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Full Length Research Paper

Antimicrobial, anti-tyrosinase and antioxidant activities of aqueous aromatic extracts from forty-eight selected herbs

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In the essential oil extraction process of herbs, the aqueous part of distillate was often been treated as waste. Thus, discovery of potentialities of the aqueous part of herbs will aid us to realize further applications of this byproduct. In this study, we examine the antimicrobial activity, anti-tyrosinase and antioxidant activities of 48 selected herbs which are suitable to be planted in Taiwan and Southeast Asia. Results shown that only 5 herbs such as *Canarium album* and *Cinnamomum japonicum* have antimicrobial activities on *Propionibacterium acne*, *Staphylococcus epidermidis* or *Malassezia furfur*. In addition, *Cinnamomum japonicum* had shown the highest activity up to 83% for the inhibition of dopachrome formation. Moreover, numerous herbs exhibited the potent antioxidant activities, including *Ocimum gratissimum*, *Canarium album*, *Melaleuca alternifolia* and *Origanum majorana*. Therefore, these results of this study may help us to recognize the potentialities of these herbal aqueous aromatic extracts in future.

Key words: Antimicrobial activity, antioxidant activity, aqueous aromatic extract, herbs, inhibition of dopachrome formation.

INTRODUCTION

Plenty of aromatic, medicinal and other herbs contain chemical compounds demonstrating anti-tyrosinase, antioxidant, antimicrobial and other functional properties. Various studies were achieved on some of these herbs in the development of functional ingredients for medical, food and cosmetic applications (Miliauskas et al., 2004; Wu et al., 2011; Yang et al., 2011). Moreover, for the past few years, the requirement for medicine, food and cosmetics containing natural plant extracts have significantly increased, which were caused by their therapeutic properties, charming fragrance, and the general opinion that they are safer than synthetic compounds (Chen et al., 2011; Corazza et al., 2009). In addition, a lot of active components from botanical origin have been shown to exhibit the therapeutic effects for numerous physiological and skin diseases (Buchness, 1998; Huang et al., 2009).

Herbs are plants that are valued for flavor, scent, or other useful qualities. For these reasons, herbs can be used for cooking, as perfumes, as medicines, and for active ingredients in cosmeceutical products (Cornara et al., 2009). Indeed, herb extracts may have antioxidant, anti-tyrosinase, anti-inflammatory, antimicrobial and

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Abbreviations: DPPH, 2,2-diphenyl-1-picrylhydrazyl; **BHA,** butylated hydroxyanisole; **BHT,** butylated hydroxytoluene; **EDTA,** ethylenediaminetetraacetic acid; **RCM,** reinforced clostridial medium; **TSB,** tryptic soy broth; **NB,** nutrient broth; **MEB,** malt extract broth; **MeOH,** methanol; **ddH_2O,** deionized distilled water.

Table 1. Selected herbs in this study.

S/N	Scientific name	S/N	Scientific name
1	*Artemisia princes*	25	*Tagetes patula*
2	*Cinnamomum camphora*	26	*Raphanus sativus*
3	*Plectranthus amboinicus* 'Variegata'	27	*Crossostephium chinense*
4	*Cinnamomum japonicum*	28	*Lonicera japonica*
5	*Mentha aquatica* 'Lime'	29	*Pelargonium grossularioides*
6	*Mentha* × *gracilis*	30	*Pelargonium graveolens*
7	*Mentha* × *piperita* 'Swiss'	31	*Plectranthus amboinicus*
8	*Micromeria thymifolia*	32	*Zanthoxylum ailanthoides*
9	*Artemisia oligoarpa*	33	*Lantana camara*
10	*Luffa cylindrica*	34	*Myristica fragrans*
11	*Nepeta cataria*	35	*Anglica sinensis*
12	*Houttuynia cardata*	36	*Tanacetum vulgare*
13	*Verbena bonariensis*	37	*Cymbopogon citrates*
14	*Eucalyptus globulus*	38	*Perilla frutescens*
15	*Agastache foeniculum*	39	*Melaleuca leucadendron*
16	*Coriandrum sativum*	40	*Rosmarinus officinalis*
17	*Laurus nobilis*	41	*Cananga odorata*
18	*Salvia dorisiana*	42	*Ocimum gratissimum*
19	*Salvia elegans*	43	*Ocimum basilicum*
20	*Salvia officinalis*	44	*Canarium album*
21	*Thymus pulegioide*	45	*Origanum majorana*
22	*Oncidium flexuosum*	46	*Hyptis suaveolens*
23	*Melaleuca alternifolia*	47	*Lavandula angustifolia*
24	*Origanum vulgare*	48	*Lavandula stoechas*

sun- protection functions; they may also enhance wound healing and act as the regulator in cellular and physiological functions (Corazza et al., 2009). Moreover, during the essential oil extraction process of herbs, the aqueous part of distillate (also referred as hydrosol) was often been treated as waste, however, there are a lot of hydrophilic active components were dissolved in aqueous part of distillate. Besides, the aromatic compounds in aqueous solutions also provide the flavor properties of its incorporated products (Tornuk et al., 2011). Therefore, discovering of potentialities of herbs' aqueous part will help the production processes to find extra applications of this byproduct.

In present study, we examine several applicative potentialities of 48 selected herbs which are suitable to be planted in Taiwan and Southeast Asia (Table 1). There are several ordinary herbs used frequently in the production of essential oils or flavor ingredients, such as species of *Artemisia*, *Cinnamomum*, *Mentha*, *Salvia*, *Thymus*, *Cymbopogon*, *Melaleuca*, *Pelargonium*, *Ocimum*, *Canarium*, *Origanum* and *Lavandula*. Besides, we also analyzed some potential herbs whereas which are not common for the production of essential oils including the species of *Luffa*, *Raphanus* and *Anglica*. Thus, to discover these herbs' biological properties, we investigated the antimicrobial, anti-tyrosinase and antioxidant activities of these herbal aqueous aromatic extracts. Furthermore, the antimicrobial activity of the herbal aqueous aromatic extracts was evaluated by the microbial inhibition zone against *Propionibacterium acne*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Malassezia furfur*. Besides, the anti-tyrosinase activity for skin whitening was analyzed through the inhibition ability of dopachrome formation. Moreover, the antioxidant activities of the herbal aqueous aromatic extracts were carried out by various antioxidant assays including 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, reducing power assay and ferrous ion chelating assay. Therefore, these results may help us to know the potentialities of these selected herbal aqueous aromatic extracts in many applications.

MATERIALS AND METHODS

Chemicals

Arbutin, vitamin C (ascorbic acid), tyrosine, DPPH, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), citric acid, ox-bile, potassium ferricyanide and trichloroacetic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium chloride, ethylenediaminetetraacetic acid (EDTA), ferrous chloride, ferrozine, Tween-40 and other chemicals were purchased from

Wako Pure Chemical Industries (Osaka, Japan). Glycerol mono-oleate was purchased from Tokyo Chemical Industry (Tokyo, Japan). Agar, reinforced clostridial medium (RCM), tryptic soy broth (TSB), nutrient broth (NB) and malt extract broth (MEB) were purchased from Difco (Detroit, MI, USA). The methanol (MeOH), ketoconazole, triclosan and zinc pyrithione were purchased from Merck (Darmstadt, Germany). Deionized distilled water (ddH_2O) for solutions and buffers was obtained from the Milli-Q system (Millipore, Bedford, MA, USA).

Preparation of aromatic extracts

Forty eight selected herbal aqueous aromatic extracts (Table 1) were provided by the Fengshan Tropical Horticultural Experiment Branch, Agricultural Research Institute (ARI, Fengshan, Kaohsiung, Taiwan, R.O.C.).

Three hundred grams of dried herb was mixed with 30 kg ddH_2O and then distilled at 100°C to collect the distillate of the 20% volume of front fraction. Subsequently, the collected cooling distillates include aqueous part and essence parts were separated using a separatory funnel. The original herbal aqueous aromatic extracts were filtered by a 0.45 µm filter and then defined as 1 arbitrary unit (a.u.)/ml.

Antimicrobial activity

The strains, *Propionibacterium acne* (BCRC 10723), *Staphylococcus aureus* (BCRC 10451), *Staphylococcus epidermidis* (BCRC 10783) and *Malassezia furfur* (BCRC 22243) were purchased from Food Industry Research and Development Institute (FIRDI, Hsinchu, Taiwan, R.O.C.) and used for antimicrobial activity assays. For the bacteria cultivation, *P. acne*, *S. aureus* and *S. epidermidis* were incubated at 37°C on the reinforced clostridial medium (RCM), tryptic soy broth (TSB) and nutrient broth (NB), respectively (Qadan et al., 2005). Besides, *M. furfur* was cultured at 30°C on the Dixon culture medium (MEB with 2% ox-bile, 1% Tween-40 and 0.25% glycerol mono-oleate) (Murai et al., 2002). For antimicrobial activity assays, a single colony was picked up and seeded in the fresh medium, and then cultivated at each temperature for 12 h. After incubation, the cultured broths were added to the correspondent agar plates and then spread out evenly on the plates.

The antimicrobial agents, triclosan, ketoconazole and zinc pyrithione, at concentrations of 5 and 10 mg/mL were used as positive control for antimicrobial activity assays. The 40 µl of each antimicrobial agent and each herbal aqueous aromatic extracts with an 8 mm diameter filter paper disc were placed individually on the surface of prepared agar plates at each temperature for 24 h. The microbial inhibition zone diameter was measured by a rule to exhibit the antimicrobial activity (Al-Hussaini and Mahasneh, 2009).

Inhibition of dopachrome formation

For the inhibition of dopachrome formation assay, 0.1 mg/ml of arbutin and kojic acid were used as the control inhibitors. The 0.9 mL phosphate buffer solution (pH 6.8) with 1 ml 0.03% (w/w) tyrosine solution and 1 ml sample (inhibitor or herbal aqueous aromatic extract) were incubated for 10 min at 37°C, and then added 0.1 mL mushroom tyrosinase (Sigma-Aldrich, St. Louis, MO, USA) solution (350 units/ml) to the mixture for a further 25 min incubation at 37°C.

The spectrophotometric analysis was performed at 475 nm and the inhibition of dopachrome formation was calculated as inhibition percentage (Song et al., 2009).

Scavenging effect on 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals

For DPPH scavenging effect, each 0.1 ml of herbal aqueous aromatic extracts diluted with 0.4 ml Tris-HCl buffer (100 mM, pH 7.4) and each 0.1 mg/mL antioxidants BHA, BHT, and vitamin C were individually mixed with 0.5 ml of methanolic solution containing DPPH radicals and the final concentration of DPPH was 0.25 mM. The mixture was shaken vigorously and left to stand for 20 min at 25°C in the dark, and the absorbance was then determined at 517 nm (Yamauchi et al., 1995).

Reducing power

The reducing power assay was measured according to earlier study with some modifications (Amarowicz et al., 2009). Each 5 mL of herbal aqueous aromatic extracts and each 0.1 mg/mL of antioxidants BHA, BHT, and vitamin C were individually mixed with 5 mL sodium phosphate buffer (0.2 M, pH 6.6) and 1 mL 1% potassium ferricyanide, and then the mixture was incubated at 50°C for 20 min. After trichloroacetic acid (1 ml, 10%, w/v) was added, the mixture was centrifuged at 4°C, 3000 rpm for 10 min. The 5 ml supernatant was mixed with 5 mL ddH_2O and 1 ml ferric chloride (0.1%). After 10 min at 25°C, the absorbance of mixture was determined at 700 nm (OD_{700}). The reducing power of vitamin C was defined as 100%.

Chelating effect on ferrous

For chelating effect on ferrous, each 1 ml of herbal aqueous aromatic extracts and each 0.1 mg/mL of EDTA and citric acid were individually mixed with 3.7 ml sodium phosphate buffer (0.05 M, pH 7.4), 0.1 ml ferrous chloride (2 mM) and 0.2 ml ferrozine (5 mM). After 10 min at 25°C, the absorbance of the mixture was measured at 562 nm. The lower absorbance indicates the higher chelating effect (Dinis et al., 1994).

Statistical analysis

Quantitative data of the present study were analyzed using Student's *t*-tests and presented as means ± S.D. for three independent experiments.

RESULTS AND DISCUSSION

Forty eight selected herbs are wildly used in many aspects including spices, foods and cosmetics, and which were suitable to be planted in Taiwan and Southeast Asia (Table 1). These herbs were distilled to collect the aqueous aromatic extracts, and the original extract was defined as 1 a.u./ml. If the extracted aromatic components in the aqueous solution were dehydrated to identify the quantity of extract, a variety of low-boiling-point aromatic compounds may remove with water simultaneously. Thus, use the volume to define the experimental quantity of extract is a appropriate method for this study (Ulusoy et al., 2009). For that reason, we utilized the original extracts for this study to discover the potentialities of these herbal aqueous aromatic extracts.

For antimicrobial activity assays, *P. acne*, *S. aureus*,

Table 2. Antimicrobial activity of some herbal aqueous aromatic extracts.

S/N	Scientific name	Inhibition zone diameter (mm)		
		Propionibacterium acne	***Staphylococcus epidermidis***	***Malassezia furfur***
3	*Plectranthus amboinicus* 'Variegata'	10.9 ± 0.4	—	—
4	*Cinnamomum japonicum*	—	—	18.3 ± 0.6
23	*Melaleuca alternifolia*	11.5 ± 0.5	—	—
31	*Plectranthus amboinicus*	10.7 ± 0.6	—	—
44	*Canarium album*	15.2 ± 0.2	21.0 ± 1.0	—
	Triclosan (10 mg/ml)	10.5 ± 0.7	18.3 ± 0.6	—
	Triclosan (5 mg/ml)	—	11.7 ± 0.3	—
	Ketoconazole (10 mg/ml)	18.8 ± 0.3	—	—
	Ketoconazole (5 mg/ml)	10.9 ± 0.7	—	—
	Zinc pyrithione (10 mg/ml)	10.1 ± 0.3	12.1 ± 0.5	23.8 ± 1.0
	Zinc pyrithione (5 mg/ml)	—	10.3 ± 0.6	11.0 ± 0.4

Each value is expressed as mean ± S.D. (n = 3). —: no inhibition.

S. epidermidis and *M. furfur* were used for the test with the diameter of inhibition zone. Triclosan, ketoconazole and zinc pyrithione were used as positive control at the concentrations of 5 and 10 mg/mL and the selected results were listed in Table 2. Besides, diameter of inhibition zone over 10 mm was defined as an antimicrobial activity-containing extract. These results shown that only 5 herbs have antimicrobial activities to *P. acne*, *S. epidermidis* or *M. furfur* (Table 2). For the diameter of inhibition zone on *P. acne*, *Plectranthus amboinicus* 'Variegata' (no. 3), *Melaleuca alternifolia* (no. 23), *Plectranthus amboinicus* (no. 31) and *Canarium album* (no. 44) have clear antimicrobial activities. In addition, results indicated that there is no herbs' aqueous aromatic extract has the antimicrobial activity to *S. aureus*. For *S. epidermidis*, only *C. album* (no. 44) exhibit a diameter of 21.0 ± 1.0 mm, which was even greater than that of the control antimicrobial agents (Table 2). Moreover, *Cinnamomum japonicum* (no. 4) was also the only one herb reveal significant antimicrobial activity to *M. furfur* (Table 2).

Major antimicrobial activities are often exhibit by compounds in the parts of ethanol extract or essential oil in plants, such as 1,8-cineole and terpinen-4-ol of *M. alternifolia* essential oil (Halcon and Milkus, 2004; Kwiecinski et al., 2009) or cinnamic aldehyde of *C. japonicum* (He et al., 2005; Juglal et al., 2002). Therefore, we can suggest that some of the antimicrobial components were dissolved in the aqueous part of plant extract. In previous research, *Plectranthus elegans* display the evident antimicrobial activities on *S. aureus*, *Bacillus subtilis* and *Streptomyces scabies* (Dellar et al., 1996). On the other hand, the aqueous aromatic extract of *P. amboinicus* 'Variegata' (no. 3) and *P. amboinicus* (no. 31) have no effect on the growth of *S. aureus* (data not show) but *P. acne* (Table 2) in this study. These might be due to the fact that the main effective antimicrobial components for *S. aureus* of various plants were not hydrophilic compounds (Ameri et al., 2011; Daniyan and Muhammad, 2008; Sukanya et al., 2009). Thus, the aqueous aromatic extract of these selected herbs in this study have no antimicrobial activity to *S. aureus*. Furthermore, it is just a few studies have reported that *C. album* (no. 44) have antimicrobial activity, however, our results indicated that the aqueous aromatic extract of *C. album* (no. 44) may obviously inhibited the growth of *P. acne* and *S. epidermidis*.

For the inhibition of dopachrome formation assay, mushroom tyrosinase was utilized to test the skin-whitening potentiality of these selected herbal aqueous aromatic extracts. 0.1 mg/ml of arbutin and kojic acid were used as the control inhibitors and the results were shown in Table 3. Results indicated that *C. japonicum* (no. 4), *Ocimum gratissimum* (no. 42), *Ocimum basilicum* (no. 43) and *C. album* (no. 44) have activities to inhibit the dopachrome formation over than 35%. Additionally, *C. japonicum* (no. 4) had shown the highest inhibition activity about 83% (Table 3), which was higher than that of 0.1 mg/ml arbutin (36.8%) and kojic acid (65.7%).

Ngoc et al. (2009) have reported that a methanol extract of the twigs of *Cinnamomum cassia* have the inhibitory activity for tyrosinase. In addition, cinnamic acid, generally found in *Cinnamomum cassia* BLUME and *Panax ginseng*, was also exhibit a anti-tyrosinase activity with an IC_{50} is 693.2 μM (Kong et al., 2008). Moreover, cinnamic acid is slightly soluble in water; therefore, it is one of the possible active compounds in aqueous aromatic extract of *C. japonicum* (no. 4). The tyrosinase inhibition activities of methanol, acetone and water extract of *Ocimum americanum* have studied by Khanom et al. (2000), and results indicated that the methanol and water extract of *O. americanum* have obvious tyrosinase inhibition activity. Hence, the inhibition activities of dopachrome formation of *O. gratissimum*

Table 3. Anti-tyrosinase and antioxidant activities of herbal aqueous aromatic extracts.

S/N	Inhibition of dopachrome formation (%)	DPPH scavenging effect (%)	Relative reducing power (%)	Chelating effect (%)
1	-10.4 ± 3.9	13.1 ± 2.5	2.9 ± 0.2	7.6 ± 0.3
2	0.9 ± 0.4	1.6 ± 0.4	3.6 ± 0.2	0.7 ± 1.1
3	9.4 ± 0.6	15.7 ± 1.6	17.1 ± 0.1	46.5 ± 5.5
4	82.9 ± 0.7	8.6 ± 0.7	8.5 ± 0.5	1.7 ± 0.3
5	1.6 ± 0.8	0.6 ± 0.3	9.7 ± 0.2	0.2 ± 0.2
6	7.5 ± 1.4	0.4 ± 0.1	3.0 ± 0.2	0.2 ± 0.6
7	2.2 ± 1.7	3.1 ± 0.8	57.7 ± 0.2	57.0 ± 4.1
8	1.7 ± 1.3	6.5 ± 1.6	9.7 ± 0.4	0.1 ± 0.2
9	6.7 ± 1.8	4.2 ± 0.7	9.3 ± 0.2	86.3 ± 1.5
10	0.9 ± 0.6	-1.1 ± 0.2	-4.3 ± 0.1	85.2 ± 2.8
11	9.8 ± 0.3	69.2 ± 2.2	9.3 ± 0.1	0.4 ± 0.5
12	1.3 ± 0.8	7.5 ± 2.8	3.3 ± 0.1	21.0 ± 2.6
13	33.9 ± 0.5	6.5 ± 1.2	9.6 ± 0.6	47.0 ± 6.6
14	5.4 ± 1.2	6.3 ± 0.6	4.0 ± 0.1	0.7 ± 0.5
15	1.9 ± 0.4	6.4 ± 3.3	5.9 ± 0.4	0.9 ± 1.4
16	0.7 ± 0.6	-2.4 ± 0.9	3.2 ± 0.6	0.2 ± 0.4
17	4.6 ± 0.5	9.8 ± 0.2	10.2 ± 0.4	0.4 ± 0.7
18	4.3 ± 1.1	2.4 ± 1.7	5.0 ± 0.3	0.5 ± 0.8
19	4.5 ± 2.6	10.2 ± 1.6	12.6 ± 3.1	19.4 ± 5.6
20	1.7 ± 0.2	0.4 ± 0.1	4.0 ± 0.1	0.3 ± 0.8
21	2.0 ± 1.2	2.6 ± 0.7	7.5 ± 0.2	0.9 ± 3.9
22	-1.9 ± 0.7	-1.7 ± 0.4	2.3 ± 0.2	0.6 ± 0.4
23	27.2 ± 2.4	5.2 ± 0.7	2.8 ± 0.3	97.3 ± 1.3
24	4.7 ± 1.2	9.6 ± 0.1	11.7 ± 0.2	0.5 ± 0.9
25	10.4 ± 1.4	4.7 ± 1.8	17.1 ± 0.5	2.0 ± 1.9
26	22.5 ± 0.6	51.4 ± 1.9	38.3 ± 0.6	19.3 ± 1.7
27	2.0 ± 0.2	6.8 ± 1.4	9.1 ± 0.1	9.1 ± 4.8
28	24.4 ± 5.3	-1.3 ± 0.4	1.5 ± 0.1	0.4 ± 0.5
29	6.8 ± 0.9	68.2 ± 2.4	70.5 ± 0.8	39.2 ± 1.7
30	3.6 ± 2.2	0.8 ± 0.1	4.1 ± 0.1	0.1 ± 0.9
31	14.9 ± 1.4	26.5 ± 1.5	21.5 ± 0.6	66.2 ± 2.4
32	-2.2 ± 0.9	0.9 ± 0.2	2.0 ± 0.4	0.3 ± 0.4
33	-3.6 ± 1.7	-0.8 ± 0.2	2.2 ± 0.1	0.2 ± 0.9
34	1.3 ± 0.8	9.8 ± 0.8	1.0 ± 0.2	0.2 ± 0.8
35	5.1 ± 0.4	4.1 ± 0.8	0.9 ± 0.3	0.1 ± 0.4
36	0.4 ± 0.1	5.9 ± 0.9	7.0 ± 0.8	8.2 ± 2.4
37	20.9 ± 1.2	15.4 ± 1.9	12.5 ± 0.3	7.4 ± 1.9
38	3.5 ± 0.8	0.2 ± 0.1	1.8 ± 0.1	8.3 ± 2.6
39	5.5 ± 2.2	8.9 ± 1.8	12.6 ± 3.1	88.6 ± 0.8
40	26.8 ± 7.9	10.8 ± 0.4	9.1 ± 1.0	11.7 ± 1.0
41	-19.8 ± 0.7	15.3 ± 1.0	13.1 ± 0.2	10.2 ± 2.0
42	53.8 ± 0.4	78.3 ± 0.2	93.0 ± 1.2	87.2 ± 6.6
43	39.2 ± 4.7	1.8 ± 0.5	14.0 ± 1.8	20.6 ± 8.4
44	35.8 ± 0.4	94.7 ± 0.3	86.8 ± 3.9	0.1 ± 0.3
45	21.8 ± 3.2	23.3 ± 0.7	17.3 ± 0.2	97.6 ± 0.7
46	24.8 ± 2.5	2.1 ± 0.8	22.2 ± 1.2	14.0 ± 1.2
47	1.9 ±1.3	1.7 ± 1.6	7.9 ± 0.4	18.4 ± 4.9
48	5.9 ±1.2	3.1 ± 0.9	8.8 ± 0.3	65.2 ± 9.1
Arb*	36.7 ±4.4	—	—	—
KA*	65.7 ± 1.5	—	—	—

Table 3. Contd.

BHA*	—	46.2 ± 0.1	72.4 ± 0.3	—
BHT*	—	31.4 ± 0.2	60.8 ± 0.1	—
VC*	—	68.7 ± 0.5	100.0 ± 0.0	—
EDTA*	—	—	—	65.1 ± 0.8
CA*	—	—	—	34.5 ± 0.1

Each value is expressed as mean ± S.D. (n = 3), —: not tested. *Controls (0.1 mg/mL), Arb: arbutin, KA: kojic acid, VC: vitamin C, CA: citric acid.

(no. 42) and *O. basilicum* (no. 43) can be forecasted in this study.

For the antioxidant activities of the herbal aqueous aromatic extracts, we used various antioxidant assays including DPPH radical scavenging assay, reducing power assay and ferrous ion chelating assay. These results were shown in Table 3.

The results of DPPH radical scavenging assay indicated that the herbal aqueous aromatic extract of *Nepeta cataria* (no. 11), *Raphanus sativus* (no. 26), *Pelargonium grossularioides* (no. 29), *O. gratissimum* (no. 42) and *C. album* (no. 44) have potent activities to scavenge DPPH radicals (Table 3). Moreover, the DPPH radical scavenging activity of *N. cataria* (no. 11), *P. grossularioides* (no. 29), *O. gratissimum* (no.42) and *C. album* (no. 44) were 69.2, 68.2, 78.3 and 94.7%, respectively (Table 3). These scavenging activity of extracts were even higher than that of 0.1 mg/ml BHA (46.2%), BHT (31.48%) and vitamin C (68.7%). Besides, for the reducing power assay, *P. grossularioides* (no.29), *O. gratissimum* (no. 42) and *C. album* (no.44) also exhibited a great relative reducing power (reducing power of vitamin C was defined as 100%) of 70.5, 93.0 and 68.6%, respectively (Table 3). Hence, the three herbs, *P. grossularioides* (no. 29), *O. gratissimum* (no. 42) and *C. album* (no. 44), have both powerful antioxidant activities of DPPH radical scavenging and reducing power. Furthermore, *Mentha* × *piperita* 'Swiss' (no. 7) has 57.7% relative reducing power (Table 3). This result of *Mentha* × *piperita* 'Swiss' (no. 7) was obviously different from that of other herbs of *Mentha* species including *Mentha aquatica* 'Lime' (no. 5) and *Mentha* × *gracilis* (no. 6). Therefore, the aqueous aromatic extract of *Mentha* × *piperita* 'Swiss' (no. 7) has a great reducing power but DPPH radical scavenging activity (Table 3).

Herbs have a large amount of natural antioxidants. Because of these natural antioxidative compounds or phytochemical antioxidants are the secondary metabolites of plants (Ghasemzadeh et al., 2010). Carotenoid, flavonoids, cinnamic acid, benzoic acid, folic acid, ascorbic acid (vitamin C) and tocopherol (vitamin E) are antioxidants produced by plants for their own sustenance (McCall and Frei, 1999). Although many natural antioxidants of plants are insoluble or slightly-soluble in water, there are still a variety of potent antioxidants may extracted by aqueous solution. Oboh et al. (2005) have reported that the potent antioxidant activities of O. *gratissimum* (no. 42) was performed by the abundant vitamin C in water extract and flavonoids/phenolic compounds in methanol extract (Mahapatra et al., 2009). Moreover, in previous study, *Canarium* species have several antioxidants including oleuropein, oleuropein aglycons, hydroxytyrosol (Morello et al., 2005) and some tannins such as procyanidins and prodelphinidins (Zhang and Lin, 2008).

For the ferrous ion chelating effect of selected herbal aqueous aromatic extracts, several herbs demonstrated the superior chelating effect and which were higher than that of 0.1 mg/ml control chelators, EDTA and citric acid. These chelating effect of *Artemisia oligoarpa* (no. 9), *Luffa cylindrica* (no. 10), *M. alternifolia* (no. 23), *Melaleuca leucadendron* (no. 39) *O. gratissimum* (no. 42) and *Origanum majorana* (no. 45) were 86.3, 85.2, 97.3, 88.6, 87.2 and 97.6%, respectively (Table 3). Although, there are several herbs displayed the excellent ferrous ion chelating effect, some of the herbal aqueous aromatic extracts revealed that no effect on ferrous ion chelating (Table 3), however, we can still find such potential herbal aqueous aromatic extracts have the ferrous ion chelating effect. In addition, the antioxidant activity of the essential oil of *M. alternifolia* (no. 23) have been reported previously, and the result was shown that essential oil of *M. alternifolia* (no. 23) exhibit a potent free radical scavenging activity whereas the ferrous ion chelating effect was not analyzed (Kim et al., 2004; Miguel, 2010). Thus, our results may also discover the new effective functions of these herbal aqueous aromatic extracts.

In summary, we analyzed the biological properties of 48 selected herbal aqueous aromatic extract. Results indicated that the *C. album* (no. 44) and *C. japonicum* (no. 4) have obvious antimicrobial activities on *P. acne*, *S. epidermidis* or *M. furfur*. Besides, *C. japonicum* (no. 4) had also shown the highest activity up to 83% for the inhibition of dopachrome formation. Moreover, numerous herbs exhibited the potent antioxidant activities, including *O. gratissimum* (no. 42), *C. album* (no. 44), *M. alternifolia* (no. 23) and *O. majorana* (no. 45). Therefore, these results of this study may help us to recognize the

potentialities of these herbal aqueous aromatic extracts in future.

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