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Contributed Paper

Potent Antioxidant Activities of Half-Sib Families of *Eucalyptus camaldulensis* Dehnh. Leaf Essential Oils Planted in Thailand and Their Antioxidative Components

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ABSTRACT

Eucalyptus essential oils are widely known to use in various application such as traditional medicine, food, cosmetic and botanical insecticide. This study is to evaluate antioxidant capacity of essential oils hydrodistillated from the leaves of six half-sib families of *Eucalyptus camaldulensis* Dehnh. planted in Nakhon Ratchasima province, Thailand. and to analyze the antioxidative component of essential oils by Gas Chromatography - Mass Spectrometry (GC/MS) method. The clear and yellowish essential oils were obtained in 1.14-2.07% yield based on the dry leaf basis. The results of the antioxidant activities revealed four *Eucalyptus* oils from leaves of half-sib families 109, 188, 208 and 209 of *E. camaldulensis* inhibited significant effect with free radical (DPPH) scavenging, IC_{50} values in the range between 0.71-1.27 (mg/mL), TEAC values ranging of 22.20-25.17 mM/g and FRAP values ranging of 291.80-676.48 mM Fe(II)/g. The high amount of phenolic terpenoids (2.18-7.25%), thymol and carvacrol together with dominant compositions of *p*-cymene (10.71-28.25%), *γ*-terpinene (5.58-40.86%) and terpene alcohols (15.21-29.19%) may cause these oils showing potent antioxidant activity. Therefore, the sample trees of these half-sib families of *E. camaldulensis* could be vegetatively propagated as clones to use their leaves as a new potential source of natural antioxidants in industrial to enhance the commercial plantation.

Keywords: *Eucalyptus camaldulensis* Dehnh., essential oil, antioxidant activity, half-sib family, terpenoids

1. INTRODUCTION

Eucalyptus camaldulensis Dehnh. (river red gum) is a tree belonging to the family of Myrtaceae which is originally distributed in Australia. Because the species is fast growing and easy adaptability, it is a plantation species in many parts of the world. In Thailand, *E. camaldulensis* has been extensively planted for pulpwood production. To increase the tree growth and wood quality for pulpwood and solid lumber production, several tree breeding programs have been developed for this species [1] where superior half-sib families were then evaluated and selected on the basis of

the superior growth performance and suitable wood properties [2]. In addition, the possibility of utilizing the leaves and their essential oils which have found various applications in everyday life are worthy of investigation from plantation-grown *E. camaldulensis* trees.

The essential oils, easily distilled from leaves, have been widely used for bactericidal, virucidal, fungicidal, antiparasitical, insecticidal, agricultural, medicinal, sanitary and cosmetic applications [3]. They are commonly used in traditional medicine for cold treatment, bronchitis, sore throat and headache [4]. *Eucalyptus* oils are used as flavor elements in foods and beverages such as ice creams, baked products, confectionaries and soft drinks. They are also used as fragrance elements in household products and cosmetics such as soaps, detergents, lotions and perfumes [5].

The essential oils of *E. camaldulensis* can be obtained from steam distillation or hydrodistillation of leaves [4, 6, 7], flowers [8], fruits [9] and seeds [10] that are known to contain a variety of volatile bioactive compounds such as monoterpenoids, sesquiterpenoids and phenylpropanoids. These oils have been reported multiple biological activities, including antibacterial [4, 11], antifungal [11, 12], larvicidal and mosquito repellent [13], acaricidal [14], antitermitic [15], antimelanogenic [16], anti-inflammatory [17], antiacetylcholinesterase [18], antidiabetic [19] and antioxidant activities [20, 21].

Nowadays, naturally occurring antioxidants have come to be preferred in cosmetic industry and food manufactures, mainly because of synthetic antioxidants such as *tert*-butyl hydroxyanisole (BHA) and *tert*-butyl hydroxytoluene (BHT) have been reported to be carcinogenic [22]. Free radicals and reactive oxygen species (ROS) are associated with ageing process, melanin synthesis [16] and several diseases such as inflammation [17], Alzheimer [28] and diabetes [19]. The antioxidant effects of *Eucalyptus* oils have been evaluated as potent activity [5, 16, 23]. Moreover, essential oils from *E. camaldulensis* have shown better antioxidant activity than some species such as *E. rudis, E. globulus, E. tereticornis, E. crebra* and *E. melanophloia* [23, 24].

The phytochemical composition of the essential oils from *E. camaldulensis*, especially from the leaves, has been widely evaluated in different varieties, planted areas and seasonal variations. Among the different *E. camaldulensis* leaves studied, two chemotypes can be distinguished: a) 1,8-cineole (eucalyptol) rich [9, 17, 25-27] and b) 1,8-cineole poor (rich in *p*-cymene [13, 19], spathulenol [5, 6]). Thus, the essential oils from different *Eucalyptus* species or the same species but different in genetic backgrounds or planting area influence different types and quantities of chemical constituents in essential oils that are very important in biological activities. However, variations in essential oils characteristics have not yet been evaluated in any existing *E. camaldulensis* breeding programs in Thailand [1, 2].

The purpose of our study is to investigate the antioxidant activity of essential oils extracted from the leaves of six half-sib families of *E. camaldulensis* Dehnh. planted in Nakhon Ratchasima province, Thailand. In addition, the relationships between the phytochemical components and antioxidant activity of essential oils are also investigated. The half-sib families of *E. camaldulensis* plants that provided essential oil as an important source of natural antioxidant are very useful which will be selected for clone production to enhance the economic plant in cosmetic, food and pharmaceutical industry.

2. MATERIALS AND METHODS

2.1 Plant Materials

The progeny test of 120 half-sib families (open pollinated) of *E. camaldulensis* selected as plus trees from Australia and Thailand based on the growth characteristics was established at Wang Nam Khieo, Nakhon Ratchasima, Thailand (14°29′N, 101°56′E) in 2006 [1]. Six half-sib families of 13-year-old *E. camaldulensis* were selected in this study to represent superior growth (families

19, 62, 188 and 208) and inferior growth (families 109 and 209) where the former is the top 20 best families and the latter is the families with their growth poorer than the 120-families mean. For each family, mature leaves were collected from a selected tree with the greatest tree diameter in July, 2019. The seed source information and growth of six half-sib families (open pollinated) of *E. camaldulensis* is shown in Table 1.

2.2 Extraction of Essential Oils

The mature dry leaves of *E. camaldulensis* samples were cut into smaller pieces and grinded with blenders. The finely ground leaves (30 g) were subjected to extraction by hydrodistillation for 1.5 h in 250 mL distilled water. The distillate was extracted with dichloromethane and dried over anhydrous sodium sulfate and the solvent was evaporated under reduced pressure. The essential oil was obtained and stored in a refrigerator until use. The oil yield was calculated as the ratio of the weight of oil to the weight of dry leaves.

2.3 Analysis of Essential Oils

The essential oils were analyzed using a gas chromatography coupled to a mass spectrometer (GC-MS) with a Shimadzu GC-MS QP2020 with electron impact ionization (70eV). A 1 ml of essential oil dissolved in dichloromethane (20 μL/mL) was injected into a split/splitless inlet at 220 °C, with a split ratio 1:50. Helium was used as the carrier gas, with a constant flow of 1 mL/min. Components were separated on SH-Rxi-5Sil MS -fused silica capillary column (30 m length x 0.25 mm ID x 0.25 mm film thickness), using the temperature programmed starting at 60 °C, increasing 3 °C / min to 180 °C, then 20 °C /min to 280 °C and holding at 280 °C for 10 min (total run time 60 min). Eluent was delivered to the mass spectrometer via a transfer line held at 280 °C. Ion source temperature was 250 °C. Data were acquired in scan mode (m/z range 45-550), with solvent delay of 2 min. The compounds were identified by comparing their mass spectra with those stored in the NIST 14 database library. The retention indices (RI) are in relation to a homologous series of n-alkanes (C_7-C_{20}) on the GC column under the same chromatographic condition. Relative amounts of components, expressed in percentages were calculated by normalization procedure according to peak area in total ion chromatogram.

2.4 Antioxidant Activity

2.4.1 Determination of total phenolic contents

The total phenolic contents (TPC) of the *Eucalyptus* oils were determined by the Folin-Ciocalteu method as described by Abdalwahab *et al.* [28] with slide modification. A 1 ml of each essential oil solution in methanol (0.5 mg/mL) was mixed with 5 mL of Folin Ciocalteu reagent (diluted tenfold) and 4 mL of sodium carbonate

Table 1. Seed source information and growth of six half-sib families of 13-year-old *E. camaldulensis*.

Family	*n*[a]	Region	Provenance	DBH[b] (cm)	H[c] (m)
19	8	Petford Region Queensland	Eccles Creek/Tributaries	17.77 ± 3.14	19.40 ± 2.04
62	7	Petford Region Queensland	Eureka Creek/Tributaries	16.17 ± 3.79	19.57 ± 2.80
109	3	Petford Region Queensland	Headwater-Emureka Creek	14.10 ± 3.00	17.05 ± 2.12
188	8	Walsh-Mitchell River Queensland	Healeys Yard	17.67 ± 2.10	19.45 ± 1.83
208	4	Northern Territory	Katherine	17.37 ± 1.62	18.20 ± 1.41
209	7	Western Australia	Lannard River	14.65 ± 6.32	16.70 ± 4.62

[a] *n*, number of trees in each family [b] diameter at breast height (1.30 m from the ground) [c] tree height.

solution (7.5%w/v). The absorbance was measured after 30 min at 765 nm by using UV–vis spectrophotometer. Amount of total phenolic contents was calculated as gallic acid equivalent from the calibration curve of gallic acid standard solutions (5-50 mg/mL) and expressed as mg of gallic acid equivalent (GAE) per g of oil. The analyses were carried out in triplicate and the average value was calculated in each case.

2.4.2 Free radical scavenging activity (DPPH assay)

The antioxidant activity of eucalyptus oils was determined on the basis of their scavenging activity of the stable DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical as previously described [24] with slight modification. The methanol solution (1 mL) of essential oils at concentrations (1-10 mg/mL) of each of them was mixed with 2 mL of DPPH methanolic solution. (0.1 mM). The mixture was incubated in dark cabinet at room temperature, and their absorbance was measured at 517 nm after 30 min with UV-vis spectrophotometer against a blank. Butylated hydroxytoluene (BHT) was used as positive control. The percentage inhibition of DPPH radicals was calculated using the following equation

$$\text{Inhibition}(\%) \text{ of DPPH radials} = (A_c - A_s / A_c) \times 100$$

where A_c is absorbance of control at 30 min and A_s is the absorbance of sample solution at 30 min. The IC_{50} (oil concentration that provides 50% inhibition of free radicals) value was calculated by using probit analysis at 95% confidence [29].

2.4.3 Trolox-equivalent antioxidant capacity (TEAC) assay

Trolox equivalent antioxidant capacity assay was conducted following the modified methodology described by Barra *et al.* [12]. Standard solutions of Trolox at different concentrations (0.2, 0.5, 1.0, 1.5 and 2 mM) were prepared in methanol in order to construct the Trolox calibration curve. Twenty mL of either Trolox standard solutions or methanol solution of essential oils (50 mg/mL) was mixed with 2 mL of DPPH methanolic solution (75 mM). The mixture was incubated in dark cabinet at room temperature and their absorbance was measured at 517 nm after 30 min with UV-vis spectrophotometer against a blank. Results were expressed as mM Trolox Equivalent (TE) per g of oil. The analyses were carried out in triplicate and the average value was calculated in each case.

2.4.4 Ferric reducing antioxidant power (FRAP assay)

The FRAP assay was determined from the method as previously described [28] with slight modification. The stock solutions included 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tri(2-pyridyl)-s-triazine) solution in 40 mM HCl and 20 mM $FeCl_3$ solution. The fresh working solution was prepared by mixing 10 ml of acetate buffer, 1 ml of TPTZ and 1 ml of $FeCl_3$. The essential oils (100 ml) were allowed to react with 900 ml of the FRAP solution for 30 min at 37°C in the dark. Colorimetric readings of the product ferrous tripyridyltriazine complex were taken at 593 nm. FRAP value was calculated from the calibration curve of ferrous sulfate standard solutions (0.05-1 mM) and expressed as mM Fe (II) per g of oil. The analyses were carried out in triplicate and the average value was calculated in each case.

3. RESULTS AND DISCUSSION

3.1 Extraction of Essential Oils

Extraction by hydrodistillation of six half-sib families of *E. camaldulensis* leaves gave the clear yellowish oils and pleasant odor. The extraction yields of *Eucalyptus* oils varied among families and ranged between 1.14-2.07% weight of oils per weight of dry leaves (Table 2). The sample of family 19 (genetic material from Eccles Creek/ Tributaries, Petford region, QLD), the best growth performance, gave the highest yield of oil while family 209 (genetic material from Lannard

Table 2. Extraction yields, total phenolic contents (TPC) and antioxidant activity of six essential oils from half-sib families of *E. camaldulensis* leaves.

Family	Yield[a] (% w/w)	TPC[b] (mg GAE/g of oil)	Antioxidant activities		
			DPPH[c], IC_{50} (mg/mL)	TEAC[d] (mM Trolox/g of oil)	FRAP[e] (mM Fe (II)/g of oil)
19	2.07	17.23 ± 0.15	2.14 ± 0.24	18.92 ± 0.84	237.42 ± 17.36
62	1.66	13.15 ± 0.21	4.39 ± 0.42	14.46 ± 0.26	204.19 ± 10.95
109	1.95	29.31 ± 0.29	1.27 ± 0.20	22.61 ± 1.30	392.22 ± 18.65
188	1.44	22.21 ± 0.31	1.18 ± 0.10	22.20 ± 1.48	291.80 ± 12.22
208	1.27	19.89 ± 1.18	1.16 ± 0.07	23.47 ± 1.25	300.69 ± 26.06
209	1.14	47.96 ± 2.15	0.71 ± 0.09	25.17 ± 1.05	676.48 ± 47.14

[a] % Weight of oil per weight of dry leaves. [b]Total phenolic content (TPC), milligram gallic acid equivalent per gram of essential oil. [c] DPPH, free radical scavenging activity. [d]TEAC, Trolox equivalent antioxidant capacity. [e]FRAP, ferric reducing antioxidant activity. Data are represented as mean ±SD of triplicate determinations, $P<0.05$. BHT was used as positive control for antioxidant activities; IC_{50} 7.28 ± 0.47 μg/mL.

River, Western Australia) with the inferior growth contained lowest amount. Our results of the oil yields, extracted from all six families are higher than those reported in previous studies, ranging from 0.2% to 0.73 % per weight of dry leaves from *E. camaldulensis* cultivated in Italy [12], Turkey [19] and Tunisia [24]. The oil yields of the superior families (19, 62 and 109) are better than that obtained from *E. camaldulensis* commercial clones planted in Kanchanaburi Province, Thailand with 0.83-1.63% yield per weight of dry leaves [20].

3.2 GC/MS Analysis of Essential Oils

The six families of *E. camaldulensis* leaves oils were further analysed by GC/MS for chemical compositions. The percentage composition of each compound was relative concentration in all analytical compounds. The seventy terpenoids, including monoterpenoids (67.09-93.88%) and sesquiterpenoids (5.05-31.82%) were identified from the six essential oils in difference compositions as shown in Table 3.

The *E. camaldulensis* leaves oils of five families 19, 62, 109, 188 and 208 had high amount of 1,8-cineole (22.75-39.08%) which is in agreement with the *Eucalyptus* leaves oils locating from the other places, Greece [9], Burkima Faso [18], Nigeria [25], Argentina [26] and Mozambique [27], while the *Eucalyptus* oils of families 209 had lower amount of 1,8-cineole (2.54%). Essential oil of family 209 was mainly composed of *γ*-terpinene (40.86%), *p*-cymene (28.25%) and 4-terpineol (7.59%). The major compounds of this oil are similar to the *Eucalyptus* oil of clone S3 from Kanchanaburi, Thailand. [20]. Unless 1,8-cineole, the other dominant compounds in essential of families 19, 62, 109, 188 and 208 were *p*-cymene (10.64-14.85%), *γ*-terpinene (5.43-14.47%), D-limonene (3.06-10.52%),*α*-terpineol (2.50-6.85%), 4-terpineol (1.34-8.10%), globulol (1.53-9.89%) and aromadendrene (1.02-7.33%). The chemical structures of all dominant compounds are shown in Figure 1.

Moreover, it was found that all the *Eucalyptus* oils contained high amount of phenolic monoterpenoids (2.17-7.25%), thymol and carvacrol (Figure 1). The phenolic compounds may cause the antioxidant activities which are in agreement with previous study [20]. In addition, all the *Eucalyptus* oils consist of high component of terpenoid alcohols (15.21-29.19%) which may also affect the antioxidant activity. This presumption

Table 3. Terpenoids composition (%) of six essential oils from *E. camaldulensis* leaves by GC/MS.

No	Compound[a]	Formula	RI[b]	Composition (%)[c]					
				19	62	109	188	208	209
1	*α*-Pinene	$C_{10}H_{16}$	959	7.39	1.48	0.95	1.16	1.08	0.36
2	*α*-Fenchene	$C_{10}H_{16}$	971	0.17	-	-	-	-	-
3	Camphene	$C_{10}H_{16}$	973	0.48	-	-	-	-	-
4	*β*-Pinene	$C_{10}H_{16}$	998	4.95	0.13	-	-	-	-
5	*β*-Myrcene	$C_{10}H_{16}$	1009	0.09	0.15	0.15	0.15	-	-
6	4-Carene	$C_{10}H_{16}$	1035	-	-	0.14	-	-	-
7	***p*-Cymene**	$C_{10}H_{14}$	1043	**10.64**	**12.04**	**14.85**	**10.71**	**14.23**	**28.25**
8	**D-Limonene**	$C_{10}H_{16}$	1048	**7.23**	**10.52**	**3.06**	**9.25**	**8.05**	1.00
9	**1,8-Cineole**	$C_{10}H_{18}O$	1052	**36.48**	**39.08**	**23.45**	**22.75**	**36.44**	2.54
10	**γ-Terpinene**	$C_{10}H_{16}$	1077	**5.86**	**5.43**	**14.47**	**9.43**	**5.58**	**40.86**
11	Terpinolene	$C_{10}H_{16}$	1104	0.19	0.10	0.45	0.23	-	0.12
12	*p*-Cymenene	$C_{10}H_{12}$	1109	0.11	-	-	-	-	-
13	Linalool	$C_{10}H_{18}O$	1119	-	-	0.12	-	-	-
14	Fenchol	$C_{10}H_{18}O$	1138	1.82	-	-	-	-	-
15	*p*-Menth-3-en-1-ol	$C_{10}H_{18}O$	1155	-	-	-	-	-	0.09
16	*trans*-Pinocarveol	$C_{10}H_{16}O$	1160	0.75	0.26	-	0.19	0.21	-
17	2-Norbornanol	$C_{10}H_{18}O$	1174	0.26	-	-	-	-	-
18	Pinocarvone	$C_{10}H_{14}O$	1181	0.38	-	-	-	-	-
19	*cis-p*-Mentha-2,8-dien-1-ol	$C_{10}H_{16}O$	1183	-	-	-	-	-	0.39
20	*δ*-Terpineol	$C_{10}H_{18}O$	1190	-	0.15	0.12	-	0.16	-
21	Borneol	$C_{10}H_{18}O$	1192	2.82	0.07	-	-	0.14	0.10
22	Citral	$C_{10}H_{16}O$	1199	0.38	0.94	-	1.26	1.09	1.02
23	**4-Terpineol**	$C_{10}H_{18}O$	1201	1.34	2.10	**8.10**	**4.60**	**2.37**	**7.59**
24	*p*-Cymen-8-ol	$C_{10}H_{14}O$	1207	-	-	0.47	-	-	0.15
25	*trans-p*-Mentha-1(7),8-dien-2-ol	$C_{10}H_{16}O$	1208	0.62	1.55	-	1.07	1.43	-
26	**α-Terpineol**	$C_{10}H_{18}O$	1216	**6.85**	**3.00**	**4.15**	**2.50**	**2.56**	0.33
27	*cis*-Sabinol	$C_{10}H_{16}O$	1233	0.48	0.37	0.65	0.38	0.62	1.28
28	Fenchyl acetate	$C_{12}H_{20}O_2$	1236	0.63	-	-	-	-	-
29	*cis*-Carveol	$C_{10}H_{16}O$	1239	-	0.29	-	0.14	0.28	-
30	*cis-p*-Mentha-1(7),8-dien-2-ol	$C_{10}H_{16}O$	1249	0.61	1.65	0.16	0.93	1.65	-
31	*trans*-Carveol	$C_{10}H_{16}O$	1253	-	0.11	-	-	0.11	-
32	Carvone	$C_{10}H_{14}O$	1263	0.08	0.17	-	-	0.18	-
33	Piperitone	$C_{10}H_{16}O$	1273	0.09	0.21	-	0.16	0.23	0.94

[a] Compounds were tentatively identified by comparison with spectra data (MS) from NIST library.
[b] Retention indices (RI) relative to n-alkanes (C_7-C_{20}) under the same condition.
[c] % composition was relative amount in all identified compounds, calculated from peak area. - ; Not detected.

Table 3. (Continued).

No	Compound[a]	Formula	RI[b]	Composition (%)[c]					
				19	62	109	188	208	209
34	**Thymol**	$C_{10}H_{14}O$	1303	**1.27**	**0.71**	**1.29**	**0.74**	**1.27**	**2.80**
35	**Carvacrol**	$C_{10}H_{14}O$	1318	**1.64**	**1.46**	**2.19**	**1.44**	**2.39**	**4.45**
36	*p*-Menth-4(8)-ene-2,5-diol	$C_{10}H_{18}O_2$	1331	-	-	-	-	-	0.32
37	2-Acetoxy-1,8-cineole	$C_{12}H_{20}O_3$	1354	0.28	0.23	-	-	-	-
38	α-Terpinyl acetate	$C_{12}H_{20}O_2$	1362	-	-	-	-	-	0.92
39	Isoledene	$C_{15}H_{24}$	1383	-	0.07	0.15	0.16	-	-
40	*α*-Copaene	$C_{15}H_{24}$	1388	-	-	-	-	0.11	-
41	Geranyl acetate	$C_{12}H_{20}O_2$	1392	-	-	-	-	-	0.10
42	α-Gurjunene	$C_{15}H_{24}$	1422	-	0.08	0.56	0.39	-	-
43	*β*-Caryophyllene	$C_{15}H_{24}$	1435	0.09	-	0.29	-	-	-
44	*γ*-Maaliene	$C_{15}H_{24}$	1443	-	-	0.10	0.15	-	-
45	Calarene	$C_{15}H_{24}$	1447	0.11	0.14	0.34	0.37	0.12	0.06
46	Aromandendrene	$C_{15}H_{24}$	1455	1.02	3.56	5.21	7.33	1.53	0.47
47	Selina-5,11-diene	$C_{15}H_{24}$	1463	-	0.17	0.22	0.32	-	-
48	Alloaromadendrene	$C_{15}H_{24}$	1478	0.33	0.97	1.24	1.63	0.44	0.08
49	γ-Gurjunene	$C_{15}H_{24}$	1491	-	0.08	0.18	0.18	-	-
50	*β*-Selinene	$C_{15}H_{24}$	1507	0.15	0.34	0.56	0.75	0.27	0.12
51	Viridiflorene	$C_{15}H_{24}$	1511	0.10	0.15	0.44	0.38	0.14	-
52	*α*-Selinene	$C_{15}H_{24}$	1515	-	0.08	0.19	0.23	-	-
53	*γ*-Cadinene	$C_{15}H_{24}$	1533	-	0.09	0.18	0.19	-	-
54	*β*-Vatirenene	$C_{15}H_{22}$	1537	-	-	0.27	0.35	-	-
55	Epiglobulol	$C_{15}H_{26}O$	1581	0.25	1.07	1.50	2.19	0.51	0.27
56	Maaliol	$C_{15}H_{26}O$	1588	-	0.41	0.56	0.79	-	-
57	Palustrol	$C_{15}H_{26}O$	1589	0.14	-	-	-	0.25	-
58	**Globulol**	$C_{15}H_{26}O$	1604	1.53	**6.10**	**7.29**	**9.89**	2.86	1.30
59	Viridiflorol	$C_{15}H_{26}O$	1613	0.28	1.21	1.41	1.94	0.41	-
60	Cubeban-11-ol	$C_{15}H_{26}O$	1615	0.16	0.52	0.58	0.76	0.25	0.12
61	Ledol	$C_{15}H_{26}O$	1623	0.07	0.30	0.36	0.45	-	-
62	Rosifoliol	$C_{15}H_{26}O$	1625	0.10	0.42	0.61	0.82	0.19	0.06
63	*α*-Eudesmol	$C_{15}H_{26}O$	1642	0.31	1.06	1.49	1.75	0.51	0.21
64	Valerianol	$C_{15}H_{26}O$	1647	-	-	-	-	1.49	0.40
65	*γ*-Eudesmol	$C_{15}H_{26}O$	1649	-	-	-	-	0.98	0.28
66	Agarospirol	$C_{15}H_{26}O$	1656	-	-	-	-	0.24	-

[a] Compounds were tentatively identified by comparison with spectra data (MS) from NIST library.
[b] Retention indices (RI) relative to n-alkanes (C_7-C_{20}) under the same condition.
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No	Compound[a]	Formula	RI[b]	Composition (%)[c]					
				19	62	109	188	208	209
67	*tau*-Cadinol	$C_{15}H_{26}O$	1658	-	-	0.17	0.19	-	-
68	*β*-Eudesmol	$C_{15}H_{26}O$	1670	0.42	0.44	-	-	**9.05**	2.31
69	*α*-Cadinol	$C_{15}H_{26}O$	1671	-	-	0.27	0.29	-	-
70	Neointermedeol	$C_{15}H_{26}O$	1673	-	0.17	0.24	0.29	-	-
	Total terpenoids			**98.93**	**99.64**	**99.19**	**98.90**	**99.44**	**99.31**
	Total monoterpenoids			**93.88**	**82.19**	**74.77**	**67.09**	**80.07**	**93.51**
	Total sesquiterpenoids			**5.05**	**17.45**	**24.42**	**31.82**	**19.35**	**5.79**
	Total terpenoid alcohols			**18.79**	**21.26**	**28.25**	**29.19**	**26.27**	**15.21**
	Total phenolic terpenoids			**2.90**	**2.17**	**3.48**	**2.18**	**3.66**	**7.25**

[a] Compounds were tentatively identified by comparison with spectra data (MS) from NIST library.
[b] Retention indices (RI) relative to n-alkanes (C_7-C_{20}) under the same condition.
[c] % composition was relative amount in all identified compounds, calculated from peak area. - ; Not detected.

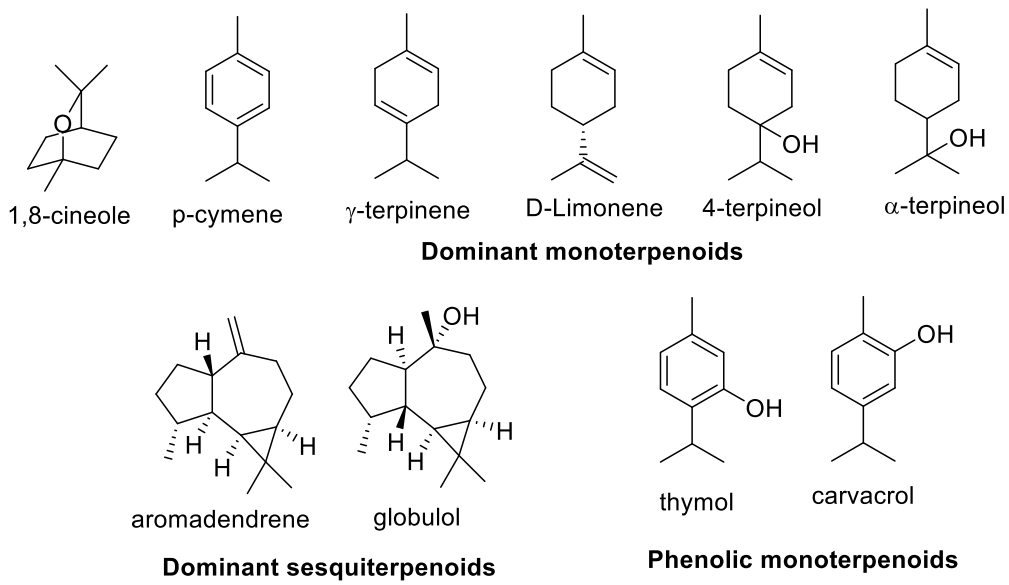

Figure 1. Chemical structures of phenolic terpenoids and dominant terpenoids identified in essential oils of *E. camaldulensis* leaves.

is supportable to those reported relevant to the potent antioxidant essential oils compose of high ratio of spathulenol (terpenoid alcohol) [21].

3.3 Antioxidant Activity of Essential Oils

Total phenolic contents (TPC) of six essential oils varied among families ranging from 13.15 ± 0.21 to 47.96 ± 2.15 mg GAE/g of oil (Table 2). The sample of family 209, one of the inferior growth families, exhibited the highest total phenolic contents, followed by family 109, whereas sample of family 62 obtained lowest amount of total phenolic contents. The results are related to total phenolic terpenoid compositions analyzed by GC/MS.

In the present study, DPPH free radical scavenging activity, Trolox equivalent antioxidant capacity (TEAC) and ferric reducing antioxidant power (FRAP) assay were used for investigation of antioxidant activities of *Eucalyptus* oils. For the DPPH assay, free radical scavenging activity of six half-sib families of *E. camaldulensis* essential oils was investigated in dose dependant mode (0.5-5.0 mg/mL). Increase in free radical scavenging activity (%) was observed with increase in concentration of each essential oil. The percent inhibition ranged from 22.28 to 86.39 % according to the tested doses and essential oils. The IC_{50} values of six essential oils varied ranged from 0.71 ± 0.09 to 4.39 ± 0.42 mg/mL (Table 2). Compared to standard synthetic antioxidant compounds like BHT, the activity of all tests oils is lower than that of the BHT standard (IC_{50} value of 7.28 ± 0.47 µg/ml). The highest free radical scavenging activity was observed in *Eucalyptus* oils of family 209, followed by those of families 208, 188, 109 and 19, respectively. While the *Eucalyptus* oil, family 62 exhibited lowest activity. The Trolox equivalent antioxidant capacity (TEAC) assay, TEAC values of six essential oils ranged from 14.46 ± 0.26 to 25.17 ± 1.05 mM/g of oil (Table 2). The highest and lowest values were observed in *Eucalyptus* oils, families 209 and 62, respectively. The antioxidant activity (TEAC) of all essential oils are similar to their DPPH assay. The FRAP assay, is presented as an accurate method for assessing "antioxidant power". FRAP values are obtained by comparing the absorbance change at 593 nm in test reaction mixtures with those containing ferrous ions in known concentration. The FRAP values of six essential oils ranged from 204.19 ± 10.95 to 676.48 ± 47.14 mM Fe (II)/g of oil (Table 2). The highest and lowest ferric reducing antioxidant power were observed in *Eucalyptus* oils, families 209 and 62, respectively. The *Eucalyptus* oil, family 109 showed strong activity next to family 209 and higher than 208, 188 and 19, respectively. The antioxidant activities of oils from the six half-sib families of *E. camaldulensis* planted in Nakhon Ratchasima province, Thailand were first reported.

Interestingly, our results showed that *Eucalyptus* oil of family 209 with inferior growth performance had the highest potent antioxidant activity with inhibition of DPPH radical (IC_{50} 0.71 ± 0.09 mg/mL), maximum values of TEAC, FRAP and TPC. The highest activity of this oil is related to the highest amount of phenolic terpenoids (thymol and carvacrol). In addition, the three *Eucalyptus* oils of families 109, 188 and 208 are also significantly stronger antioxidant activity than those of the families 19 and 62. The strong activity of these three oils are related to the high amount of phenolic terpenoids and terpenoid alcohols.

Comparison these activities and major constituents (*p*-cymene and *γ*-terpinene) found that *Eucalyptus* oils of family 209 contained the highest amount of *p*-cymene and *γ*-terpinene indicating the strongest activity. In the same way, oils of families 109, 188 and 208 showing stronger activities compared with those of the families 19 and 62 had higher amount of both compounds than other two oils. This result is similar to those reported about the potent antioxidant essential oils compose of higher amount of *p*-cymene [12, 19]. Moreover, both *γ*-terpinene and *p*-cymene could be probably converted to thymol and carvacrol (great antioxidant active compounds) by terpene

synthases [30].

In comparison with previously reported for *E. camaldulensis* leaves planted in the other sites, the antioxidant effect of four essential oils, families 109, 188, 208 and 209 (IC_{50} 0.71-1.27 mg/mL) were more active than essential oils of *E. camaldulensis* leaves of three commercial clones, planted in Kanchanaburi, Thailand (IC_{50} 1.75-12.62 mg/mL) [20]. In addition, the antioxidant activity of these four essential oils were more effective than essential oils of *E. camaldulensis* leaves growing in Ouagadougou [17] and Burkina Faso [18], but less active than those growing in Pakestani [5, 23], Egypt [21] and Tunisia [24]. This difference in activity of essential oils could be contributed to the difference in phytochemical components, affected by genetic background, planting sites, plantation management and climatic factors.

4. Conclusions

The six essential oils were hydrodistillated from dry leaves of six half-sib families of *E. camaldulensis* Dehnh., planted in Nakhon Ratchasima province, north-eastern part of Thailand. The clear and yellowish essential oils were obtained in 1.14-2.07% yield based on the dry leaf basis. Evaluation of antioxidant activities of these oils showed variations among *E. camaldulensis* families and essential oil of family 209 had the highest potent antioxidant activity. In addition, the other three essential oils from leaves of families 109, 188 and 208 also exhibited stronger antioxidant activity than the other two essential oils. The chemical identification of four oils that exhibited potent activity found phenolic compounds, thymol and carvacrol together with dominant composition of *p*-cymene, *γ*-terpinene and terpenoid alcohols such as 4-terpineol, *α*-terpineol and globulol. All of these compounds may cause significant antioxidant activity. The results indicate that these essential oils could be used as a new potential source of natural antioxidants for the therapeutic benefits, food and cosmetic industries. Overall, four potential half-sib families of *E. camaldulensis*, families 109, 188, 208 and 209, could be selected for leaf oil production. Nevertheless, the sample trees of the families 188 and 208 with superior growth performance could be vegetatively propagated as clones mainly for wood production and for leaf oil production as minor products to enhance the commercial plantation of *E. camaldulensis*.

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References

[1] Sungtong T. and Diloksumpun S., *Thai J. Forest.*, 2011; **30(3)**: 1-13.

[2] Ishiguri F., Diloksumpun S., Tanabe J., Ohshima J., Iizuka K. and Yokota S., *Int. Wood Prod. J.*, 2017; **8(1)**: 36-40. DOI 10.1080/20426445.2016.1261528.

[3] Bakkali F., Averbeck S., Averbeck D. and Idaomar M., *Food Chem. Toxicol.*, 2008; **46**: 446-475. DOI 10.1016/j.fct.2007.09.106.

[4] Asiaei E.O., Moghimipour E. and Fakoor M.H., *Jundishapur J. Nat. Pharm. Prod.*, 2018; **13(4)**: e65050. DOI 10.5812/jjnpp.65050.

[5] El-Ghorab A., El-Massry K.F., Anjum F.M., Shahwarb M.K. and Shibamoto T., *J. Essent. Oil Bear. Pl.*, 2009; **12(3)**: 262-272. DOI 10.1080/0972060X.2009.10643719.

[6] Knezevic P., Aleksic V., Simin N., Svircev E., Petrovic A. and Mimica-Dukic N., *J. Ethnopharmacol.*, 2016; **178**: 125-136. DOI 10.1016/j.jep.2015.12.008.

[7] Ndiaye E.H.B., Diop M.B., Gueye M.T., Ndiaye I., Diop S.M., Fauconnier M.L. and Lognay G., *J. Essent. Oil Res.*, 2018; **30(2)**: 131-141. DOI 10.1080/10412905.2017.1420554.

[8] Giamakis A., Kretsi O., Chinou I. and

Spyropoulos C.G., *Phytochemistry*, 2001; **58**: 351-355. DOI 10.1016/s0031-9422(01)00193-5.

[9] Tsiri D., Kretsi O., Chinou I.B. and Spyropoulos C.G., *Flavour Fragr. J.*, 2003; **18**: 244-247. DOI 10.1002/ffj.1220.

[10] Olawore N.O. and Ololade Z.S., *Chem. Sci. J.*, 2017; **8(1)**: 148. DOI 10.4172/2150-3494.1000148.

[11] Sabo V.A. and Knezevic P., *Ind. Crop. Prod.*, 2019; **132**: 413-429. DOI 10.1016/j.indcrop.2019.02.051.

[12] Barra A., Coroneo V., Dessi S., Cabras P. and Angioni A., *Nat. Prod. Commun.*, 2010; **5(2)**: 329-335. DOI 10.1177/1934578X1000500232.

[13] Cheng S.S., Huang C.G., Chen Y.J., Yu J.J., Chen W.J. and Chang S.T., *Bioresors. Technol.*, 2009; **100**: 452-456. DOI 10.1016/j.biortech.2008.02.038.

[14] Ghasemi V., Moharramipour S. and Tahmasbi G., *Exp. Appl. Acarol.*, 2011; **55**: 147-154. DOI 10.1007/s10493-011-9457-1.

[15] Siramon P., Ohtani Y. and Ichiura H., *J. Wood Sci.*, 2009; **55**: 41-46. DOI 10.1007/s10086-008-0990-4.

[16] Huang H.C., Ho Y.C., Lim J.M., Chang T.Y., Ho C.L. and Chang T.M., *Int. J. Mol. Sci.*, 2015; **16**: 10470-10490. DOI 10.3390/ijms160510470.

[17] Bayala B., Bassole I.H.N., Gnoula C., Nebie R., Yonli A., Morel L., Figueredo G., Nikiema J.B., Lobaccaro J.M.A. and Simpore J., *PLOS One*, 2014; **9(3)**: e92122. DOI 10.1371/journal.pone.0092122.

[18] Kiendrebeogo M., Coulibaly A.Y., Nebie R.C.H., Zeba B., Lamien C.E., Lamien-Meda A. and Nacoulma O.G., *Braz. J. Pharmacogn.*, 2011; **21(1)**: 63-69. DOI 10.1590/S0102-695X2011005000008.

[19] Basak S.S. and Candan F., *J. Iran. Chem. Soc.*, 2010; **7(1)**: 216-226. DOI 10.1007/BF03245882.

[20] Siramon P. and Ohtani Y., *J. Wood Sci.*, 2007; **53**: 498-504. DOI 10.1007/s10086-007-0887-7.

[21] Salem M.Z.M., Ashmawy N.A., Elansary H.O. and El-Settawy A.A., *Nat. Prod. Res.*, 2015; **29(7)**: 681-685. DOI 10.1080/14786419.2014.981539.

[22] Dinis T.C.P., Madeira V.M.C. and Almeida L.M., *Arch. Biochem. Biophys.*, 1994; **315**: 161-169. DOI 10.1006/abbi.1994.1485.

[23] Ghaffar A., Yameen M., Kiran S., Kamal S., Jalal F., Munir B., Saleem S., Rafiq N., Ahmad A., Saba I. and Jabbar A., *Molecules*, 2015; **20**: 20487-20498. DOI 10.3390/molecules201119706.

[24] Sliti S., Ayadi S., Kachouri F., Khouja M.A., Abderrabba M. and Bouzouita N., *J. Mater. Environ. Sci.*, 2015; **6(3)**: 743-748.

[25] Oyedeji A.O., Ekundayo O., Olawore O.N. and Koenig W.A., *J. Essent. Oil Res.*, 2000; **12**: 102-104. DOI 10.1080/10412905.2000.9712053.

[26] Leicach S.R., Garau A.M., Guarnaschelli A.B., Grass M.A.Y., Sztarker N.D. and Dato A., *J. Plant Interact.*, 2010; **5(3)**: 205-210. DOI 10.1080/17429145.2010.483744.

[27] Pagula F.P., Baser K.H.C. and Kürkçüoglu M., *J. Essent. Oil Res.*, 2000; **12**: 333-335. DOI 10.1080/10412905.2000.9699530.

[28] Abdalwahab S.I., Mariod A.A., Taha M.M.E., Zaman F.Q., Abdelmageed A.H.A., Khamis S., Sivasothy Y. and Awang K., *Arab. J. Chem.*, 2017; **10**: 131-135. DOI 10.1016/j.arabjc.2014.02.001.

[29] Finney D.J., *Probit Analysis*, Cambridge University Press, Cambridge, England, 1952.

[30] Crocoll C., Asbach J., Novak J., Gershenzon J. and Degenhardt J., *Plant Mol. Biol.*, 2010; **73**: 587-603. DOI 10.1007/s11103-010-9636-1.