4 spore/mL of fungus suspension (50 µL) was added in all column wells with the final volumes being 100 µL per well. The positive control used fungal inoculation only and negative control used the blank without fungus in the two last wells. Finally, the mixture was incubated at 25° C for 72 hours and then, the growth of fungus was observed. The MIC was then determined to have the lowest AEo concentration.

2.7 Determination of cytoxicity and anticancer activities:

Green fluorescent protein (GFP) detection was used in the investigation of the cytotoxicity of AEo. This method was done by Hun *et al*., 1999 with some modifications. Vero cells of African green monkey kidney cell line were used to evaluate the efficiency of AEo. The sample was prepared in 0.5% DMSO, the amount of AEo solution 5 µL was transferred into 96-wells micro titer plate. Next, 190 µL of the GFP-expressing Vero cells line ($3.3x10^4$ cells/mL) was added. Then, the mixture was incubated at 37° C and 5% CO_2 for 4 days. After that, the fluorescence signal detection was measured by SpectraMax M5 Multi-mode Microplate Reader at excitation and emission wavelengths of 458 and 535 nm. The result was reported in terms of IC_{50} in the percentage unit (≤50% cell growth included). Ellipticine (1.06 mg/mL) and DMSO (0.5%) were used as positive and negative controls, respectively. Resazurin microplate assay (REMA) was investigated to assess the anticancer activity of AEo on KB-Oral cavity cancer, NCI-H187-Small cell lung cancer including MCF7-Breast cancer as well. This method was performed according to the procedure of Brien *et al.*, 2000. All of logarithmic growth phase cancer cells were harvested and diluted at concentrations of $7x10^4$ cells/mL for KB-Oral cavity cancer and $9x10^4$ cells/mL for NCI-H187-Small cell lung cancer as well as MCF7-Breast cancer, respectively. Next, 5 µL of each suspension cancer cells were mixed with 5 µL of the diluted AEo which was prepared in 0.5% DMSO in micro titer plate 384-wells. Then, the mixture was incubated at 37° C and 5% CO_2 (3 days for KB-Oral cavity cancer and MCF7-Breast cancer while 5 days for NCI-H187-Small cell lung cancer). After that, 12.5 µL of resazurin solution (62.5 µg/mL) was added into each well and continuously incubated (37° C) for 4 hours. Finally,

measurement of the fluorescence signal was done by using SpectraMax M5 Multi-mode Microplate Reader at excitation and emission wavelengths 530 nm and 590 nm, respectively. The result was calculated and interpreted in terms of percentage unit of IC_{50} (≥50% Inhibition included). Ellipticine (0.822 µg/mL and 0.781 µg/mL) and Doxorubicin (0.593 µg/mL and 0.087 µg/mL) were used as positive controls of two cancer cells (KB-Oral cavity cancer and NCI-H187-Small cell lung cancer) while Tamoxifen (9.51 µg/mL) and Doxorubicin (9.20 µg/mL) were used for positive control of MCF7-Breast cancer. In addition, 0.5% DMSO was used as negative control in all experiments.

3. Results and discussion

Anethum graveolens L. seeds showed 1.47% of AEo yield which was extracted by hydro-distillation. The chemical components of AEo were identified by using GC-MS technique, the retention indices and their percentages of composition are presented in Table 1. The results showed that AEo composed of 18 constituents. Major component groups revealed monoterpene hydrocarbon (38.83%) and oxygenated monoterpene (38.83%) and aromatic ether groups (16.67%). The highest percentage of relative content revealed *D*-carvon (32.94%), carvone (20.73%), dill apiol (19.64%) and limonene (18.08%) as majority of components. Comparative to previous research, the result found that the chemical composition showed similar in chemical pattern but different in relative quantity of chemical compounds found in the essential oil. The essential oil of *A. graveolens* L. seed that grew in Romania and Estonia exhibited two components, carvone (45.9-75.2%) and limonene (18.4-21.6%), as major (Radulescu *et al.,* 2010; Vokk *et al.,* 2011). Some research indicated that *D*-carvone (36.09%), Limonene (19.89%), dill apiol (16.83%), *E*-dihydrocarvone (7.36%) and *Z*-dihydrocarvone (6.59%) were present as major compounds (Mahmoodi *et al.,* 2012). Besides, Embong and co-worker (1977) reported that carvone, limonene and phellandrene revealed up to 90% as main compounds in *A. graveolens* L. seed that cultivated in Alberta Canada (Embong *et al.,* 1977). The difference of their chemical compositions may depend on many factors such as cultivation, growth stage, weather condition, etc. (Hay *et al.,* 1988; Svoboda and Hay, 1990). However, most chemical constituents found from the essential oil were mainly terpenes which were generally several of mono-, di- and sesqui-terpene (Hay *et al.,* 1988; Svoboda and Hay, 1990). Terpenes or terpinoids have shown activities on antimicrobial, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, anti-inflammatory and immunomodulatory properties and also have been found to be useful in prevention and therapy of several diseases including cancer (Paduch *et al.,* 2007). Moreover, they all act as natural insecticides which can be used as insecticidal substances for protecting agriculture products (Theis and Lerdau, 2003). The information of chemical constituents of the essential oils can be used for quality controlling of the raw materials use in further process for pharmaceutical product development.

Table 1 Chemical compositions of AEo

No.	Rt.	Relative content (%)	Compound	Groups	KI[a]	KI[b]
1	5.527	0.09	Sabinene	Monoterpene hydrocarbon	979	979
2	5.812	0.07	*β*-Myrcene	Monoterpene hydrocarbon	992	992
3	6.231	0.47	*1,5,8-p*-Menthatriene	Monoterpene hydrocarbon	1010	1007
4	6.695	0.19	*β*-Cymene	Monoterpene hydrocarbon	1029	1026
5	6.809	**18.08**	Limonene	Monoterpene hydrocarbon	1033	1037
6	7.586	0.06	*γ*-Terpinen	Monoterpene hydrocarbon	1061	1062
7	8.63	0.13	*p*-Cymenene	Monoterpene hydrocarbon	1095	1096
8	10.269	0.04	*cis*-Limonene oxide	Oxygenated monoterpene	1141	1140
9	12.583	0.07	Dill ether	Oxygenated monoterpene	1194	1193
10	13.024	5.81	*trans*-Dihydrocarvone	Oxygenated monoterpene	1204	1202
11	13.306	**20.73**	Carvone	Oxygenated monoterpene	1213	1208
12	13.819	0.39	Isodihydrocarveol	Oxygenated monoterpene	1228	1223
13	14.335	0.67	Carveol	Oxygenated monoterpene	1242	1246
14	14.825	**32.94**	*D*-Carvone	Oxygenated monoterpene	1256	1254
15	23.18	0.08	Myristicin	Aromatic ether	1529	1523
16	23.846	0.05	*β*-Asarone	Aromatic ether	1556	1559
17	24.858	0.16	Diethyl Phthalate	Aromatic ester	1595	1585
18	25.635	**19.64**	Dill apiol	Aromatic ether	1629	1625
		100.00				

Notes: [a] Relative retention indices: *n*-alkanes (C_8-C_{20}) as reference points were used for relative retention indices calculation.

[b] Relative retention indices from reference which were previously reported (Adam, 1995; NIST web book).

The quantity of total phenolic content of AEo was monitored by spectrophotometer, using Folin-Ciocalteu reagent and compared with gallic acid standard. The result was calculated and reported in terms of gallic acid equivalence (GAE mg/mL). As well, the antioxidant activities were proved by DPPH, ABTS and FRAP assays. The estimations of antioxidant scavengers were compared with trolox standard and reported in terms of trolox

equivalence antioxidant capacity (TEAC mg/mL). The results clearly indicated that the total phenolic content and antioxidant activities were present in AEo. The amount of GAE was 4.5746 mg/mL and also showed TEAC values on DPPH, ABTS and FRAP assays which were 52.5391, 1.5936 and 0.5469 mg/mL, respectively (Table 2). The total phenolic content corresponds to chemical composition of essential oil that contained phenols group in molecules (Houlihan *et al.,* 1985; Wu *et al.,* 1982) which resulted in potential of sample to antioxidant and antimicrobial activities (Nakiatini and Kikuzaki, 1987). Shahidi and Wanasumdara in 1992 reported that one group of phytochemicals, i.e., phenolic compound, could act as a radical scavenger in medicinal plants and had been used to prevent various diseases. AEo showed higher antioxidant activity on DPPH, followed by ABTS and FRAP assay, respectively. The difference of anti-oxidative values on their assay may be due to the ability of bioactive compounds in AEo. Cao and co-worker in 2009 described that the oxygenated monoterpene and monoterpene hydrocarbon are the principal antioxidant compounds in the essential oil from plant. Then, various factors like stereoselectivity of the radicals or the solubility of the tested sample in different testing systems and functional groups present in bioactive compounds have been reported to affect the capacity of the sample to react and quench different radicals (Adedapo *et al.,* 2008). However, the result indicated that AEo showed ability of antioxidant in all assays which related to the mechanisms of hydrogen atom and electron transfer and also showed capability of reducing agent. Quantity of total phenolic compound and antioxidant potential of AEo have shown the efficiency of *A. graveolens* L. seed which proves the advantage and usefulness of folk wisdom.

Table 2 Total phenolic content and antioxidant activities of AEo.

Sample	**Total phenolic content (GAE mg/mL)**	**Antioxidant activities (TEAC mg/mL)**		
		DPPH assay	**ABTS assay**	**FRAP assay**
AEo	4.5746 ±0.0046	52.5391 ±0.0088	1.5936 ±0.0012	0.5469 ±0.0047

The diameter zone of inhibitions and the lowest concentration of AEo had an effect on the visible growth of microorganisms as shown in Table 3. The results presented that AEo exhibited against all microorganisms except *P. aeruginosa*, the size of inhibition zones ranged from 16-30 mm. Width of zone of AEo-suppressed *S. aureus* was equal to gentamicin (20 mm vs. 20 mm) standard and also showed the widest zones on *A. flavus* and *C. albicans* (30 mm, 27.5 mm) which were wider than ketoconazole standard (17 mm, 23 mm). The MIC of AEo ranged from 5.99-59.47 µg/mL, with the lowest concentration of AEo at 5.99 µg/mL which could restrain the gram-positive and gram-negative bacteria (*S. aureus* and *E. coli*). The AEo exhibited higher activity to restrain microorganism than the previous research of Nanasombat and Wimuttigosol in 2011 which reported that the essential oil from seed of *A. graveolens* L. revealed MIC to *S. aureus, E. coli* and *A. flavus* at 6, 10 and 2 mg/mL. Then, capability of AEo against *C. albicans* (29.78 µg/mL) corresponds with earlier study which reported that this essential oil showed activity of inhibition in the six isolates of *C. albicans* (MIC = 0.626 µL/mL) (Zeng *et al.,* 2011). The antimicrobial activity of AEo may be due to major components that contained in essential oil. Previous researches reported that *D*-carvone and limonene have exhibited strong antifungal activity against *Aspergillus niger, Saccharomyces cerevisiae* and *Candida albicans* (Jirovetz *et al.,* 2003; Stavri and Gibbons, 2005). Besides, dill apiole showed specific inhibitor to inhibit aftoxinx G1 production by *Aspergillus parasiticus* (Razzaghi-Abyaneh *et al.,* 2007). Former reports indicated that the membrane structure of microbial strains was damaged by their lipophobic character of cyclic monoterpene which permeates from aqueous phase into membrane structure and consequently increased membrane fluidity and expansion and also inhibition of a membrane-embedded enzyme inhibition (Andrews *et al.,* 1980; Sikkema *et al.,* 1995). In addition, Faleiro in 2011 compiled and summarized the action of antimicrobial agent of essential oil as follows: one of characteristics of essential oil that is important to antimicrobial activity is hydrophobicity character which increases cell membrane permeability and causes leaking of cell constituent. The results can estimate the capability of *A. graveolens* L. seed corresponding to the fermented food preservation in some regions of Thailand.

Table 3 Zone of inhibition and minimum inhibitory concentration of AEo.

Sample	**Zone of inhibition (mm)**					**Minimum inhibitory concentration (µg/mL)**				
	S	***E***	***P***	***C***	***A***	***S***	***E***	***P***	***C***	***A***
AEo.	20	16	In	27.5	30	5.99	5.99	ND	29.78	59.47
Gentamicin	20	25	20	ND	ND	0.31	0.63	1.25	ND	ND
Ketoconazole	ND	ND	ND	23	17	ND	ND	ND	15.63	7.81

In= Inactive, ND= Not detected,

The cytoxocity evaluation of AEo was carried out by GFP detection using African green monkey kidney Vero cells. The anticancer activities were tested by REMA against KB-Oral cavity cancer, NCI-H187-Small cell lung cancer including MCF7-Breast cancer. The results are presented in Table 4. AEo showed non-cytotoxicity

whereas it could inhibit KB-Oral cavity cancer (IC_{50} = 0.054%) and MCF7-Breast cancer (IC_{50}= 0.053%) except NCI-H187-Small cell lung cancer. The result corresponds with the former report that summarized *A. graveolens* L., belonging to family Apicieace, has also been reported as anticancer (Zheng *et al.,* 1992). Additionally, Sharopov and co-worker in 2013 reported that essential oil from aerial part of *A. graveolens* L. showed cytotoxicities for Hela (IC_{50}= 93 μg/mL), Caco-2 (IC_{50}= 216 μg/mL) and MCF-7 cell lines (IC_{50}= 67 μg/mL). Besides, the major components of AEo have been described about the potential for anticancer activity such as limonene which is a well-estabilished chemopreventive and therapeutic agent against many tumor cells (Crowell, 1999; Fabian, 2001; Kris-Etherton *et al.,*2002). Then, carvone has been shown to prevent chemically-induced lung and forestomach carcinoma development (Wattenberg *et al.,* 1989). This information may be beneficial for clinical studies and also could be used as a guide for the high advantages in the use of herbs for application of pharmaceutical product development.

Table 4 Cytoxicity and anticancer activity of AEo.

Sample	Cytotoxicity (IC_{50} %)	Anticancer activity		
		KB-Oral cavity	**MCF7-Breast**	**NIC-H187-Small cell lung**
AEo[a]	In	0.054	0.053	In
Elipticine[b]	0.716	0.958	ND	0.781
Doxorubicin[b]	ND	0.836	9.31	0.087
Tamoxifen[b]	ND	ND	7.12	ND

a=(IC_{50} %), b= μg/mL, In= Inactive, ND= Not detected

4. Conclusion

The essential oil of *A. graveolens* L. seeds revealed *D*-carvone, carvone, dill apiol and limonene as major components with slightly different in relative content with former reports (Delaquis *et al.,* 2002; Embong *et al.,* 1977; Jirovetz *et al.,* 2003; Mahmoodi *et al.,* 2012; Radulescu *et al.,* 2010; Vokk *et al.,* 2011). However, several of mono-, di-, sesqui-terpenes were found in the essential oil of plants and the different groups of chemicals in the essential oil, depending on many conditions, i.e., the growth stage, weather condition, etc. (Hay *et al.,* 1988; Svoboda and Hay, 1990). The biological activity of this essential oil distinctly showed positive effect. AEo exhibited high total phenolic content (GAE= 4.5746 mg/mL) and antioxidant activities in each method of DPPH, ABTS and FRAP assays with TEAC= 52.5391, 1.5936 and 0.5469 mg/mL respectively. The higher anti-oxidative efficiency correlates with the quantity of total phenolic compound in plants (Mirghani *et al.,* 2012). The potency of *A. graveolens* L. seeds corresponds to the antimicrobial activity because the essential oil consisted of terpenes which affect the levels of inhibition of microorganism (Aligiannis *et al.,* 2001; Bardar *et al.,* 2004; Panizzi *et al.,* 1993; Sivropoulou *et al.,* 1996). AEo clearly showed potent activity against *S. aureus* and *E. coli* at the MIC= 5.99 μg/mL and also showed ability against *C. albicans* (MIC= 29.78 μg/mL) and *A. flavus* (MIC= 59.47 μg/mL). The antimicrobial activity against the microorganisms maybe due to effective compounds of oxygenated monoterpene group, i.e., carvone, *D*-carvone and *tran*-dihydrocarvone, which are present in this essential oil. Moreover, AEo exhibited non-cytotoxicity in normal cell. On the other hand, it promoted anticancer activity on KB-Oral cavity cancer and MCF7-Breast cancer. This activity provides an important role for clinical studies and may be advantageous in other directions about the use of medicinal herbs that are grown in Thailand to high advantage.

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