Original Article

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Antityrosinase and antioxidant activities of essential oils of edible Thai plants

Kiattisak Saeio, Wantida Chaiyana, Siriporn Okonogi*

Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand.

ABSTRACT: This work was undertaken to explore antityrosinase and antioxidant activities of twenty essential oils of edible Thai plants. Antityrosinase activity against mushroom tyrosinase was examined by means of the dopachrome method using L-dopa as an enzymatic substrate. The essential oil of *Cymbopogon citratus* had the highest level of antityrosinase activity, followed by that of *Ocimum canum* with enzymatic inhibition of 69 ± 4 and 66 ± 3%. GC-MS revealed that geranial and neral were the two most abundant components of their chemical compositions. Antioxidant activity was gauged by the free radical scavenging activity test and ferric reducing/antioxidant power assay. The essential oil of *Ocimum sanctum* had the highest level of antioxidant activity, followed by the essential oil of *Ocimum gratissimum*. These results led to the conclusions that the essential oils of edible Thai plants exhibit important biological activities and are a promising choice as natural active ingredients because of their antityrosinase and antioxidant activities.

Keywords: Essential oil, antityrosinase, antioxidant, geranial, neral

1. Introduction

Plants have been used in cooking, cosmetics, perfumery, and medicine since ancient times (*1*). Certain parts of edible plants are added to the diet because they can produce flavors and enhance the palatability of food. Many natural compounds extracted from plants exhibit important biological activities. Among the diverse natural compounds, essential oils extracted from aromatic plants are attracting particular attention. Essential oils are complex natural mixtures of volatile secondary metabolites, isolated from different parts of plants, and are also responsible for the fragrant and biological properties of medicinal plants (*2*). The multi-component systems in the essential oils consist mainly of low molecular weight terpenoids (*3*). These small compounds allow easy transport across cell membranes to induce different biological activities (*4*). The essential oils are recognized as having several therapeutic applications and have vast pharmacological effects (*5,6*). Recently, essential oils from edible plants have attracted greater interest due to their availability, fewer side effects or toxicity, and biodegradability (*2*). However, a comparative study of important activities of edible plants has yet to be conducted. The aim of the present study was to investigate and compare antioxidant and antityrosinase activities of essential oils extracted from several often consumed edible Thai plants. The chemical profile of the potential oils was also determined in order to analyze the constituents existing in the oils.

**Address correspondence to:*
Dr. Siriporn Okonogi, Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand.
e-mail: sirioko@chiangmai.ac.th

2. Materials and Methods

2.1. *Plant materials*

Eighteen edible plants, *Centella asiatica* Urban., *Polyscias fruticosa* Harms., *Eupatorium odoratum* Linn., *Cymbopogon citratus* Stapf., *Ocimum sanctum* Linn., *Ocimum canum* Sims., *Ocimum gratissimum* Linn., *Melissa officinalis* L., *Ocimum basilicum* Linn., *Cinnamomum bejolghota* (Buch. Ham.) Sweet, *Piper sarmentosum* Roxb., *Polygonum odoratum* Lour., *Citrus hystrix* DC., *Citrus aurantifolia* Swing., *Citrus maxima*, *Alpinia galanga* (Linn.) Swartz., *Zingiber officinale* Roscoe, and *Zingiber cassumunar* Roxb., were collected from local farms located in Chiang Mai, Thailand in January 2010. All plants were authenticated and their voucher specimens were deposited in the Herbarium of the Faculty of Pharmacy, Chiang Mai University, Thailand.

2.2. *Chemicals and enzymes*

Trolox, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,4,6-tri(2-pyridyl)-s-

triazine (TPTZ), anhydrous sodium sulphate (Na_2SO_4), sodium carbonate (Na_2CO_3), potassium persulfate ($K_2S_2O_8$), ferrous sulfate ($FeSO_4 \cdot 7H_2O$), and ferric chloride ($FeCl_3 \cdot 6H_2O$) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Mushroom tyrosinase and L-dopa were purchased from Fluka Chemical Co. (Japan). Sodium dihydrogen orthophosphate dehydrate, sodium chloride, and calcium chloride were obtained from Fisher Chemicals (Loughborough, UK). Di-Sodium hydrogen orthophosphate dehydrate was obtained from Ajax Finechem (NSW, Australia). Methanol and ethanol were of analytical grade and obtained from Merck (Darmstadt, Germany).

2.3. *Distillation of essential oils*

The essential oils from a total of twenty plant samples were obtained by hydrodistillation for 3 h using a Clevenger-type apparatus. The yield of each essential oil was determined. These oils were dried over anhydrous sodium sulphate and kept in light-protected containers at 4ºC until analysis. The density of each essential oil was analyzed using a pycnometer.

2.4. *GC-MS*

The essential oils isolated were analyzed using GC-MS. The GC-MS analysis was performed on Agilent 6890 gas chromatograph in electron impact (EI, 70 eV) mode coupled to an HP 5973 mass selective detector and fitted with a fused silica capillary column (HP-5MS) supplied by HP, USA (30.0 m × 250 mm i.d., 0.25 mm film thickness). The analytical conditions were: carrier gas, helium (ca. 1.0 mL/min); injector temperature, 260ºC; oven temperature, 3 min isothermal at 100ºC (no peaks before 100ºC after first injection), 3 ºC/min to 188ºC, and then 20 ºC/min to 280ºC (3 min isothermal); and detector temperature, 280ºC. Programmed-temperature Kováts retention indices (RI) were obtained by GC-MS analysis of an aliquot of the volatile oil spiked with an n-alkanes mixture containing each homologue from n-C11 to n-C27. Identification of the compounds was based on a comparison with a mass spectra database (WILEY&NIST) and spectroscopic data. The percentage of each component was calculated based on the total area of all peaks obtained from the oil.

2.5. *Antityrosinase activity test*

The antityrosinase activity of the oils was determined using the modified dopachrome method with L-dopa as a substrate (*7*). Assays were conducted in a 96-well microtiter plate. Test samples were dissolved in 50% DMSO. Each well contained 40 µL of sample, 80 µL of phosphate buffer solution (PBS) (0.1 M, pH 6.8), 40 µL of tyrosinase (200 units/mL), and 40 µL of L-dopa (2 µM). The microplate reader was read at absorbance of 450 nm. Each sample was accompanied by a blank that had all of the components except L-dopa. Results were compared with a control consisting of 50% DMSO in place of the sample. The percentage tyrosinase inhibition was calculated as follows:

$$\text{Tyrosinase inhibition (\%)} = 100 \times (\Delta A_{Control} - \Delta A_{Sample})/\Delta A_{Control}$$

2.6. *Free radical scavenging activity test*

A free radical scavenging activity test or ABTS assay was performed according to the method described by Tachakittirungrod *et al*. (*8*) with some modifications. An ABTS free radical solution was prepared by mixing 7 mM ABTS with 2.45 mM potassium persulphate ($K_2S_2O_8$) in a ratio of two to three. After incubation in the dark for 16 h, the ABTS free radical solution was then diluted with 20-fold ethanol to obtain absorbance of 0.7 ± 0.1 units at 750 nm. The ABTS free radical solution (180 µL) was mixed with 20 µL of each sample. The disappearance of ABTS free radicals was determined by measuring the decrease in absorbance at 750 nm at the end of 5 min using a 96-well microplate reader. Solutions of Trolox with known concentrations were used to construct a calibration curve. Each sample was measured at 4 concentrations to plot % inhibition *versus* concentration. The results were expressed in terms of Trolox equivalent antioxidant activity (TEAC).

2.7. *Ferric reducing/antioxidant power assay*

The ferric reducing/antioxidant power (FRAP) assay was carried out according to the method described by Okonogi *et al*. (*9*). This assay measures the reducing properties of antioxidants based on the reduction of ferric ion. Therefore, ferrous sulfate ($FeSO_4$) was used for calibration. Briefly, a freshly prepared FRAP solution contained 50 mL of 0.3 M acetate buffer (pH 3.6) plus 5 mL of 10 mM TPTZ solution in 40 mM HCl (previously prepared) and 5 mL of 20 mM ferric chloride. After mixing 180 µL of FRAP solution with 20 µL of each sample, the ferric reducing ability was measured using a 96-well microplate reader at the end of 5 min at an absorbance of 595 nm. The results were reported as equivalent capacity (EC) indicating the ability to reduce ferric ions, expressed as mM $FeSO_4$ equivalents per mg of the oil. Each experiment was done in triplicate.

2.8. *Statistical analysis*

The experiments were done in the triplicate and all data are indicated as mean ± standard deviation. The antityrosinase activity is presented as % enzymatic inhibition. Antioxidant activity in the form of free radical scavenging and ferric reducing/antioxidant

power is presented in terms of TEAC and EC values, respectively. Individual differences were evaluated by one-way ANOVA (post-hoc test). In all cases, $p < 0.05$ indicated a significant difference.

3. Results and Discussion

3.1. *Yield and density of essential oils*

The essential oils obtained were clear liquids with a light yellow color except *C. asiatica* and *E. odoratum*, which had no color. The yield of essential oils ranged from 0.005% to 1.393% (w/w), as shown in Figure 1. The highest content was obtained from the leaves of *C. hystrix*. The whole *C. asiatica* plant had the lowest oil content and this might be because of its nature as a succulent. The density of each essential oil analyzed by the conventional method ranged from 0.761 to 0.941 g/mL, as shown in Table 1.

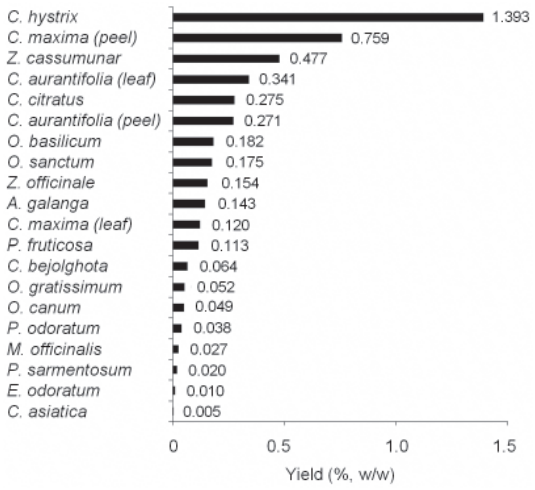

Figure 1. Yield (%, w/w) of essential oils.

3.2. *Antityrosinase activity test*

Tyrosinase, also known as polyphenol oxidase, is a copper-containing enzyme widely distributed in microorganisms, animals, and plants. This enzyme is mainly implicated in two distinct reactions of melanin biosynthesis, including monophenolase and diphenolase activity. After L-tyrosine (monophenol) is hydroxylated by catalysis of tyrosinase, the hydroxylation product named L-dopa (diphenol) is further oxidized into the corresponding *o*-quinone by catalysis of the same enzyme (*10,11*). Tyrosinase catalyzes melanin biosynthesis in human skin, resulting in epidermal hyperpigmentation that leads to various dermatological disorders such as melasma, freckles, and age spots (*12*). Because they inhibit enzymatic oxidation, tyrosinase inhibitors have become increasingly important in medicines (*11*) and cosmetics (*13*) to prevent hyperpigmentation. The antityrosinase activities of twenty essential oils are shown in Figure 2. The highest level of inhibitory activity, 69 ± 4%, was from the essential oil of *C. citratus*, which was followed closely by the inhibitory activity, 66 ± 3%, of the essential oil of *O. canum*.

The chemical composition of these two oils was further investigated by GC-MS and the results are shown in Table 2. Twelve volatiles were identified in the *C. citratus* oil, comprising 89.5% of the total composition. Most of this oil was monoterpene, which comprised up to 85.2% of the oil. The most abundant compositions were geranial (42.0%) and neral (32.1%). These results are in accordance with previously published data on *C. citratus* essential oils (*14,15*). For *O. canum*, thirteen volatiles representing 94.7% of the total oil were identified. Geranial (35.1%) and neral (27.4%) were the major components of this oil. The

Table 1. Density of essential oils of plants used in this study

Plant species	Family	Plant part	Density (g/mL)
Centella asiatica Urban.	Apiaceae	Whole plant	0.920
Polyscias fruticosa Harms.	Araliaceae	Leaf	0.808
Eupatorium odoratum Linn.	Asteraceae	Whole plant	0.900
Cymbopogon citratus Stapf.	Gramineae	Stem	0.886
Ocimum sanctum Linn.	Lamiaceae	Leaf	0.941
Ocimum canum Sims.	Lamiaceae	Whole plant	0.810
Ocimum gratissimum Linn.	Lamiaceae	Leaf	0.833
Melissa officinalis Linn.	Lamiaceae	Leaf	0.883
Ocimum basilicum Linn.	Lamiaceae	Leaf	0.910
Cinnamomum bejolghota (Buch. Ham.) Sweet	Lauraceae	Leaf	0.795
Piper sarmentosum Roxb.	Piperaceae	Leaf	0.813
Polygonum odoratum Lour.	Polygonaceae	Whole plant	0.761
Citrus hystrix DC.	Rutaceae	Leaf	0.823
Citrus aurantifolia Swing.	Rutaceae	Leaf	0.812
		Fruit peel	0.840
Citrus maxima (Burm.) Merr.	Rutaceae	Leaf	0.826
		Fruit peel	0.835
Alpinia galanga (Linn.) Swartz.	Zingiberaceae	Rhizome	0.887
Zingiber officinale Roscoe.	Zingiberaceae	Rhizome	0.856
Zingiber cassumunar Roxb.	Zingiberaceae	Rhizome	0.900

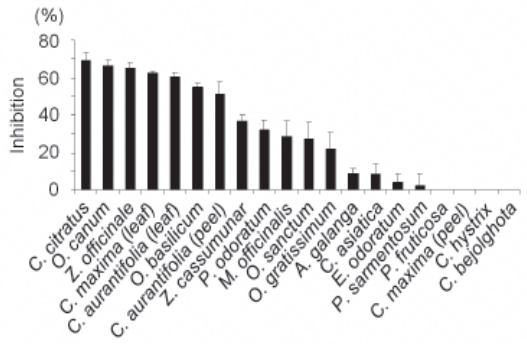

Figure 2. Tyrosinase inhibitory activities of essential oils.

Table 2. Chemical composition of the essential oil of *C. citratus* and *O. canum*

Retention time (min)	Component	Component percentage	
		C. citratus	*O. canum*
3.48	*trans*-beta-ocimene	0.58	
4.07	*n*-undecene	1.4	
4.26	linalool		5.51
5.32	D-camphor	0.49	
5.75	(+)-borneol	0.35	
6.24	alpha-terpineol		0.94
7.09	nerol		4.16
7.25	neral	32.07	27.35
7.55	geraniol	5.21	3.69
8.13	geranial	42.01	35.08
11.05	eugenol		3.09
11.38	piperitenone oxide	2.25	
11.53	decanoic acid	0.79	
11.72	alpha-copaene	1.4	
13.25	caryophyllene		4.87
13.71	alpha-*trans*-bergamotene		1.57
14.41	alpha-humulene		1.29
15.37	germacrene D		1.92
17.51	*cis*-alpha-bisabolene		4.58
18.97	caryophyllene oxide		0.64
20.66	beta-maaliene	0.94	
21.02	alpha-cadinol	2.04	
	Total	89.53	94.69

components of linalool (5.5%), caryophyllene (4.9%), *cis*-alpha-bisabolene (4.6%), and nerol (4.2%) were also present. Of 13 volatiles, monoterpene compounds accounted for most of the *O. canum* oil, comprising 79.9%. The essential oil of *O. canum* has been reported to have a good lemony flavor and high citral content (*16-18*).

3.3. *Antioxidant activities*

Multiple reactions and mechanisms are reportedly involved in antioxidant processes (*19*), *e.g.* free radical scavenging and reducing activity. ABTS and FRAP assays are tests based on different mechanisms of antioxidant action. ABTS indicates the ability of natural antioxidants to scavenge free radicals and is expressed as the TEAC value while FRAP determines the total reducing capacity of the test compound and is expressed as the EC value. Therefore, these two methods were used to investigate the mechanism of antioxidant action of the test samples.

The ABTS test was first introduced in 1993 (*20*) to test biological samples and was then widely used to test the antioxidant activity of other samples. The principle of this method is to monitor the decay of the radical-cation ($ABTS^{\bullet+}$) resulting from the oxidation of ABTS. Since $ABTS^{\bullet+}$ is soluble in both aqueous and organic solvents and is not affected by ionic strength, this method was widely used to indicate the antioxidant activity of samples (*21*). The quantity of $ABTS^{\bullet+}$ consumed due to the reaction is expressed as the TEAC value although TEAC of an individual antioxidant is really the number of the $ABTS^{\bullet+}$ consumed per molecule of antioxidant. The TEAC values of twenty essential oils are shown in Figure 3A. Essential oils obtained from different plant species and plant parts were found to have different potentials. The two highest TEAC values were 1.06 ± 0.01 mM/mL and 1.05 ± 0.01 mM/mL and were from the essential oils of *O. sanctum* and *O. gratissimum*, respectively.

The ferric reducing/antioxidant power (FRAP) assay was originally developed in 1996 (*22*) to measure reducing power in plasma, but the assay has also subsequently been adapted and used to assay antioxidants in many botanicals. This method is based on the ability of the test sample to reduce Fe(III) to Fe(II). In the presence of TPTZ, this reduction is accompanied by the formation of a colored complex with Fe(II). The reducing power of twenty essential oils is shown in Figure 3B in terms of EC. The two highest EC values were 3.74 ± 0.01 mM/mL and 3.26 ± 0.01 mM/mL and were from essential oils of *O. sanctum* and *O. gratissimum*, respectively.

These results clearly indicate that the essential oils of both *O. sanctum* and *O. gratissimum* possess antioxidant activity with respect to mechanisms of both free radical scavenging and reducing activities. Other essential oils were further investigated for these mechanisms. The correlation between TEAC and EC values was plotted and the results are shown in Figure 4. A strong correlation (R^2 = 0.964) was obtained, indicating that most of the essential oils possessed antioxidant mechanisms in the form of both free radical scavenging and reducing power. This result coincided with that of previous studies indicating that the antioxidant compounds from plants generally possess both mechanisms of action (*21*). A study by Nantitanon *et al*. (*23*) found a strong correlation (R^2 of 0.894) between the free radical scavenging activity and reducing power of guava leaf extracts. In addition, Khonkarn *et al*. (*24*) revealed that there was a linear relationship (R^2 = 0.979) between TEAC and EC values of the different fractionated extracts of peels from the three fruits of rambutan, mangosteen, and coconut.

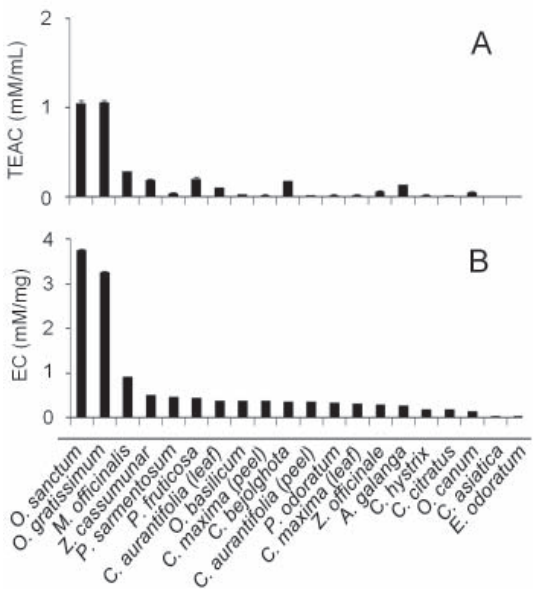

Figure 3. Free radical scavenging activity (A) and ferric reducing/antioxidant power (B) of essential oils.

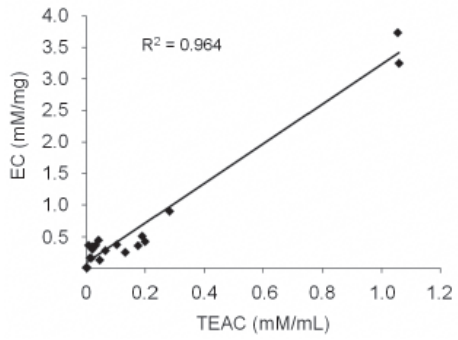

Figure 4. Correlation between free radical scavenging activity and reducing power of essential oils (R^2 = 0.964).

4. Conclusion

Among 20 essential oils from edible Thai plants, the essential oil of *C. citratus* had the highest level of antityrosinase activity, followed by that of *O. canum* with enzymatic inhibition of 69 ± 4 and 66 ± 3%. GC-MS revealed that geranial and neral were the two most abundant components of their chemical compositions. Antioxidant activity was gauged by the free radical scavenging activity test and ferric reducing/antioxidant power assay. The essential oil of *O. sanctum* had the highest level of antioxidant activity, followed by the essential oil of *O. gratissimum*. These results led to the conclusions that the essential oils of several edible plants exhibit important biological activities and are a promising choice as natural active ingredients because of their antityrosinase and antioxidant activities.

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