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Extraction Essential Oils from *Ocimum Gratissimum* L. , *Ocimum Basilicum* L. and *Rosmarinus Officinalis* L. Cultivated in Vietnam Using Steam Distillation Method

Nguyen N. Hai,[a] Phan N. Duy,[a] Tran T. T. Tra,[a] Nguyen B. Viet,[b] Nguyen T. N. Lan,[a] Mai H. Cang[a]*

[a]*Department of Natural Products, Faculty of Chemical Engineering and Food Technology, Nong Lam University of Ho Chi Minh city, zone 6, Linh Trung ward, Thuduc city, Ho Chi Minh city, Vietnam*

[b]*Department of Chemical Engineering, Faculty of Chemical Engineering and Food Technology, Nong Lam University of Ho Chi Minh city, zone 6, Linh Trung ward, Thuduc city, Ho Chi Minh city, Vietnam*

Abstract

In this study, essential oils (EO) of *Ocimum gratissimum* L., *Ocimum basilicum* L. (basil) and *Rosmarinus officinalis* L. (rosemary) originated in Vietnam were extracted by using steam distillation method. GC/MS analysis indicated that the major components in *O. gratissimum* EO: Eugenol (68.7 %), cis-*β*-Ocimene (8.2 %), D-Germacrene (11.7 %) and others. *O. basilicum* EO: Estragole (84.8 %), trans-*α*-Bergamotene (2.7 %), Linalool (1.9 %) and *Rosemarinus officinalis* EO: D-*α*-Pinene (22.1 %), Eucalyptol (17.0 %) and Verbenone (14.9 %). The results showed that the antioxidant ability of *O. gratissimum* EO by DPPH method and ABTS method have the highest value of IC_{50}. In DPPH method, IC_{50} of *O. gratissimum* EO is 5.1 (µg/ml), IC_{50} of *O. basilicum* EO is 18.2 (µg/ml), IC_{50} of *Rosemarinus officinalis* EO is 42.0 (µg/ml), which are lower than that of Vitamin C (IC_{50} = 4.8 (µg/ml) as control sample. In ABTS method, IC_{50} of *O. gratissimum* EO = 2.9 (µg/ml), IC_{50} of *O. Basil* EO = 10.4 (µg/ml), IC_{50} of *Rosemary* EO = 42.8 (µg/ml), which are lower than that of Vitamin C (IC_{50} = 2.3 (µg/ml)) as control sample.

Keywords: Ocimum gratissimum L; Ocimum basilicum L; Rosmarinus officinalis L; essential oils.

1. Introduction

Essential oils are aromatic, volatile liquids extracted from plant organs such as flowers, seeds, leaves, etc. For thousand years, essential oils are always the important materials for food cosmetic and pharmaceutical industry. One of the most popular source of essential oils are the plants of Lamiaceae family such as *Ocimum gratissimum* L., *Ocimum basilicum* L., and *Rosmarinus officinalis* L. These plants have a long history application as medicinal herbs and spices (to enhance the flavor and organoleptic properties). Their essential oil are well known for specific odor and a high antioxidant capacity [1]. Previous studies also revealed that these essential oils are rich in phenolic and terpenoid compounds including eugenol, estragole and eucalyptol. These bioactive compounds are not only useful for the cosmetic products but also functional food and drugs due to their potential health benefits such as antimicrobial, anti-bacterial, anti-fungal, astringent, antioxidation, and anti-inflammatory properties [2]. For example, Basil essential oils were applied to inhibit some strains of intestinal diseases such as *Escherichia coli*, *Salmonella typhimurium*, *Shigella*, *Bacillus cereus* [3]. For *Rosmarinus* and *Gratissimum* essential oils, they can contribute into preventing the growth of both bacteria and fungus such as *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger* [4-7].

Nowadays, steam distillation is the most common technology to extract and recover essential oils from *Lamiaceae* plants [5, 8-10]. Besides, some assisting technologies including ultrasound and microwave were also applied to shorting extraction time and improve the recovery yield [11]. However, the extraction conditions of essential oils from Lamiaceae plants were not well-studied and the purification step was often ignored. This not only affects the chemical profiles of extracted essential oils but also the quality of product. In fact, essential oils extracted using steam distillation method often contains some unwanted minor compounds which can have negative effect on the customer health [12]. Therefore, the purification is

*Corresponding author e-mail: maihuynhcang@hcmuaf.edu.vn; (Mai H. Cang).

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a necessary step to enhance the quality of essential oils. Various methods can be applied to purify essential oils including hydro-distillation, crystallization and second distillation [11-15] but the combination of these methods has been rarely studied.

The aim of this study, therefore, was to apply steam distillation method and various post-treatments (including hydro-distillation, crystallization, precipitation and second distillation) to extract and purify essential oils from three Vietnamese Lamiaceae plants (*Ocimum gratissimum* L., *Ocimum basilicum* L., and *Rosmarinus officinalis* L.*)*. The chemical composition profile of obtained essential oils was analysed using GC-MS and their antioxidant activity was estimated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay.

2. Material and methods

2.1. Materials

Ocimum gratissimum plants and rosemary plants were collected from Highland provinces, Vietnam while basil plants were collected from Ho Chi Minh City, Vietnam. The moisture content of plant materials are 83.0 %, 89.4 % and 73.9 %, respectively. They were washed with water, drained, then stored in a fridge (4 °C). Chemical compounds (DPPH, ABTS, salt) were in analytical grade and were provided by Sigma- Aldrich Ltd.

2.2. Essential oil extraction and purification

Plant materials were mixed with distilled water at different ratios (1:3, 1:4 and 1:5 g/mL). Esential oils were extracted from the pureer using the steam distillation method at 100 °C for 2-6 hrs. Steam distillation is a method that uses water to remove the essential oil from the plant material. Heat is applied to the water, which produces steam. The steam rises and moves through a chamber holding the plant material. The temperature of the system must remain within a strict range; too low and the essential oils will not be distilled, too high and there is risk of damaging the essential oils or collecting unwanted, non-aromatic compounds. Since the steam generator is outside of the distillation unit, the ambient temperature at which the material to be distilled is located is kept below 100°C and the occurrence of impairments due to the heat effect can be prevented or reduced.

For essential oil purification, four methods were applied including using anhydrous sodium sulfate, crystallization, sedimentation and second distillation.

Purification using anhydrous sodium sulfate: 1mL distillate mixture was blended with 1g anhydrous sodium sulfate for 5 min. Then, the essential oil was collected from the upper phase of mixture.

Purification using the crystallization method: 1mL distillate mixture was crystallized at 0-5 °C to remove water.

Purification using the sedimentation method: Distillate mixture was stored at ambient temperature for 24 hrs. Then the essential oil was collected from the upper phase of mixture.

Purification using the second distillation method: 1st distillate mixture was mixed with water at ratio of 1/10 (v/v) and was distilled at 100 °C for 180 min. Then, the essential oil was collected from the upper phase of 2nd distillate mixture.

All the experiments were repeated three times and average values were expressed. To compare the efficiencies of experiments, obtained essential oil, after being unhydrated using sodium sulfate was evaluated for the essential oil quantity (mL/g dry weight of material). The essential oils product were stored into a dark, sealed vials for further analysis.

2.3. GC/MS analysis

Chemical composition of essential oils was determined by GC/MS analysis using GC Agilent 6890 N instrument coupled with HP5-MS column and MS 5973 inert [16]. Briefly, 25µL essential oil was diluted into 1mL *n*-hexane before injected into system. The pressure of head column was of 9.3 psi and the flow rate of gas was 1.5 mL/min while the injector temperature was 250 ^{o}C. Oven temperature was initiated at 50 ^{o}C for 2 min before was heated to 80 ^{o}C, 150 °C, 200 °C and 300 °C with the rates of 2 ^{o}C /min, 5 ^{o}C /min, 10 ^{o}C /min and 20 ^{o}C /min, respectively. Finally, samples were held at 300 °C for 5 min. To identify chemical compounds, obtained data were compared with the database of Wiley and NIST libraries.

2.4. Determination of antioxidant activity

The free radical scavenging activity of samples was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays (Ref). The DPPH stock solution was prepared by dissolving 24 mg of DPPH in 100 mL methanol. This solution was kept in dark room for 24 hrs before using. Then, dilute the sample in methanol absolute to an appropriate concentration. 0.5 mL of samples was mixed with 1.5 mL of DPPH stock solution and the mixtures were incubated at 25 ^{o}C (in the dark room) for 30 min. The absorbance of sample was recorded at 517 nm using a spectrophotometer (Cary 60, Agilent, Germany).

For ABTS, stock solution was prepared by adding 10 mL of 7.4 mM ABTS solution in 10 mL of 2.6 mM

$K_2S_2O_8$. This mixture was diluted in methanol with a ratio of 1:60. Then, 0.5 mL of samples with different concentrations was mixed with 1.5 mL of ABTS methanol solution and was kept in dark for 30 min at room temperature. The absorbance of sample was recorded at 734 nm using a spectrophotometer (Cary 60, Agilent, Germany).

For both DPPH and ABTS assay, the scavenging activity (%) was calculated by ((Ablank – Asample)/Asample) x 100. In which, methanol absolute was used as the blank sample. The antioxidant activity of samples is expressed as the IC50 which is the requirement concentration of samples to cause a 50 % decrease in the initial DPPH/ABTS concentration.

2.5. Statistical analysis

All experiments were conducted triplicate. The results were perfomed as the mean ± standard deviation. Statistical analysis was perfomed using software Statgraphics (v15, Statgraphics Technologies, USA). ANOVA and Tukey comparision were used to evaluate the effect of extraction and purification conditions on the recovery yield of essential oils and a value of $p < 0.05$ was considered as statistically significant.

3. Results and discussion

3.1. Essential oils extracted by steam distillation method

The effect of extract conditions on the obtained amount of essential oils from *Ocimum gratissimum* L., *Ocimum basilicum* L., and *Rosmarinus officinalis* L. were summarized in Fig. 1. Firstly, one-way ANOVA showed that both the material: solvent ratio and the distillation time affected significantly the essential oil extraction of all investigated plant materials ($p < 0.05$). In general, the amount of extracted essential oil was improved when increasing the material: solvent ratios as well as prolonging the distillation time but the efficiency of extraction also depended on the type of plant materials. For example, the essential oil yield extract of *Ocimum gratissimum* L. was enhanced approximately of 25 % when increasing material: solvent ratio of this plant from 1:3 to 1:5. However, for *Ocimum basilicum* L. and *Rosmarinus officinalis* L., the maximum yield extract was obtained when the ratio between plant materials and solvent was of 1:4 (v/w).

Because the steam distillation method is the combination of various transport phenomena including diffusion, osmosis, dissolution, and steam attraction, the distillation time often plays an important role in the separation efficiency of essential oil. In the first period, the material is exposed to saturated steam and the diffusion is quite easily. As prolonging distillation, the content of essential oils inside and outside the cell will reach to the equilibrium and therefore the amount of extracted essential oil will not be improved significantly ($p < 0.05$). According to Figure 1b, the appropriate time for extracting essential oils from *Ocimum gratissimum* L., *Ocimum basilicum* L. and *Rosmarinus officinalis* L. using steam distillation method is of 4 hrs.

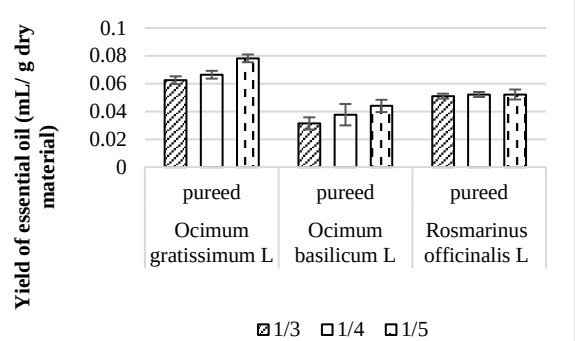

Fig. 1a

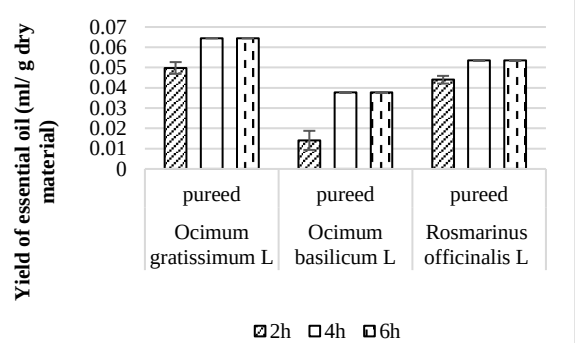

Fig. 1b

Fig. 1. The effect of material : solvent ratio (a) and distillation time (b) on the yield of essential oils.

Table 1. The characterization of essential oil with different purification method.

Properties	Methods	Essential oils		
		***Ocimum gratissimum* L.**	***Ocimum basilicum* L.**	***Rosmarinus officinalis* L.**
Color (Lab)	Hydro-distillation	Lightness (L): 34.9± 1.2 Yellowness (b): 16.4±0.7 Redness (a): 17.9±0.6	L: 39.6± 0.8 b: 12.5±0.5 a: 15.0±0.3	L: 32.9± 1.2 b: 16.4±0.7 a: 17.9±0.6
	Crystallization	L: 35.2± 0.9 b: 14.5±0.7 a: 14.3±0.5	L: 40.2± 1.8 b: 10.5±0.5 a: 12.2±0.5	L: 34.3± 1.7 b: 15.0±0.7 a: 15.3±0.2
	Precipitation	L: 35.9± 1.2 b: 13.8±0.7 a: 13.9±0.5	L: 41.0± 85 b: 10.2±0.5 a: 11.5±0.8	L: 33.2± 1.4 b: 13.0±0.4 a: 14.6±0.6
	2nd distillation	L: 36.3± 1.1 b: 12.8±0.6 a: 12.9±0.5	L: 41.7± 0.9 b: 9.8±0.5 a: 10.5±0.7	L: 35.4± 0.9 b: 12.7±0.8 a: 13.5±0.8
	Combination	L: 37.3± 0.8 b: 11.4±0.7 a: 11.9±0.6	L: 46.2± 1.2 b: 9.2±0.4 a: 9.6±0.4	L: 36.3± 0.8 b: 11.5±0.2 a: 12.5±0.9
Odor	Hydro-distillation	Leaf scent, slightly sweet, soft smell.	Leaf scent, slightly spicy	Fragrant, soft smell, slightly spicy.
	Crystallization	Leaf scent, slightly sweet.	Leaf scent, soft smell.	Fragrant, fresh, slightly spicy.
	Precipitation	Leaf scent, slightly sweet, soft smell.	Leaf scent, soft smell.	Fragrant, fresh, slightly spicy.
	2nd distillation	Leaf scent, soft smell.	Leaf scent, soft smell.	Fragrant, soft smell, slightly spicy.
	Combination	Soft smell, no leaf scent.	Soft smell, no leaf scent.	Fragrant, slightly spicy, no leaf scent.

3.2. Purification process

The effect of purification methods on the recovery yield and the quality of essential oils is depicted in Fig. 2 and Table 1. In general, the type of plant material does not affect significantly the recovery yield of purified oils ($p > 0.05$). Among methods, the purification using hydro-distillation and crystallization give the highest recovery yield (> 90 %) followed by precipitation and 2nd distillation method.

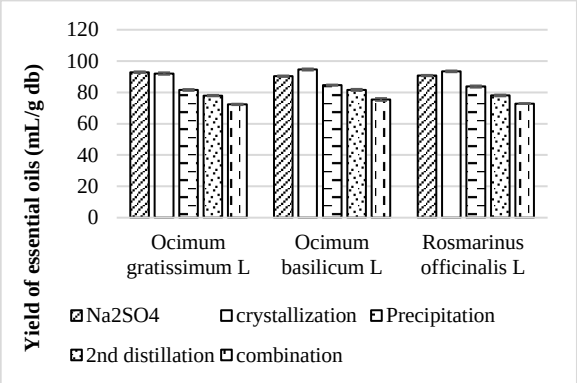

Fig. 2. The effect of purification methods on recovery yield of essential oils.

As hypothesed, the combination method has a lowest recovery yield (around 70 %) and the accumulation loss of each step in this method can up to 20 %. In a contrast, the quality of essential oils purified by this method is the highest while hydro-distillation and crystallization method often lead to a lower quality essentials oils. For example, the leaf scent is still remaining in the oils purified by the later methods accompanying with a dark color. For essential oils purified by the combination method, they have a bright color without the leaf scent.

3.3. Chemical composition and antioxidant activity

Purified essential oils from *Ocimum gratissimum* L., *Ocimum basilicum* L., and *Rosmarinus officinalis* L. (using the combination method) were analysed by GC/MS and the results were shown in Fig. 3. Besides, the chemical components of essential oils are summarized in Table 2.

Table 2. Chemical composition of essential oil extracts

***Ocimum gratissimum* L.**		***Ocimum basilicum* L.**		***Rosmarinus officinalis* L.**	
Compound	**(%)**	**Compound**	**(%)**	**Compound**	**(%)**
cis-*β*-Ocimene	8.2	Eucalyptol	0.7	D-*α*-Pinene	22.1
Eugenol	68.7	*trans*-*β*-Ocimene	0.8	Camphene	3.5
α-Copaene	1.9	Linalool	1.9	L-*β*-Pinene	1.9
β-Bourbonene	0.7	Estragole	84.8	*β*-Myrcene	1.1
levo-*β*-Elemene	0.6	Fenchyl acetate	0.6	*α*-Terpinene	0.6
Caryophyllene	4.4	Cyclohexane	0.5	*β*-Cymene	0.9
D-Germacrene	11.7	*trans*-*α*-Bergamotene	2.7	D-Limonene	2.2
-	-	*trans*-*α*-Bergamotene	2.7	Eucalyptol	16.987
-	-	*γ*-Cadinene	0.9	*γ*-Terpinene	1.0
-	-	-	-	Cyclohexene	1.0
-	-	-	-	Linalool	2.7
-	-	-	-	Camphor	3.7
-	-	-	-	Camphol	4.4
-	-	-	-	*δ*-Terpineol	0.6
-	-	-	-	Isocamphopinone	1.1
-	-	-	-	4-Terpineol	1.6
-	-	-	-	*α*-Terpineol	2.6
-	-	-	-	Myrtenol	0.6
-	-	-	-	Verbenone	14.9
-	-	-	-	Geraniol	4.4
-	-	-	-	L-*α*-bornyl acetate	3.9
-	-	-	-	Caryophyllene	1.8
-	-	-	-	*β*-Caryophyllene oxide	0.6

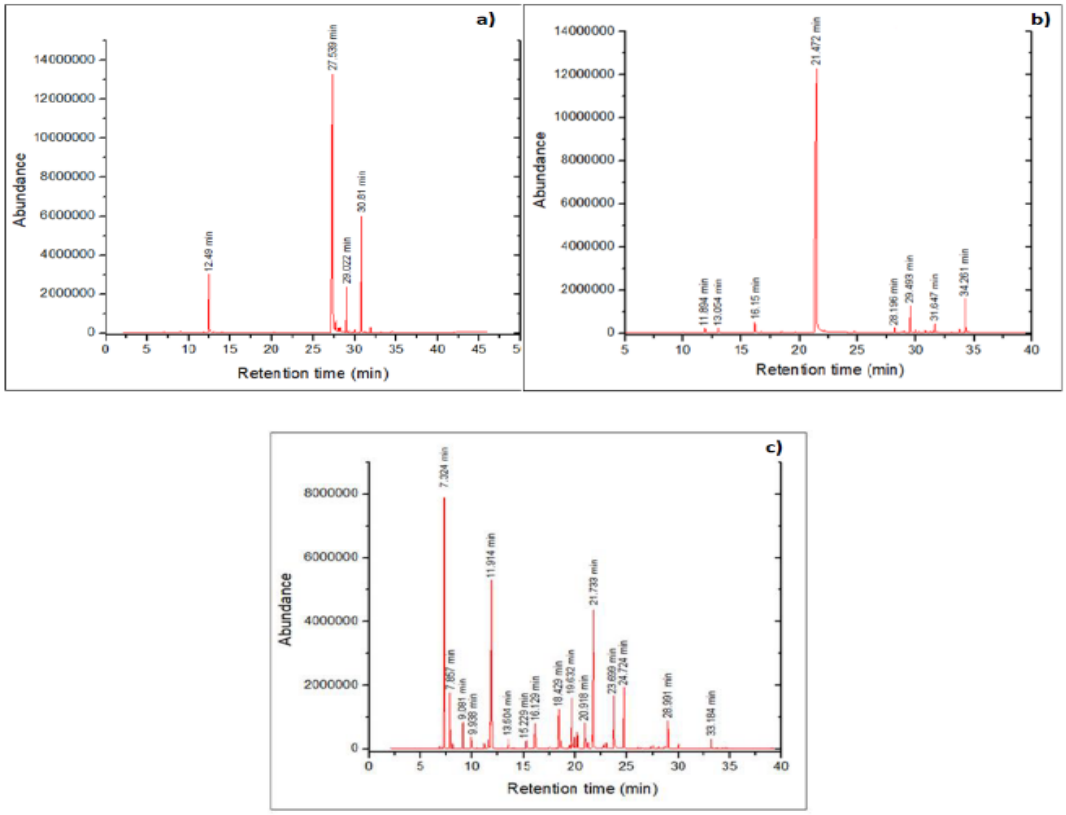

Fig. 3. GC/MS analysis results of compounds present in the *Ocimum gratissimum* L. (a), *Ocimum basilicum* L. (b) and *Rosmarinus officinalis* L. (c) essential oils.

Firstly, the essential oil extracted from *Ocimum gratissimum* L. is the combination of 26 different compounds including Eugenol (68.7 %), β-Ocimene (8.2 %), Caryophyllene (4.4%) and D-Germacrene (11.7 %). Eugenol, a phytogenic bioactive component is frequently found in diversified herbal plants. Raja et al. (20150 report that prominent sources of eugenol are clove buds (180mg/g), cinnamon bark (3.52 mg/g), tulsi leaves (4.2–4.97 mg/g), turmeric oils (2.1 mg/g), clover pepper fruit (36 mg/g), betel pepper (17.85 mg/g) [17]. In addition, several other aromatic herbs including basil, bay, marjoram, mace and nutmeg are also claimed to have significant quantity of eugenol. Among these plant sources, clove and cinnamon are considered as the prosperous provenances of eugenol containing 45–90% and 20–50% eugenol correspondingly. The concentration of eugenol in different parts of plants varies with season. Eugenol has been approved to encompass numerous beneficial aspects against a capacious spectrum of life threatening indispositions including oxidative stress, inflammation, hyperglycemia, elevated cholesterol level, neural disorders and cancer. In addition, eugenol has also shown strong potential as an antimicrobial agent against wide ranges of pathogenic and spoilage causing microorganisms [17].

For *Ocimum basilicum* L., its essential oil has 29 different compounds and mainly includes Estragole (84.8 %), trans-α-Bergamotene (2.7 %) and Linalool (1.9 %). Estragole is a natural constituent of a number of aromatic plants and their essential oil fractions including tarragon (60-75%), sweet basil (20-43%), sweet fennel (5-20%), sweet fennel (1%), anise star (5-6%) [18]. Estragole has many biological effects, including antioxidant and antimicrobial activities.

Lastly, the essential oil of *Rosmarinus officinalis* L. has 40 chemical coumpounds containing 11 major constituents such as D-*α*-Pinene (22.1 %), Eucalyptol (17.0 %), Verbenone (14.9 %), Geraniol (4.4 %), Camphol (4.4 %), L-*α*-bornyl acetate (4.0 %), Camphor (3.7 %), Camphene (3.5 %), Linalool (2.7 %), *α*-Terpineol (2.5 %) and D-Limonene (2.2 %). These results are in line with previous studies of essential oils extracted from *Ocimum gratissimum* L., *Ocimum basilicum* L. and *Rosmarinus officinalis* L. [4, 11, 16, 19]. In comparison between plant materials, essential oils extracted from *Rosmarinus officinalis* L. has the most complicated chemical composition profile. Most constituents of this essential oil have a concentration below 25 %. For *Ocimum gratissimum* L. and *Ocimum basilicum* L., their essentials are mainly dominated by Eugenol (> 68 %) and Estragole (> 84 %), respectively.

DPPH and ABTS tests were applied to evaluate the antioxidant activity of essential oils extracted and purified from the *Ocimum gratissimum* L., *Ocimum basilicum* L. and *Rosmarinus officinalis* L. The antioxidant activity was evaluated based on IC_{50} value and the results concentration was summarized in Table 3. It can be seen that the essential oil of *O. gratissimum* L. has the highest free radical scavenging activity. The IC_{50} values of DPPH and ABTS test of this essential oils are 5.1 (µg/ml) and 2.9 (µg/ml). In the combination with Table 2, the antioxidant activity of this oil should be from eugenol, a phenolic compound owning a high antioxidant capacity [20].

Table 3. IC_{50} Value of essential oils and Vitamin C

Essential oils	IC_{50} Value (µg/ml)	
	DPPH	ABTS
Ocimum gratissimum L.	5.1	2.9
Ocimum basilicum L.	18.2	10.4
Rosmarinus officinalis L.	41.9	42.8
Vitamin C	4.8	2.3

4. Conclusion

In this study, essential oils from *Ocimum gratissimum* L., *Ocimum basilicum* L. and *Rosmarinus officinalis* L. were extracted by using steam distillation method. The obtained essential oils were evaluated for chemical composition and the antioxidant activity. Thought gas chromatography/mass spectrometry (GC/MS), twenty-six components were identified in the *Ocimum gratissimum* L. essential oils, the most abundant component is Eugenol (68.7 %). Next, twenty-nine components were identified in the Ocimum basilicum L. essential oils, the most abundant component is Estragole (84.8 %). The last, fourty components were identified in the *Rosmarinus officinalis* L. essential oils and the most abundant component is D-*α*-Pinene (22.1 %). For antioxidant activity, the essential oil of *O. gratissimum* had the highest free radical scavenging activity with DPPH and ABTS methods among three essential oils. The IC50 values were 5.1 (µg/ml), 2.9 (µg/ml) for DPPH and ABTS, respectively. The results suggested that high potential use of *O. gratissimum* essential oil in research and development of pharmaceutical products and functional foods.

Conflicts of interest

There are no conflicts to declare.

Acknowledgments

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