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Chemical compositions, antioxidant and antibacterial activities of essential oils from *Anethum graveolens* L. and *Trachyspermum roxburghianum* (DC.) Craib grown in Thailand

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Abstract

The essential oils from the aerial part of *Anethum graveolens* L. and *Trachyspermum roxburghinum* (DC.) Craib were obtained by hydro-distillation. Gas chromatography-mass spectrometry (GC-MS) was employed for the identification of chemical components. Folin-Ciocalteu colorimetric method, DPPH, ABTS and FRAP assays were used to determine total phenolic content and to evaluate antioxidant potential. Agar-well diffusion and agar-dilution methods were used to investigate antibacterial activity. The results indicated that α-phellandrene (61.57%), β-phenandrene (10.39%) and dill ether (8.23%) represented as the major components of *A. graveolens* while sabinene (28.60%) and α-terpinolene (24.20%) including 3-*n*-butylphathalide (23.34%) were the main compounds of *T. roxburghinum*. The essential oils of both plants showed high total phenolic content (GEA= 1.7948-3.0971 mg/mL) and exhibited potent antioxidant activities in DPPH (TEAC= 19.3119 and 357.9297 mg/mL), ABTS (TEAC= 4.6031 and 13.4242 mg/mL) and FRAP assays (TEAC= 0.8327and 27.4173 mg/mL). Moreover, they had an effect on both gram-positive bacteria with MIC 2.66-11.88 µg/mL and gram-negative bacteria with MIC 23.76-85.77 µg/mL, respectively.

Keywords: *Anethum graveolens* L., *Trachyspermum roxburghinum* (DC.) Craib, essential oil, antioxidant activity, antibacterial activity.

1. Introduction

Pak Chi Lao and Pak Chi Rai are the local names of *Anethum graveolens* L. and *Trachyspermum roxburghinum* (DC.) Craib, respectively. They are members of family Umbelliferae (Apiaceae) [1] which are a rich source of essential oils and have been extensively cultivated in northern and northeastern regions of Thailand as vegetables. Thai folk wisdom has shown that the seeds of this family can be used to prevent spoilage of fermented food. As well, Thai traditional formularies use some Umbelliferous seeds as an ingredient in Thai tradition medicines, such as "Ya Tart Buun Jop and Ya Hom Intajak" which are used to treat the symptoms of dizziness, vomitting and as a cardiotonic drug. In fact, the uses of the aerial parts of plants have been widely practised to provide flavor in local cuisine, e.g., Mok pla: steamed fish in banana leaf, and several coconut milk-based curries that contain fish or prawns [2]. In addition, it is used as an edible vegetable. Therefore, these plants can be used to eliminate fishy aromas and influence the flavor of food and benefits for health because some phenolic compounds in plants have antioxidant activity. Many ailments such as cancer, cardiovascular disease, inflammation-related diseases, neurodegenerative diseases (Alzheimer's and Parkinson's disease) etc. are caused from free radicals which are dangerous molecules that can be generated in human body [3]. Natural sources of antioxidant substances have been discovered from fruits and vegetables, even a small amount of free radical scavenger can prevent and reduce reactive species of free radicals in the human body [4-5]. In Thailand, both plants are widely used in household while the scientific information of plants is still lacking. The study concerning the usefulness of these plants has shown the utility and potential of plants corresponding to phytonutrient consumption. Then, the examination on antimicrobial activity from these sources has been aimed to assess the potential and the uses of medicinal plants to their highest advantage because the natural antimicrobial agents could be a safe alternative for food preservation and human remedies. The aim of this research was to study the chemical compositions, antioxidant potential and antibacterial activity of essential oils

from aerial part of *A. graveolens* and *T. roxburghinum* that are widely grown in Thailand.

2. Material and Methods:

2.1 Plant materials

A. graveolens and *T. roxburghinum* were callected from Khon Kaen and Lampang Provinces of Thailand during November 2011 to January 2012. The identification of plant materials 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 and 8).

2.2 Essential oil extraction

The essential oils from the aerial part of plants were extracted by hydro- distillation, using a Clevenger-type apparatus for 2 hours [6]. The essential oils were then dried with anhydrous sodium sulfate and placed in brown glass vials to protect from light and also stored at 4° C for further analysis.

2.3 Determination of total phenolic content

Some modification of the Folin-Ciocalteu colorimetric method [7] was used for examination of the total phenolic content of essential oils. The sample solution of 250 µL was mixed with 2.5 mL of the Folin-Ciocalteau reagent (1:10 distillation water), then 2 mL of 7.5% Na_2CO_3 was added into the mixture and incubated in the dark at room temperature. After that, the mixture was measured at 765 nm by spectrophotometer (SHIMADZU® UV-2450). Gallic acid standard was used to compare and the results were reported in terms of Gallic acid equivalents value (GAE mg /mL).

2.4 Determination of antioxidant activities

2.4.1 The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay: The DPPH radical scavenging assay was determined according to the procedure of Wu *et al*. [8]. The 96-well micro titer plate was used and measured by multimode detector spectrophotometer (Beckman® Coulter/DTX880). Sample solution with different concentrations, about 20 µL, were added into 96-well micro titer plate and mixed with 180 µL of DPPH (6×10^{-5} mol/L) radical solution, the absorption was detected at not more than 1.00±0.02 at the wavelength 517 nm. Then, the mixture was shaken and left in the dark at room temperature for 30 minutes. After that, the absorbance was measured at the same wavelength. The percentage inhibition of antioxidant capacity was calculated as the formula:

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

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).

2.4.2 The 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radial scavenging assay: The ABTS radial scavenging method was modified from Re *et al*. [9]. The procedure prepared the ABTS radical cation from the reaction at the ratio of 2:1 of 7 mM ABTS radical solution in water and 2.45 mM potassium persulfate solution. Then, the mixture was stored in the dark at room temperature for 12 hr, the ABTS radical solution was diluted with ethanol to the absorbance value of 0.70±0.05 at the wavelength 734 nm. Then, 20 µL of the different concentrations of sample solution were added into test tubes and mixed with 80 µL of ethanol and 2 mL of ABTS radical solution. The mixture was left at room temperature for 5 minutes. After that, the mixture solution absorbance was detected at 734 nm. The percentage inhibition was calculated and compared with Trolox standard using the same formula as above.

2.4.3 Ferric reducing antioxidant power (FRAP) assay: The FRAP method was monitored using modifications of the method of Benzie and Strain [10]. The working solution as FRAP reagent was freshly prepared using the mixture of 200 mL 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). Then, FRAP reagent was incubated at 37° C before using. The sample investigation was as follows: 10 µL of sample solution was transferred into 96-well micro titer plate and mixed with 190 µL of FRAP reagent, the mixture solution was set aside in the dark

at room temperature for 30 minutes. Then, the absorbance was measured at the wavelength 593 nm by multimode detector spectrophotometer (Beckman® Coulter/DTX880). The result was compared with linear equation of Trolox standard at 50-1000 µM of concentration and interpreted in terms of Trolox equivalence antioxidant capacity value (TEAC mg/mL).

2.5 Determination of antimicrobial activity

2.5.1 Bacterial strains: Three species of microorganisms: *Staphylococcus aureus* ATCC25923, *Pseudomonas aeruginosa* ATCC27853 and *Escherichia coli* ATCC25922 were used in this examination. These bacterial strains were obtained from the culture collection of the Department of Medical Technology, Faculty of Associated Medical Sciences, Chiang Mai University. The cell concentrations of each bacteria was adjusted to match with the turbidity of McFarland No. 0.5 standard approximately 10^8 CFU/mL which was prepared and suspended in trypticase soy broth (TSB) agar.

2.5.2 Agar-well diffusion test: Agar-well diffusion method was used to test microbial sensitivity to antibiotics. The method was described from Bouhdid [11] with some modification as follows: the test was done by the mixture of melted agar and cell suspension (10^8 CFU/mL) in the ratio 10:1 mL. The first layer of solid medium was poured into petridish and the steriled 12 mm-diameter aluminum rings were placed on the layer. Then, the same mixture medium was transferred over the first layer and left until it became medium-solidified and the rings were removed. After that, 100 µL of pure essential oils were poured into the well and incubated at 37° C for 24 hours. The zones of inhibition were measured in millimeters. Gentamicin (75 µg/mL) was used as positive control.

2.5.3 Agar-dilution test: Agar-dilution technique was used for determination of minimum inhibitory concentration (MIC) of essential oils. The procedures were followed from NCCLS [12] with some modification. The method of all tests was arranged in *trypticase soy agar* (TSA) mixed with the serial dilution of essential oils, ranging from 0.00-12.5%. The media mixtures were transferred into a 24-well micro titer plate. Then, 2 µL of each bacterial suspension (10^4 CFU) was dropped on the surface. After that, the micro plates were incubated at 37° C for 24 hr and the growth of microorganism was observed for MIC.

2.6 Determination of chemical composition

GC-MS analysis of essential oils (0.5% in ethanol) was performed on a Shimadzu GCMS-QP 2010 Plus. A J&W Scientific, USA capillary column DB-5 MS with length 30 m, i.d. 0.25 mm, film thickness 0.25 µm of 5% phenylmethypolysiloxane was used with helium (99.99%) as a carrier gas at the flow rate of 1.00 mL/min. The temperature program was initially created from 60° C to 90° C with the rate of 5° C/min, next ramped to 95° C with the rate 1° C/min, then ramped to 180° C with the rate 5° C/min, after that ramped to 185° C with the rate 1° C/min and finally ramped to 200° C with the rate 10° C/min (5 min hold). The injector temperature was set at 180° C. Mass spectrometry was run in the electron impact mode (EI) at 70 eV with ion source temperature of 200° C. The mass spectra were recorded at the range m/z 40-400 amu in the full-scan acquisition mode. The volatile components were identified by matching with WILLEY 7 library as well as compared with Kovat retention indices (KI) of those provided in the information literature.

3. Results and discussion

The essential oils of *A. graveolens* and *T. roxburghinum* were 0.05% and 0.14% of yields, respectively. The chemical components and their relative content of essential oils are shown in Table 1. The essential oils of two plants in this study were distinctly different in chemical compositions. *A. graveolens* essential oil consisted of 17 chemical components while *T. roxburghinum* essential oil was found to contain 39 chemical compositions. The transparent essential oil of *A. graveolens* consisted of monoterpene hydrocarbon 64.71% as major groups which were α-phellandrene (61.57%), β-phellandrene (10.39%) and dill ether (8.23%), represented as major components similar to previous report by Vokk *et al*. [13] and Kazemi *et al*. [14]. *T. roxburghinum* composed of major component groups of monoterpene hydrocarbon 35.74% and sesquiterpene hydrocarbon 25.53%. Sabinene (28.60%) and α-terpinolene (24.20%) including 3-n-bytylphthalide (23.34%) the latter was related to phthalide and their corresponding dihydro, tetrahydro and dimer analogues which are found in several plants of the Umbelliferae family [15], represented as major compounds. This is the first report of volatile components from aerial part of *T. roxburghinum* grown in Thailand, in earlier information, Chowdhury and co-worker (2009) reported only chemical compounds from leaf [16]. However, both essential oils showed terpens as the major compound group which correspondingly had commonly been found in plant essential oil [17]. Terpenoids were described for significant biological activities such as antibacterial, anti-malarial, anti-inflammatory and anti-cancer [18]. Generally, essential oils of plants consisted of several of mono-, di-, sesqui-terpenes, the mixture of

essential oils contained different majority groups, chemical composition and relative content which depend on many conditions, i.e., cultivation, the growth stage, climate, etc. [14, 19].

Table 1 Chemical compositions of essential oils from aerial parts of *A. graveolens* and *T.roxburghinum.*

No.	Rt.	Compound	KI[a]	KI[b]	Groups	Relative content (%)	
						A. graveolens	*T. roxburghinum*
1	4.592	*α*-Thujene	931	931	Monoterpene hydrocarbon	0.37	0.14
2	4.753	*α*-Pinene	940	940	Monoterpene hydrocarbon	1.88	0.16
3	5.520	Sabinene	979	979	Monoterpene hydrocarbon	0.32	**28.60**
4	5.649	*β*-Pinene	985	985	Monoterpene hydrocarbon	0.28	0.13
5	5.802	*β*-Myrcene	992	992	Monoterpene hydrocarbon	0.73	**3.30**
6	6.236	*α*-Phellandrene	1010	1011	Monoterpene hydrocarbon	**61.57**	0.29
7	6.482	*α*-Terpinene	1021	1018	Monoterpene hydrocarbon	-	0.22
8	6.683	*p*-Cymene	1029	1029	Monoterpene hydrocarbon	2.12	0.19
9	6.792	Limonene	1033	1032	Monoterpene hydrocarbon	**4.49**	1.29
10	6.852	*β*-Phellandrene	1035	1032	Monoterpene hydrocarbon	**10.39**	0.96
11	6.894	*cis*-Ocimene	1037	1037	Monoterpene hydrocarbon	-	1.25
12	7.187	*trans*-*β*-Ocimene	1047	1047	Monoterpene hydrocarbon	-	0.16
13	7.573	*γ*-Terpinene	1061	1062	Monoterpene hydrocarbon	0.15	1.03
14	8.036	*trans*-Sabinenhydrate	1077	1075	Oxygenated monoterpene	-	0.14
15	8.434	*α*-Terpinolene	1089	1089	Monoterpene hydrocarbon	0.26	**24.20**
16	10.677	*cis*-Thujanol	1151	1149	Oxygenated monoterpene	-	0.19
17	11.188	Dictyotene	1163	1155	Hydrocarbon	-	0.26
18	12.288	Terpinen-*4*-ol	1188	1186	Oxygenated monoterpene	-	0.24
19	12.552	Dill ether	1193	1187	Oxygenated monoterpene	**8.23**	-
20	12.602	*p*-Cymen-*8*-ol	1194	1194	Oxygenated monoterpene	-	0.2
21	19.000	*α*-Copaene	1379	1380	Sesquiterpene hydrocarbon	-	0.12

22	19.380	*β*-Cubebene	1390	1390	Sesquiterpene hydrocarbon	-	0.12
23	20.312	Caryophyllene	1423	1423	Sesquiterpene hydrocarbon	-	**3.42**
24	21.229	*β*-Farnesene	1457	1458	Sesquiterpene hydrocarbon	-	0.32
25	21.329	Humulene	1460	1462	Sesquiterpene hydrocarbon	-	0.35
26	22.032	Germacrene D	1485	1485	Sesquiterpene hydrocarbon	0.72	0.15
27	22.253	*β*-Selinene	1492	1490	Sesquiterpene hydrocarbon	-	**3.62**
28	22.440	*α*-Selinene	1499	1494	Sesquiterpene hydrocarbon	-	0.85
29	22.625	*α*-Farnesene	1506	1506	Sesquiterpene hydrocarbon	-	0.23
30	23.012	*δ*-Cadinene	1522	1524	Sesquiterpene hydrocarbon	-	0.19
31	23.176	Myristicin	1529	1532	Aromatic ether	0.48	-
32	23.331	(+)-Nerolidol	1535	1534	Oxygenated sesquiterpene	-	0.92
33	24.111	Nerolidol	1566	1566	Oxygenated sesquiterpene	-	0.75
34	24.253	Guaiol	1572	1571	Oxygenated sesquiterpene	-	0.17
35	24.699	Caryophyllene oxide	1589	1589	Oxygenated sesquiterpene	-	0.19
36	24.842	Diethyl Phthalate	1594	1585	Aromatic ester	0.22	0.21
37	25.598	Dill apiol	1627	1625	Aromatic ether	6.76	1.02
38	25.911	Alloaromadendrene epoxide	1641	1641	Oxygenated sesquiterpene	-	0.11
39	26.557	Juniper camphor	1668	1675	Oxygenated sesquiterpene	0.16	-
40	26.839	*(E)-3*-Butylidene phthalide	1680	1677	Aromatic ester	-	0.38
41	26.907	Apiol	1683	1685	Aromatic ether	-	0.16
42	28.221	*3-n*-Butylphthalide	1740	1815	Aromatic ester	-	**23.34**
						99.52	99.57

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

[b] Relative retention indices from reference which were previously reported [20-21].

The quantity of total phenolic content in the aerial part essential oils from two edible plants are presented in

Table 2. Since various researches focus studies only on seeds plants, this examination presents amount of phenolic compounds found from aerial part essential oils of both plants. The studies clearly showed that the essential oil of *T. roxburghinum* had higher total phenolic content than *A. graveolens*, the amounts of GAE were 3.0971 and 1.7948 mg/mL, respectively. The results suggested that both plants consist of phenolic compounds in each part that can support the consumption of consumer. The phenolic compound is one group of phytochemical substances which have been used for prevention of various diseases and exhibit a wide range of biological activities such as antioxidant, antimicrobial, anti-inflamatory, anti-allergenic, etc. [23-25]. It can act as a free radical scavenger and is responsible for antioxidant activity in medicinal herbs [26]. The quantity of phenolic compounds corresponds to structure of phenols group that contained in essential oil [27-28]. Then, the aromatic compounds and monoterpene with hydroxyl functional groups have good antioxidant effects [29].

Table 2 Total phenolic content and antioxidant activities of essential oils from the aerial part of *A. graveolens* and *T. roxburghinum*.

Sample	**Total phenolic content** (GAE mg/mL)	**Antioxidant activities** (TEAC mg/mL)		
		DPPH	**ABTS**	**FRAP**
A. graveolens	1.7948±0.0018	19.3119±0.0044	4.6031±0.0096	0.8327±0.0643
T. roxburghinum	3.0971±0.0018	357.9297±0.0187	13.4242±0.0102	27.4173±0.0099

The antioxidant potential of two essential oils is presented in Table 2. The properties of antioxidant scavenger and reducing antioxidant power were proved on DPPH, ABTS and FRAP assays. The results indicated that both essential oils exhibited anti-oxidative activity in all methods. The essential oil of *T. roxburghinum* was shown to be higher in antioxidant activities than *A. graveolens*. The effects of antioxidant potential presented highest activity on DPPH, ABTS and FRAP assay which were 357.9297, 13.4242 and 27.4173 mg/mL, respectively. The capability of antioxidant activity is related to total phenolic compound in medicinal herbs, that is, the higher amount of the phenolic content will lead to higher anti-oxidative efficiency [30]. Besides, their major components consisted in both essential oils are monoterpenes and oxygenated monoterpene which corresponds to Cao and co-worker (2009) who described these compounds (oxygenated monoterpene and monoterpene hydrocarbon) that they are the principal antioxidant substances in the essential oil from plants [31]. The methods (DPPH, ABTS and FRAP) are used to evaluate capability of antioxidant potential of samples. Although the characterizations of mechanism on DPPH and ABTS assay were similar, they also showed significant differences in reaction to antioxidant substances, then various factors, i.e., stereolecectivity of the radicals, the solubility of the tested sample in different testing and functional groups present in bioactive compounds have been reported to affect the capacity to react and quench different radicals of sample [26,32]. Some compound which has $ABTS^{\bullet+}$ scavenging activity may not show DPPH scavenging activity [33]. For FRAP assay, result reflected only the antioxidant reducing potential [34]. The correlation between total phenolic contents and reducing antioxidant including scavenging free radicals were described in the report of Fu *et al.* [35]. However, this result can be summarized that both essential oils from aerial part of *A. graveolens* and *T. roxburghinum* had capability of radicals scavenger and they showed ability of reducing agent together.

The diameter of inhibition zone and the MIC of essential oils had an effect on the visible growth of microorganisms as shown in Table 3. The results showed that the essential oils of *A. graveolens* exhibited against all gram-positive and gram-negative bacteria. The zones of inhibition ranged from 16.50-23.00 mm with the widest zones of suppression on *S. aureus* and *E. coli* being equal to the standard gentamicin and the MIC presented at the range of 11.88-47.52 µg/mL. This activity was similar to the previous research which reported that essential oil from leaf of this plant inhibited *S. aureus, P. aeruginosa, E. coli, Candida albicans* and *Aspergillus flavus* [14]. For *T. roxburghinum*, it showed inhibition zone on *S. aureus* (24.00 mm) and *E. coli* (16.75 mm) except *P. aeruginosa*. This essential oil showed strong inhibition at the lowest concentration as 2.66 µg/mL to *S. aureus*, and exhibited against *E. coli* at 85.77 µg/mL. The antibacterial activity of both plants resulted from their essential oil which related to their major components including phenolic compound. Both plants showed terpenes as major components group which have been found to be active against a variety of microorganism and related to their functional groups and hydroxyl group of phenolic compounds including the presence of delocalized electrons as important element for their antimicrobial action [36-37]. However, these results can support the utilization of these plants in the folk wisdom which used some Umbelliferous plants to prevent spoilage of fermented food. Furthermore, it can also be used as a natural substance in food preservation.

Table 3 Zone of inhibition and minimum inhibitory concentration of essential oils from the aerial part of *A. graveolens* and *T. roxburghinum*.

Sample	Zone of inhibition (mm)			Minimum inhibitory concentration (μg/mL)		
	S. aureus	*E. coli*	*P. aeruginosa*	*S. aureus*	*E. coli*	*P. aeruginosa*
A. graveolens	20.00	23.00	16.50	11.88	23.76	47.52
T. roxburghinum	24.00	16.75	Inactive	2.66	85.77	ND
Gentamicin	20.00	25.00	20.00	0.31	0.63	1.25

ND: not detected

4. Conclusion

Both essential oils of plants used in this study clearly showed the chemical constituents comprises of 17 components in *A. graveolens* which were α-phellandrene, β-phellandrene and dill ether as major components. *T. roxburghinum* essential oil consisted of 39 constituents, with sabinene, α-terpinolene and *3-n*-bytylphthalide was found as a majority components. The amount of phenolic compounds, *T. roxburghinum* had higher total phenolic content than *A. graveolens* essential oil, corresponding to higher antioxidant potential of this herb. Moreover, the antibacterial activity of *A. graveolens* showed inhibition of all bacteria while *T. roxburghinum* was effective against *S. aureus* and *E.coli* except *P.aeruginosa*. The information of biological potential has supported the phytonutrient consumption of two edible plants. The use of fresh aerial parts of plants indirectly provides an advantage because both plants are a good source of phenolic compounds and free radical scavenger including reducing antioxidant power and also these essential oils of plants could be alternatively applied to food flavor and other production. Moreover, the antibacterial activity of these plants can be used to guide further study for preventing the poisoning in food. Then, the natural antimicrobial agents could be a safe alternative for food preservation. This information provides an important role for clinical studies and may be advantageous in other directions in the utilization of two medicinal plants that are cultivated in Thailand. Next, our research will investigate the antibacterial activity that is related to food fermentation from two edible plants (*A. graveolens* and *T. roxburghinum*).

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