Research Article

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Essential Oil Composition and *in Vitro* Anti-aging Potential of *Elettariopsis wandokthong* Rhizome

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Abstract

The objectives of this study were to determine the essential oil composition and the anti-aging potential of essential oil and extracts from *E. wandokthong* rhizome. The essential oil was obtained by hydro-distillation of the fresh rhizome and its composition was identified by Gas Chromatography coupled to Mass Spectrometry system (GC-MS). Five major components, α -gurjunene (22.31%), alloaromadendrene (18.20%), 4-allyloxyimino-2-carene (5.83%), fenchyl acetate (5.44%), and γ -cadinene (5.19%), were identified in *E. wandokthong* essential oil. Moreover, the fresh rhizome and hydrodistillation residue were each extracted with ethanol, 50% ethanol-water, and water. The anti-aging potential of the essential oil and of the different extracts was evaluated for their total phenolic content, antioxidant, anti-tyrosinase, and keratinocyte proliferation promoting activities. The *E. wandokthong* rhizome extract provided the highest efficacy in all assays. Moreover, the antioxidant, anti-tyrosinase, and keratinocyte proliferation residue extracts were reduced about 2 times compared to those of the fresh rhizome. The results indicated that *E. wandokthong* rhizome essential oil and extracts have potential to use as fragrant and active ingredient in cosmetic products.

Keywords: Anti-aging, Antioxidant, Anti-tyrosinase, *Elettariopsis wandokthong*, Essential oil, Keratinocyte proliferation

1 Introduction

Aromatic herbs are frequently used in traditional medicine because of their essential oils and bioactive compounds. Essential oils are the mixtures of volatile and lipophilic substances, formed by the secondary metabolism of plants and characterized by a pleasant odor most of them presented [1]. Plant essential oils have been known to exhibit biological activities such as antioxidant, anti-inflammatory, antimicrobial, and anticancer activities [2]–[5]. Essential oils are valuable natural products that used as raw materials in many fields including perfumes, cosmetics, aromatherapy, phytotherapy, and nutrition [6].

The genus of Elettariopsis belong to the family Zingiberaceae which 6 species were found in Thailand [7]. Elettariopsis wandokthong Picheans and Yupparach, is a new species of perennial rhizomatous herbs that is widely distributed in Thailand [8]. *E. wandokthong* has creeping slender rhizome with pseudostem at intervals

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and white flower with yellowish apex. In Thailand, *E. wandokthong* is commonly known as Wan dokthong and Wan maha seneh that is believed to process a magical power, good luck charm attracting customer and increasing sex appeal [8]. Moreover, essential oil from rhizomes of *E. wandokthong* also used as one of the ingredients for making magical herbal charming oil or lip balm, believing that it will help attracting the targeted opposite sex, particularly women [8].

Many previous studies reported about Elettariopsis species for chemical components and antibacterial activities of essential oil such as *E. curtisii* [9], [10], *E. slahmong* [11] and *E. wandokthong* [12]. However, there are no reported about essential oil composition and anti-aging potential of extracts from rhizome of *E. wandokthong*. The aim of this study was to investigate the chemical composition of essential oil from the fresh rhizome of *E. wandokthong* by using GC-MS. Moreover, the organic solvents (ethanol, 50% ethanol in water and water) were evaluated extracting effect on total phenolic content, ferric reducing antioxidant power, DPPH radical scavenging, nitric oxide inhibition, and superoxide dismutase, anti-tyrosinase activities, and keratinocytes' proliferation promoting.

2 Materials and Methods

2.1 Plant material

The fresh rhizome of *E. wandokthong* was collected from Nongkhai, Thailand. The rhizomes were washed with tap water, air dried and then cut into small pieces.

2.2 Isolation of essential oil

Essential oil from fresh rhizome of *E. wandokthong* was extracted by hydrodistillation for 4.5 h using a Clevenger apparatus. Then, the essential oil was separated from hydrolyte and stored at 4°C in the dark until used. The essential oil was diluted in ethanol with the ratio of oil to ethanol 1:10 by volume before analyzed.

2.3 GC-MS analysis of essential oil

The identification of the component of essential oils was performed by Gas Chromatography/Mass Selective Detector (GC-MS) (GC 6890N; MS 5973N, Agilent technologies, USA) with a HP-5MS agilent column (30 m × 0.25 mm diameter, 0.25 μ m film thickness). Injector temperature was 250°C. Oven temperature was programmed holing at 60°C for 5 m min in, heating to 250°C at 3°C/min, and keeping the temperature constant at 250°C for 10 min. Helium was used as a carrier gas at a constant flow of 1.0 mL/min. The injection volume of sample was 1.0 μ L. The MS scan parameters included electron impact ionization voltage of 70 eV, a mass range of 50–550 m/z. The identification of components of the essential oil was based on comparison of their mass spectra with those stored in NIST08 library.

2.4 Preparation of extracts

The fresh rhizome and residue of hydrodistillation of *E. wandokthong* were extracted with three different solvents (ethanol, 50% ethanol in water and water). One gram of plant material was extracted with 10 mL of each solvent for 24 h. The extracts were filtered through Whatman® No.1 filter paper and collected at 4°C until analyzed.

2.5 Determination of Total Phenolic Content (TPC)

The total phenolic content was analyzed using the Folin-Ciocalteu assay [13]. Briefly, the $20 \,\mu\text{L}$ of extracts were mixed with $20 \,\mu\text{L}$ of Folin-ciocalteu reagent, $125 \,\mu\text{L}$ of 7% sodium bicarbonate and $50 \,\mu\text{L}$ of DI water. The mixture was incubated at room temperature for 60 min and then measured the absorbance at 750 nm using microplate reader (Biochrom, USA). The total phenolic content was expressed as mg gallic acid equivalents (mg GAE/mL extract).

2.6 Determination of antioxidant activities

2.6.1 Ferric Reducing Antioxidant Power (FRAP)

The ferric reducing power was determined according to the ferric reducing power method [13]. The extracts $(25 \,\mu\text{L})$ were added with 50 μ L of 1% potassium ferrocyanide and stand at room temperature for 60 min. Then, the mixture was added 25 μ L of 10% trichloro acetic acid and 75 μ L of DI water. The absorbance was measured at 700 nm. Then, the mixture was added 25 μ L of 0.1% ferric chloride and the absorbance was measured at 700 nm again. The reducing power of samples was

determined by compared with the standard ascorbic acid calibration curve and expressed as ascorbic acid equivalents (mg AAE/ml extract).

2.6.2 DPPH radical scavenging activity (DPPH)

The scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals was determined according to Vichit and Saewan [13]. The 5 μ L of extract was added with 195 μ L of 0.1 M DPPH solution. The mixture was stand at room temperature for 30 min and measured absorbance at 515 nm. The DPPH scavenging activity was calculated using the Equation (1),

$$= [(A_{control} - A_{sample})/A_{control}] \times 100$$
(1)

2.6.3 Nitric Oxide inhibition (NO)

Nitric oxide inhibition was assessed using Griess reagent system [14]. The culture medium of treated cells with 50% extract and 1 μ g/mL lipopolysachharide was combined with sulfanilamide solution and incubated for 5 min at room temperature. Then, N-1-napthylethylenediamine dihydrochloride solution was added and incubated for 5 min. The absorbance of the solution was measured at 540 nm. The percentage of nitric oxide inhibition was calculated using Equation (2),

Inhibition of nitric oxide (%)
=
$$[(A_{control} - A_{sample})/A_{control}] \times 100$$
 (2)

2.6.4 Superoxide dismutase activity (SOD)

The SOD activity in supernatants was measured by using SOD assay Kit-WST [14]. The supernatant of treated cells with extract was collected. WST working solution and enzyme working solution were added and then mixed thoroughly. The mixture was incubated at 37°C for 20 min and measured absorbance at 450 nm. The SOD activity was calculated using Equation (3),

SOD activity (%) =
$$[(A_{control} - A_{sample})/A_{control}] \times 100$$
 (3)

2.7 Anti-tyrosinase activity (ATR)

Anti-tyrosinase assays were determined by using a modified dopachrome method with L-DOPA as the

substrate [15]. Extract was added to 0.1 M phosphate buffer (pH 6.8) and 1 mM L-DOPA. The mixture was incubated for 10 min at 37°C. Then, 200 units/mL mushroom tyrosinase was added into the sample solution and incubated for 15 min at 37°C. Absorbance was measured at 475 nm and calculated using Equation (4),

Inhibition of tyrosinase (%)

$$= [(A_{control} - A_{sample}) / A_{control}] \times 100$$
(4)

2.8 Keratinocytes' Proliferation Promoting (KPP)

Keratinocytes' proliferation promoting was determined by method of Vichit & Saewan [14]. The extract was added to human epidermal keratinocyte culture and incubated for 72 h at 37°C in a 5% CO_2 humidified incubator. Then, the culture medium was removed and 0.1 mg/mL MTT solution was added. After 4 h of incubation, dimethylsulfoxide was added and incubated for a further 30 min. The absorbance was measured at 570 nm and the percentage of keratinocytes' proliferation was calculated using Equation (5),

Keratinocytes' proliferation (%)
=
$$[(A_{sample} - A_{control})/A_{control})] \times 100$$
 (5)

2.9 Statistical analysis

The obtained data were performed in triplicate and statistically analyzed using the SPSS program version 21 for Windows (SPSS Inc., Chicago, IL, USA), and the differences were considered significant when p < 0.05. The comparison of data between each sample was analyzed by using One Way Analysis of Variance (ANOVA).

3 Results and Discussion

3.1 GC-MS analysis of essential oil

Hydrodistillation of fresh rhizome of *E. wandokthong* produced clear light yellow oil which presented yield of 1.56%. Chemical component of essential oil was determined by using GC-MS and presented in Figure 1 and Table 1. The analysis of the essential oil revealed 54 compounds which representing 98.77% of the total essential oil. Five major components which were presented in Figure 2; α -gurjunene (22.31%),

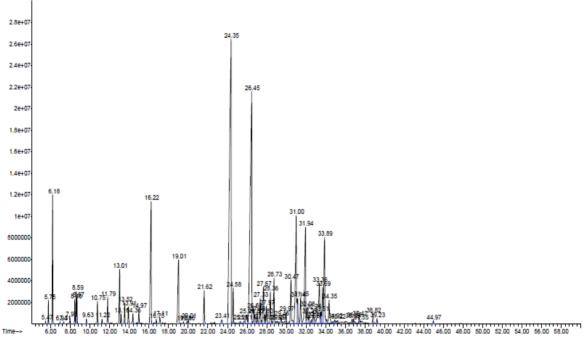


Figure 1: GC-MS chromatogram of essential oil from E. wandokthong rhizome.

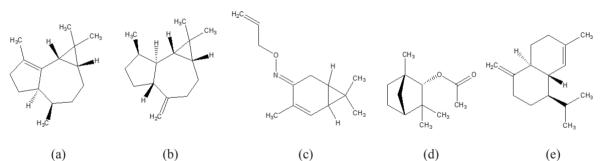


Figure 2: The major components of essential oil from *E. wandokthong* rhizome : (a) α -gurjunene, (b) alloaromadendrene, (c) 4-allyloxyimino-2-carene, (d) fenchyl acetate, and (e) γ -cadinene.

alloaromadendrene (18.20%), 4-allyloxyimino-2-carene (5.83%), fenchyl acetate (5.44%), and γ -cadinene (5.19%) were observed in the essential oil. However, this experimental results were different from previous report of Theanphong *et al.* [12] who found three major compounds in essential oil of *E. wandokthong* fresh rhizome including camphene (22.51%), fenchyl acetate (9.11%) and 1, 8-cineole (8.12%). Moreover, the odor of *E. wandokthong* rhizome essential oil was strongly sweet odor which has a potential to use as natural fragrant in cosmetic products.

Table 1: Chemical components of essential oil from*E. wandokthong* rhizome

Compounds		Formula	MW (g/mol)	%
1	Tricyclene	$C_{10}H_{16}$	136.23	0.13
2	α-Pinene	$C_{10}H_{16}$	136.23	0.62
3	Camphene	$C_{10}H_{16}$	136.23	2.92
4	β-Pinene	$C_{10}H_{16}$	136.23	0.09
5	<i>p</i> -Cymene	C10H14	134.22	0.56
6	Limonene	C10H16	136.23	0.78
7	Eucalyptol	C10H18O	154.25	0.54

<i>E. wandokthong</i> rhizome (Continued)						
	Compounds	Formula	MW (g/mol)	%		
8	γ-Terpinene	C10H16	136.23	0.12		
9	Fenchone	C ₁₀ H ₁₆ O	152.23	0.58		
10	Linalool	C ₁₀ H ₁₈ O	154.25	0.14		
11	Fenchol	C ₁₀ H ₁₈ O	154.25	0.74		
12	Camphor	C ₁₀ H ₁₆ O	152.23	1.77		
13	Terpinenol	C ₁₀ H ₁₈ O	154.25	0.60		
14	Isoborneol	C ₁₀ H ₁₈ O	154.25	0.58		
15	Borneol	C10H18O	154.25	0.57		
16	α -Terpineol	C10H18O	154.25	0.40		
17	Fenchyl acetate	$C_{12}H_{20}O_2$	196.29	5.44		
18	Thymol methyl ether	C ₁₁ H ₁₆ O	164.24	0.17		
19	Tricyclodecane	C10H16	136.23	0.25		
20	Isobornyl acetate	$C_{12}H_{20}O_2$	196.29	3.07		
21	Carracrol	C ₁₀ H ₁₄ O	150.22	0.09		
22	1,2-diethyl-o-Xylene	C12H18	162.27	0.14		
23	α -Terpinyl acetate	C ₁₂ H ₂₀ O ₂	196.29	1.08		
24	β-Elemene	C15H24	204.35	0.25		
25	α-Gurjunene	C15H24	204.35	22.31		
26	γ-Gurjunene	C15H24	204.35	1.63		
27	Caryophyllene	C15H24	204.35	1.01		
28	δ -Selinene	C15H24	204.35	0.15		
29	α-Caryophyllene	C15H24	204.35	0.58		
30	Alloaromadendrene	C15H24	204.35	18.20		
31	γ-Selinene	C15H24	204.35	0.37		
32	Germacrene D	C ₁₅ H ₂₄	204.35	0.35		
33	β -Panasinsene	C15H24	204.35	1.01		
34	β-Cubebene	C ₁₅ H ₂₄	204.35	0.10		
35	Bicyclogermacrene	C15H24	204.35	1.57		
36	α-Muurolene	C15H24	204.35	0.27		
37	γ-Muurolene	C15H24	204.35	0.37		
38	Dehydroisolongifolene	C15H22	202.34	0.62		
39	γ-Cadinene	C15H24	204.35	5.19		
40	δ -Cadinene	C15H24	204.35	2.01		
41	Calamenene	C15H22	202.34	0.08		
42	α-Calacorene	C ₁₅ H ₂₀	200.33	0.23		
43	y-Elemene	C ₁₅ H ₂₄	204.35	0.36		
44	Palustrol	C ₁₅ H ₂₆ O	222.37	1.87		
45	4-Allyloxyimino-2-carene	C ₁₃ H ₁₉ NO	205.30	5.83		
46	Globulol	C ₁₅ H ₂₆ O	222.37	0.82		
47	Ledol	C ₁₅ H ₂₆ O	222.37	4.86		
48	α -Humulene epoxide II	C ₁₅ H ₂₄ O	220.35	0.73		
49	Spathulenol	C ₁₅ H ₂₄ O	220.35	0.25		
50	α-Cadinol	C ₁₅ H ₂₆ O	222.37	2.94		
51	α-Copaene	C ₁₅ H ₂₄	204.35	0.41		
52	Jasmone	C ₁₁ H ₁₆ O	164.24	1.66		
53	Isolongifolanone	C ₁₅ H ₂₄ O	220.35	1.18		
		C ₁₅ H ₂₂ O	218.33	0.18		

Table 1: Chemical components of essential of	from				
E. wandokthong rhizome (Continued)					

3.2 Total Phenolic Content (TPC)

The test samples of this experiment were essential oil and extracts from E. wandokthong rhizome. The essential oil was obtained from hydrodistillation of fresh rhizome. The extracts were prepared form the fresh rhizome and the residue of hydrodistillation by extracting with ethanol, 50% ethanol in water, and water. Total phenolic content in extracts from E. wandokthong rhizome was determined by using Folin-Ciocalteu assay and expressed as gallic acid equivalents (mg GAE/mL). The results were shown in Table 3. The essential oil showed low total phenolic content (0.02 mg GAE/mL). The rhizome extracts showed 0.10–0.30 mg GAE/mL for fresh rhizome extracts and 0.03-0.14 mg GAE/mL for hydrodistillation residue extracts. The highest phenolic content was observed in 50% ethanol in water for both fresh rhizome (0.30 mg GAE/mL) and residue of hydrodistillation (0.14 mg GAE/mL).

3.3 Antioxidant activities

The essential oil and extracts of *E. wandokthong* were determined antioxidant activities by using 4 assays including Ferric Reducing Power (FRAP), DPPH radical scavenging (DPPH), Nitric Oxide inhibition (NO) and Superoxide Dismutase (SOD) activities. The results of antioxidant activities were summarized in Table 2. All of antioxidant activity assays showed in same tend of the results. All extract samples were 5 times higher antioxidant activities than the essential oil. The 50% ethanol in water provided the highest antioxidant activities in all assays. Moreover, the fresh rhizome extracts were 2 times higher antioxidant activities than hydrodistillation residue extracts.

3.4 Anti-tyrosinase activity (ATR)

Anti-tryrosinase activity or melanin biosynthesis inhibitory assay are useful to evaluate skin whitening agents. In this study, anti-tyrosinase assay was determined by using a dopachrome method with L-DOPA as the substrate and reported as percentage of tyrosinase inhibition. Among all the test samples, 50% ethanolic extract of fresh *E. wandokthong* rhizome was found to be the most potent anti-tyrosinase activity (72.80%), followed by ethanolic extract of fresh rhizome (41.50%), and 50% ethanolic extract

Material	Solvent	FRAP	DPPH	NO	SOD
Wraterial		(mg AAE/mL)	(% inhibition)	(% inhibition)	(% activity)
Essential oil		0.06±0.00g	13.59±3.24 ^f	8.67±0.39 ^g	12.20±1.86 ^g
P 1 1	EtOH	0.49±0.02 ^b	66.80±6.84 ^b	61.13±2.20 ^b	65.23±5.30 ^b
Fresh rhizome extract	50% EtOH	1.09±0.01ª	83.75±3.99ª	81.34±2.63ª	78.37±3.34ª
extract	Water	0.43±0.01°	32.04±0.12°	47.65±1.07°	27.74±5.28°
D' d'Il d' l' l	EtOH	0.15±0.00°	26.58±3.12 ^d	25.02±0.23°	32.87±2.45 ^d
Distillation residue extract	50% EtOH	0.23±0.00 ^d	62.92±1.91 ^b	37.59±1.25 ^d	48.68±2.15°
extract	Water	0.12±0.01 ^f	17.11±2.41°	13.85±2.30 ^f	25.30±1.53 ^{e, f}
Reference antioxidants (0.1 mg/ml Ascorbic acid)		-	37.12±2.44	29.56±3.27	60.34±1.34

Table 2: Antioxidant activities of *E. wandokthong* essential oil and extracts from fresh rhizome and residue of hydrodistillation

Note: Values represent means \pm sd with different letters within the same column being significantly different (p < 0.05).

Table 3: Total phenolic content, anti-tyrosinase and keratinocyte proliferation promoting activities of

 E. wandokthong essential oil and extracts from fresh rhizome and residue of hydrodistillation

Material	Solvent	TPC (mg GAE/mL)	ATR (% inhibition)	KPP (% proliferation)
Essential oil		$0.02{\pm}0.01^{d}$	4.61±1.34 ^e	3.44±1.27 ^d
	EtOH	0.14±0.01 ^b	41.50±0.65 ^b	13.90±0.16 ^b
Fresh rhizome extract	50 % EtOH	0.30±0.02ª	72.80±2.00ª	19.16±0.90 ^a
	Water	0.10±0.01°	34.61±2.32°	11.50±0.35 ^b
Di cili ci i i	EtOH	$0.04{\pm}0.00^{d}$	26.60±3.91 ^d	6.36±0.18°
Distillation residue	50 % EtOH	0.14±0.00 ^b	37.98±4.50 ^{b, c}	11.59±0.69 ^b
CALLACT	Water	$0.03{\pm}0.00^{d}$	5.83±1.35°	3.56±0.59 ^d

Note: Values represent means \pm sd with different letters within the same column being significantly different (p < 0.05).

of residue rhizome (37.98%) (Table 3). The essential oil of *E. wandokthong* rhizomes were low activity as 4.61%. The 0.5 mg/mL of kojic acid was taken as reference anti-tyrosinase activity that showed very high activity as 91.85%. A linear correlation comparing the anti-tyrosinase activity and phenolic content showed very high positive correlation (r = 0.926). These results showed that the concentration of phenolic compounds in *E. wandokthong* extracts was related with anti-tyrosinase activity. The result was similar to that obtained by Alam *et al.* [16] who reporting the phenolic compounds can bind to the active site on tyrosinase and changed conformation to low activity through hydroxyl groups.

3.5 Keratinocytes' Proliferation Promoting (KPP)

The keratinocytes' proliferation promoting activity is used to prove wound healing and anti-aging properties of natural products due to the fact that keratinocytes are the major cell type in the epidermis and have a critical role in the complex process of wound healing [14]. The promoting of keratinocytes proliferation was performed on human epidermal keratinocyte and detected by using MTT assay. All samples of *E. wandokthong* rhizomes showed keratinocytes proliferation promoting activity in range of 3.44-9.16 % (Table 3). Amongst them, the 50 % ethanolic extract of fresh *E. wandokthong* rhizome had the highest activity (19.16 %). In addition, there was a high positive correlation between total phenolic content and keratinocytes' proliferation promoting (r = 0.893), indicating that phenolic compounds may be the major chemical constituent required for the activity. Phenolic compounds are effective skin cell renewal and anti-aging properties that are a promising target for cosmetics [17].

4 Conclusions

The present study is the first report of the essential oil components, antioxidant, anti-tyrosinase and keratinocytes' proliferation promoting activities of *E. wandokthong* rhizomes growing in Thailand. The GC-MS analysis of essential oil showed high diversity of identified 54 compounds. The bioactivity evaluation demonstrates that the extracts of *E. wandokthong* rhizome were higher significantly activities than essential oil. The 50% ethanol in water was the interesting solvent to extract active constituents from *E. wandokthong* rhizome. Moreover, *E. wandokthong* rhizome essential oil has a potential to use as natural fragrant in cosmetic products due to its strongly sweet odor. The *E. wandokthong* rhizome extract has a potential to use as active ingredient for antioxidant, whitening and anti-aging skin in cosmetic products.

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