J Wood Sci (2007) 53:498–504
DOI 10.1007/s10086-007-0887-7

© The Japan Wood Research Society 2007

ORIGINAL ARTICLE

**Pornpun Siramon · Yoshito Ohtani**

# Antioxidative and antiradical activities of *Eucalyptus camaldulensis* leaf oils from Thailand

Received: November 13, 2006 / Accepted: February 7, 2007 / Published online: June 16, 2007

**Abstract** The leaf essential oils (six samples) from three clones of *Eucalyptus camaldulensis* Dehnh. were characterized by gas chromatography-mass spectrometry. Radical scavenging and antioxidant properties were investigated by the 1,1-diphenyl-2-picylhydrazyl (DPPH) assay and the $\beta$-carotene bleaching test. It was found that the whole essential oil and its fractions had significant antioxidant effects when they were tested by each method. In the DPPH assay, the *E. camaldulensis* leaf oils showed $IC_{50}$ inhibitory concentrations in the range of 1.75–12.62 mg/ml. In the $\beta$-carotene bleaching test, the $IC_{50}$ valued were in the range of 14.30–118.55 $\mu$g/ml.

**Key words** Leaf essential oil · *Eucalyptus camaldulensis* · Antioxidant activity · DPPH · $\beta$-Carotene bleaching test

## Introduction

In recent years, natural extracts have been in high demand from the manufacturers of foods, cosmetics, and pharmaceuticals due to the growing interest of consumers in ingredients from natural sources. Increasing concern about potentially harmful synthetic additives has also contributed to the demand. Synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butlylated hydroxytoluene (BHT), have restricted use in food because they have been reported to be carcinogenic. Some countries, such as Japan and some European countries, have not permitted the use of tertiary butyl hydroquinone (TBHQ), the most potent synthetic food antioxidant and other countries may ban it in the future. Therefore, the search for new natural antioxidant sources has been greatly intensified.[1]

In light of the differences among the wide number of test systems available, the results of a single assay can give only a suggestion of the antioxidant properties of essential oils toward food matrices and must be interpreted with some caution. Moreover, the chemical complexity of essential oils, often a mixture of dozens of compounds with different functional groups, polarity, and chemical behavior, could lead to scattered results, depending on the test employed. Therefore, an approach with multiple assays in screening work is highly advisable. Among the plethora of methods that can be used for the evaluation of antioxidant activity: trolox-equivalent antioxidant capacity (TEAC), total radical-trapping antioxidant parameter (TRAP), low-density lipoprotein oxidation (LDL), N,N-dimethyl-p-phenylenediamine (DMPD), ferric reducing ability of plasma (FRAP), oxygen radical absorbance capacity (ORAC), Photochemiluminescence (PCL), 1,1-diphenyl-2-picylhydrazyl (DPPH), and $\beta$-carotene bleaching. Very a few of them (TEAC, DPPH, PCL), however, are useful for determining the activity of both hydrophilic and lipophilic species.[2]

The pulp and paper industry in Thailand has increased the total domestic paper production capacity year by year. *Eucalyptus camaldulensis* Dehnh. is the most famous *Eucalyptus* species for cultivation in Thailand. It is mainly planted for use as pulpwood and is utilized as the main raw material even at ages of 3–5 years because of the high growth rate. During the process of papermaking, large amounts of waste such as leaves are disposed of. Therefore, the possibility of utilizing the leaves is worthy of investigation.

In this study, the components of *E. camaldulensis* leaf oils and their antioxidant properties were investigated by using the DPPH assay and the $\beta$-carotene bleaching test.

P. Siramon · Y. Ohtani (✉)
Department of Forest Science, Faculty of Agriculture, Kochi University, B-200 Monobe, Nankoku, Kochi 783–8502, Japan
Tel. +81-88-864-5143; Fax +81-88-864-5143
e-mail: ohtani@kochi-u.ac.jp

Part of this report was presented at the 18th meeting of the Chugoku-Shikoku Branch of the Japan Wood Research Society, November 2006

## Materials and methods

### Chemicals and plant materials

Butylated hydroxyanisole (BHA), $\beta$-carotene, chloroform, 1,1-diphenyl-2-picylhydrazyl (DPPH), ethanol, ethyl acetate, hexane, linoleic acid, silica gel 60 (70–230 mesh), $\alpha$-pinene, $\alpha$-terpineol, terpinen-4-ol, and Tween 40 were purchased from Nacalai Tesque (Kyoto, Japan). Carvacrol, 1,8-cineole, *p*-cymene, thymol and $\alpha$-tocopherol were purchased from Wako (Osaka, Japan). Thymoquinone and $\gamma$-terpinene were purchased from Tokyo Kasei Kogyo (Tokyo, Japan).

Three clones S1, S2, and S3 of *Eucalyptus camaldulensis* Dehnh. were selected through several generations based on their pulp yields and adaptability to circumstance, etc. by Siam Forestry, the former two are mainly utilized now. The leaves (S1, S2, S3) of 3 clones (total of six samples, namely S1-1, S1-2, S2-1 S2-2, S3-1, and S3-2) were collected during different periods in afforested areas of the company in Kanchanaburi Province, Thailand. For example, S1-1 and S1-2 denote S1 clones that were sampled initially and then a second time, respectively.

### Isolation, column chromatographic separation and gas chromatography-mass spectrometry analysis of Eucalyptus leaf oil

About 1 kg of *E. camaldulensis* fresh leaves was extracted by water and steam distillation for 5 h (until no more essential oil was obtained). The essential oils were collected, dried over anhydrous sodium sulfate, and stored in sealed vials at low temperature before analysis.

One gram of essential oils was fractionated by glass columns packed with silica gel. The columns were eluted with 200 ml of hexane, followed by 200 ml of chloroform, and 200ml of ethyl acetate, respectively.

The essential oils were analyzed by gas chromatography-mass spectrometry (GC-MS) on a system consisting of a GC-17A gas chromatograph (Shimadzu, Kyoto, Japan) coupled to a QP5050A mass spectrometer (Shimadzu) with a fused-silica capillary column TC-1 (0.25 mm i.d. × 15 m, 0.25 $\mu$m film thickness; GL Sciences). GC-MS was performed using the following conditions: carrier gas He; flow rate 20.6 ml/min; splitless injection; injection volume 1.0 $\mu$l; injection temperature 230°C; oven temperature programmed from 30°C (5 min hold) to 100°C at 10°C/min (5 min hold), and from 100°C to 230°C at 15°C/min (5 min hold); interface temperature 230°C; electron-impact ionization at 70 eV.

Identification of the compounds was achieved by using the National Institute of Standards and Technology (NIST) database library, and most compounds such as *p*-cymene, $\gamma$-terpinene, $\alpha$-pinene, 1,8-cineole, terpinen-4-ol, $\alpha$-terpineol, carvacrol, thymol, thymol acetate, and thymoquinone were directly compared with the authentic compounds. Chemical compositions of the oils were calculated based on the peak areas of the GC chromatograms.

### Determination of free radical scavenging activity by DPPH method

Free radical scavenging capacity (DPPH method) of the samples was determined by the degree of bleaching of the stable DPPH. An ethanol solution (1 ml) of a test sample was added to a solution (10 ml) of 0.25 mM DPPH in ethanol. The solution was rapidly mixed and was kept in a water bath at 30°C for 30 min. The absorbance of the mixture was measured at 515 nm on an ultraviolet-visible (UV-VIS) spectrophotometer. $\alpha$-Tocopherol and BHA were used as the references. The scavenging activity on the DPPH radical was expressed as inhibition percentage using the following equation:

$$\%\text{Inhibition} = [(B - S)/B] \times 100$$

where $B$ and $S$ are the absorbances of the blank and the sample, respectively. The $IC_{50}$ value (the concentration of substrate that causes 50% loss of the DPPH activity) of each oil sample was also evaluated.

### Determination of the antioxidant activity by $\beta$-carotene bleaching method

Antioxidant activities of the oil samples were determined using the $\beta$-carotene bleaching test.[2] Approximately 10 mg of $\beta$-carotene was dissolved in 10 ml of chloroform. The carotene–chloroform solution of 0.2 ml was pipetted into a flask containing 20 mg of linoleic acid and 200 mg of Tween 40. Chloroform was removed using a rotary evaporator at 40°C for 5 min and 50 ml of distilled water was added slowly to the residue with vigorous agitation in order to form an emulsion. A 4.8-ml aliquot of the emulsion was added to a tube containing 0.2 ml of the sample solution and the absorbance at 470 nm was immediately measured against a blank that was an emulsion without $\beta$-carotene. The tubes were placed in a water bath at 50°C and the oxidation of the emulsion was monitored spectrophotometrically by measuring absorbance at 470 nm for 60 min. Control samples contained 0.2 ml of ethanol instead of the oil samples. BHA and $\alpha$-tocopherol were used as the references. The antioxidant activity was expressed as the inhibition percentage with reference to the control after a 60-min incubation using the following equation:

$$AA = 100(DR_C - DR_S)/DR_C,$$

where AA is the antioxidant activity; $DR_C$ is the degradation rate of the control $\{DR_C = [\ln(a/b)/60]\}$; $DR_S$ is the degradation rate in presence of the sample $\{DR_S = [\ln(a/b)/60]\}$; where a is the absorbance at time 0; b is the absorbance at 60 min. The $IC_{50}$ value of each oil sample was also evaluated.

### Statistical analyses

Each experiment was performed several times and the resulting data were analyzed by statistical software. Relative

**Table 1.** Tree ages, dates of harvesting, and oil yields of *Eucalyptus camaldulensis* leaf samples

| Sample code | Tree age | Harvesting date | Season type | Yield (%)[a] |
|---|---|---|---|---|
| S1-1 | 8 months | 9 September 2005 | Early rainy season | 1.15 |
| S1-2 | 2 years | 19 September 2005 | Mid-rainy season | 1.45 |
| S2-1 | 4 years | August 2004 | Dry season | 0.83 |
| S2-2 | 3 years | 19 September 2005 | Mid-rainy season | 1.38 |
| S3-1 | 6 years | 9 September 2005 | Early rainy season | 1.46 |
| S3-2 | 6 years | 19 September 2005 | Mid-rainy season | 1.63 |

[a] Of dry leaf

**Table 2.** Yields of *E. camaldulensis* oil components fractionated by column chromatography

| Sample | Yield (%)[a] | | |
|---|---|---|---|
| | Hexane fraction | Chloroform fraction | Ethyl acetate fraction |
| S1-1 | 26.35 | 40.12 | 0.53 |
| S1-2 | 32.93 | 30.73 | 0.88 |
| S2-1 | –[b] | – | – |
| S2-2 | 33.87 | 49.01 | 0.74 |
| S3-1 | 60.37 | 10.97 | 0.72 |
| S3-2 | 66.09 | 7.48 | 0.71 |

[a] Percentage (w/w) based on the starting oil (steam distillate)
[b] No data for S2-1 due to small quantity of sample

**Table 3.** Chemical composition of six *E. camaldulensis* leaf oils

| Sample | *p*-Cymene (%) | γ-Terpinene (%) | α-Pinene (%) | 1,8-Cineole (%) | Terpinen-4-ol (%) | α-Terpineol (%) | Carvacrol (%) | Thymol (%) | Total (%) |
|---|---|---|---|---|---|---|---|---|---|
| S1-1 | 40.19 | 17.53 | 0.97 | 36.67 | 1.98 | 2.66 | – | – | 100.00 |
| S1-2 | 31.78 | 28.45 | 3.81 | 30.20 | 2.13 | 2.26 | – | – | 98.63 |
| S2-1 | 87.28 | 6.59 | – | – | 1.30 | – | 1.28 | 3.23 | 99.68 |
| S2-2 | 49.02 | 13.98 | – | 36.19 | 0.81 | – | – | – | 100.00 |
| S3-1 | 41.45 | 53.87 | – | 0.76 | 3.52 | – | – | 0.40 | 100.00 |
| S3-2 | 31.07 | 65.84 | – | – | 2.52 | – | – | – | 99.43 |

Data based on chromatogram peak areas. Dashes indicate that the analyte was not detected

standard deviations were obtained and analyses of variance were performed. All computations were done using the statistical software Excel Statistics 2006 for Windows, Social Survey Research Information.

## Results and discussion

### Yields and chemical compositions of *Eucalyptus camaldulensis* leaf oils

The water and steam distillation of *E. camaldulensis* leaves gave the clear yellowish oils in yields that ranged between 0.83% and 1.63% of the dry leaves. The ages, dates of harvesting, seasons, and oil yields of the samples are shown in Table 1. Differences in the oil yields were statistically significant at the 5% level. The sample clone S3-1 and S3-2 gave the highest oil yields, 1.46% and 1.63%, respectively. Samples S1-2, S2-2, and S3-2 that were collected in the middle of the rainy season (with heavy rain) gave higher oil yields than the same clones when sampled in a different season. Sample S2-1, which was collected in the dry season, gave the lowest oil yield (0.83%). The oil yields of *Eucalyptus* leaves have been reported to be relatively stable against seasonal conditions,[3,4] but *Eucalyptus* species are usually grown in relatively dry areas, so the effect of rain has rarely been investigated. The oil yield was found to be higher in the rainy season than in the dry season or early in the rainy season (with little rain); similar results were reported for *E. camaldulensis* Dehnh. leaf oils of Thailand origin.[5]

The oils were divided by silica-gel column chromatography into three fractions by elution with hexane, chloroform, and ethyl acetate, respectively. This procedure fractionated the oil based on solvent polarity. The yield of each fraction is shown in Table 2. The oils of the clone S3 were abundant in the hexane fraction. The oils of the leaves sampled in the rainy season contained more hexane fractions and less chloroform fractions. The ethyl acetate fractions were wholly very small.

Determination of the percentage composition of each oil sample was based on peak area normalization of GC-MS total ion chromatograms. The results for the six oils are shown in Table 3. The analysis data of hexane and chloroform fractions for each oil are shown in Table 4. The data for the ethyl acetate fractions are not shown due to the

**Table 4.** Chemical composition of hexane and chloroform fractions taken from five *E. camaldulensis* leaf oils

| Sample | Hexane fraction | | | | Chloroform fraction | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *p*-Cymene (%) | γ-Terpinene (%) | α-Pinene (%) | Total (%) | 1,8-Cineole (%) | Terpinen-4-ol (%) | α-Terpineol (%) | Carvacrol (%) | Thymol (%) | Thymol acetate (%) | Thymoquinone (%) | Aromadendren (%) | 1,3-Cyclohexadiene, 2-methyl-5-(1-methyl ethyl)-, monoepoxide (%) | Total (%) |
| S1-1 | 59.01 | 31.17 | 2.47 | 92.65 | 78.26 | 6.56 | 7.52 | 4.97 | – | – | – | – | – | 97.31 |
| S1-2 | 50.62 | 36.27 | 6.17 | 93.06 | 80.89 | 8.24 | 7.22 | – | – | – | – | – | – | 96.35 |
| S2-2 | 69.21 | 28.62 | – | 97.83 | 94.26 | 4.17 | 1.57 | – | – | – | – | – | – | 100.00 |
| S3-1 | 43.46 | 56.54 | – | 100.00 | 7.46 | 46.36 | 1.31 | 15.16 | 9.75 | 3.72 | 1.31 | 1.62 | 7.66 | 94.35 |
| S3-2 | 34.29 | 64.97 | – | 99.26 | 6.76 | 57.06 | 1.13 | 10.13 | 5.46 | – | 1.20 | 2.02 | 4.61 | 88.37 |

Data based on chromatogram peak areas. Dashes indicate that the analyte was not detected

low quantities found and low contribution to antioxidant activity. The hexane fractions were mostly composed of monoterpene hydrocarbons and the chloroform fractions contained oxygenated monoterpenes and phenolic compounds. The major component of S2-1 sampled in the dry season was found to be *p*-cymene and the major component of S3-1 and S3-2 sampled in the rainy season was found to be γ-terpinene. Similar seasonal change of the major component of *E. camaldulensis* leaf oils has been reported.[5] The quantity of 1,8-cineole, a typical component of *Eucalyptus* oil, varied widely. The variations in the chemical ingredients of *E. camaldulensis* leaf oils occur according to tree age, sample size,[6] and the leaf harvesting period. It has also been reported that the composition of essential oils from several plants could vary significantly depending on species, chemotypes, geographical origin, season, and extraction procedures.[7,8]

## DPPH radical scavenging method

Relatively stable organic radical DPPH has been widely used in the determination of the antioxidant activity of single compounds as well as that of different plant extracts. The method is based on the reduction of alcoholic DPPH solutions in the presence of a hydrogen-donating antioxidant. DPPH solutions show a strong absorption band at 515 nm to give a deep violet color. The absorption vanishes and the resulting decolorization is stoichiometric with respect to the degree of reduction. The remaining DPPH, measured after a certain time, corresponds inversely to the radical scavenging activity of the antioxidant.[9]

The method was used to evaluate the free radical scavenging activity of *E. camaldulensis* leaf oils. The DPPH radical scavenging activities (% inhibition) of various concentrations of *E. camaldulensis* leaf oils are shown in Fig. 1. The leaf oils of S1 had lower radical scavenging activities than S2 and S3. S2-1 and S3-1 showed higher activities among the *Eucalyptus* oil samples by this method. This may be due to the relatively high contents of the phenolic

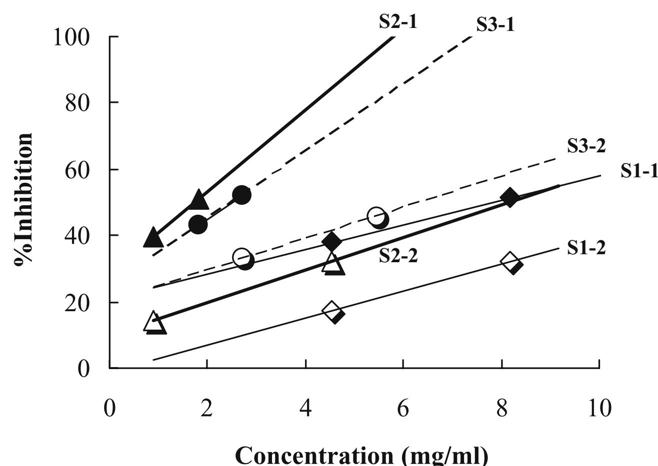


**Fig. 1.** Free radical scavenging activities of *Eucalyptus camaldulensis* leaf oils by 1,1-diphenyl-2-picylhydrazyl (DPPH) method

**Table 5.** Radical scavenging activity of *E. camaldulensis* leaf oils and their fractions as measured by 1,1-diphenyl-2-picylhydrazyl (DPPH) method

| Sample | % Inhibition | | |
|---|---|---|---|
| | Steam distillate[a] | Hexane fraction | Chloroform fraction |
| S1-1 | 16.20 | 1.50 | 17.69 |
| S1-2 | 6.26 | 1.72 | 13.79 |
| S2-1 | 39.58 | –[b] | –[b] |
| S2-2 | 14.20 | 1.50 | 11.12 |
| S3-1 | 29.49 | 1.95 | 65.85 |
| S3-2 | 15.39 | 1.45 | 53.84 |

Data given as percentage inhibition at concentration of 0.91 mg/ml
[a] Before fractionation
[b] Data unavailable due to small quantity of S2-1 sample

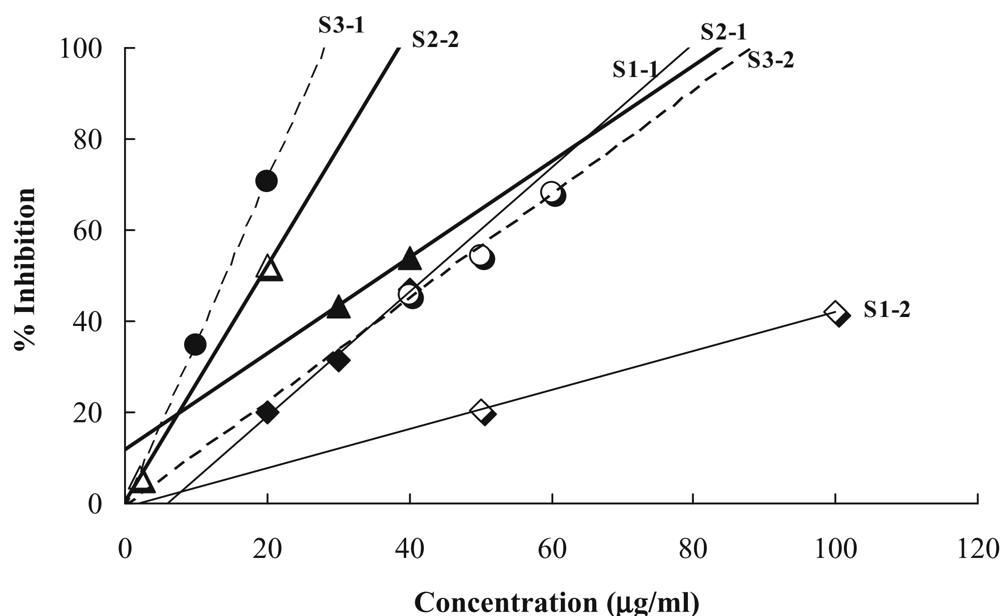


**Fig. 2.** Antioxidant activities of *E. camaldulensis* leaf oils by $\beta$-carotene bleaching method

compounds, carvacrol and thymol (Tables 3 and 4). Antioxidant activities of *p*-cymene, $\gamma$-terpinene, and 1,8-cineole are very poor, but those of thymol and carvacrol are very high.

The hexane fraction of every sample showed very poor radical scavenging activity, but each chloroform fraction showed relatively higher activity (Table 5). The chloroform fractions contained more polar components like phenolic compounds (Table 4), which may be the reason why these fractions had higher antioxidant activity.

### $\beta$-Carotene bleaching method

The $\beta$-carotene bleaching method is based on the loss of the yellow color of $\beta$-carotene due to its reaction with radicals that are formed by linoleic acid oxidation in an emulsion. $\beta$-Carotene undergoes rapid discoloration in the absence of an antioxidant. The rate of $\beta$-carotene bleaching was determined by measuring the absorbance at 470 nm. The presence of different antioxidants can hinder the extent of $\beta$-carotene bleaching by neutralizing the linoleate free radical and other free radicals formed in the system. This method is widely used because $\beta$-carotene shows strong biological activity and is a physiologically important compound. Furthermore, $\beta$-carotene is used as a coloring agent in beverages and its discoloration would markedly reduce the quality of those products.[10] This fact is used in the evaluation of antioxidant activity of the *E. camaldulensis* leaf oil. Figure 2 shows the antioxidant activities (% inhibition) of various concentrations of *E. camaldulensis* leaf oils according to the $\beta$-carotene bleaching method. S3-1 and S2-2 showed higher antioxidant activity among the *Eucalyptus* oil samples for this method. S1-2 showed the lowest activity in both the DPPH method and the $\beta$-carotene method, but S1-1 showed relatively high activity in the $\beta$-carotene method.

### Comparison of the two methods

As previously described,[10] the use of multiple methods is necessary in the assessment of antioxidant activity because their specificity and sensitivity may be slightly different between the antioxidative substances. Furthermore, real

**Table 6.** Comparison of $IC_{50}$ inhibitory concentration values by DPPH and $\beta$-carotene bleaching methods

| Sample | $IC_{50}$ by DPPH method (mg/ml) | $IC_{50}$ by $\beta$-carotene bleaching method (μg/ml) |
|---|---|---|
| BHA | $11.46 \times 10^{-3}$ | 0.16 |
| $\alpha$-Tocopherol | $24.19 \times 10^{-3}$ | 0.19 |
| Thymol | 0.65 | 2.83 |
| Carvacrol | 0.70 | 3.05 |
| S1-1 | 7.80 | 42.60 |
| S1-2 | 12.62 | 118.55 |
| S2-1 | 1.75 | 36.29 |
| S2-2 | 8.18 | 19.16 |
| S3-1 | 2.52 | 14.30 |
| S3-2 | 6.34 | 44.63 |

BHA, butylated hydroxyanisole

food systems generally consist of multiple phases in which lipid and water coexist with some emulsifiers. Hence, an antioxidant assay that uses a heterogeneous system such as an oil–water emulsion is also required.[11] One of the major factors influencing antioxidant activity in real food systems is the presence of water, because antioxidants partition between the lipid and aqueous phases, and hydrophilic antioxidants are often less effective in oil–water emulsions than lipophilic antioxidants. To accurately measure the potential for antioxidant activity in foods, individual models such as bulk oil and emulsions should be studied to allow the antioxidant activity to be assessed.[8,12] Two different model systems, the DPPH and $\beta$-carotene bleaching methods, were used in this study. Comparison of $IC_{50}$ values by these two methods are shown in Table 6.

In the results for the DPPH method, the antioxidant activities decreased according to the following order: S2-1 > S3-1 > S3-2 > S1-1 > S2-2 > S1-2. On the other hand, the $\beta$-carotene bleaching method indicated the antioxidant activities according to the following order: S3-1 > S2-2 > S2-1 > S1-1 > S3-2 > S1-2. It was found that if the polar compounds (ascorbic acid, rosmarinic acid, caffeic acid, etc.) were tested only by the $\beta$-carotene bleaching method,[11] they would be considered to be weak antioxidants. However, the strong antioxidant activities of these compounds can be proven by the other testing methods.[12] This means that the $\beta$-carotene bleaching method tends to underestimate the antioxidant activity of the polar compounds, which may explain why S2-1 showed relatively low activity even though it had relatively high levels of the polar substances, carvacrol and thymol etc. The $IC_{50}$ values of thymol and carvacrol were 0.65 and 0.70 mg/ml by the DPPH method, and 2.83 and 3.05 $\mu$g/ml by the $\beta$-carotene bleaching method, respectively (Table 6). Other compounds, such as *p*-cymene, $\gamma$-terpinene, and 1,8-cineole, showed very low activities when measured with the DPPH method, but showed somewhat higher activities when measured with the $\beta$-carotene bleaching method. The $\beta$-carotene bleaching method has been found to indicate relatively higher antioxidant activities for nonpolar compounds. This phenomenon is described as the "polar paradox."[11–13]

The DPPH method is faster than the $\beta$-carotene bleaching method and it can be helpful in investigation of novel antioxidants for rapid estimation and preliminary information of radical scavenging abilities. The antioxidant activities of various foods have been accurately and rapidly determined using DPPH, which can be used for both solid and liquid samples and is also not specific for any particular antioxidant alone.[2,14,15] The method is sensitive and requires only a small quantity of sample,[9] which made it the more favorable method for this study.

It was confirmed that the *E. camaldulensis* leaf oil possessed fair antioxidant properties. The antioxidative effects estimated by the DPPH method are mainly due to the presence of phenolic compounds like thymol and carvacrol, and these compounds are crucial for the antioxidant activity of *E. camaldulensis* leaf oil. The contents of these compounds also varied from sample to sample, and may have caused the differences in the antioxidative properties of the leaf oils.

The results indicate that *E. camaldulensis* leaf oil could be used as a source of natural antioxidants for the food industry. It will be interesting to further examine the application of this leaf oil as a source of natural antioxidant additives.

**Acknowledgments** The authors thank Siam Forestry Co. Ltd., Kanchanaburi Province, Thailand, for providing the *Eucalyptus* leaf samples.

## References


1. Suja KP, Jayalekshmy A, Arumughan C (2005) Antioxidant activity of sesame cake extract. J Food Chem 91:213–219
2. Sacchetti G, Maietti S, Muzzoli M, Scaglianti M, Manfredini S, Radice M, Bruni R (2005) Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. J Food Chem 91:621–632
3. Dunlop PJ, Bignell CM, Hibbert DB (2000) Use of gas chromatograms of essential leaf oils to compare clones of *Eucalyptus camaldulensis*. Biochem Syst Ecol 28:383–391
4. Nicolle D, Dunlop PJ, Bignell CM (1998) A study of the variation with time of the compositions of the essential leaf oils of 16 *Eucalyptus* species. Flavour Frag J 13:324–328
5. Channu C (2001) Efficacy of essential oil from Eucalyptus (*Eucalyptus camaldulensis* Dehnh.) leaf on some stored-product insect pests. Masters thesis, Faculty of Agriculture, Kasetsart University

6. Coppen JW (2002) Eucalyptus—the genus *Eucalyptus*. Taylor and Francis, New York, pp 350–353
7. Suhr KI, Nielsen PV (2003) Antifungal activity of essential oils evaluated by two different application techniques against rye bread spoilage fungi. J Appl Microbiol 94:665–674
8. Decker EA, Warner K, Richard MP, Shahidi F (2005) Measuring antioxidant effectiveness in food. J Agric Food Chem 53:4303–4310
9. Blois MS (1958) Antioxidant determinations by the use of a stable free radical. Nature 181:1199–1200
10. Sakanaka S, Tachibana Y, Okada Y (2005) Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinoha-cha). J Food Chem 89:569–575
11. Kulisic T, Radonic A, Katalinic V, Milos M (2004) Use of different methods for testing antioxidative activity of oregano essential oil. J Food Chem 85:633–640
12. Koleva II, van Beek TA, Linssen JPH, de Groot A, Evstatieva LN (2002) Screening of plant extracts for antioxidant activity: a comparative study on the three testing methods. Phytochem Anal 13: 8–17
13. Maisuthisakul P, Pongsawatmanit R, Gordon MH (2006) Antioxidant properties of Teaw (*Cratoxylum formosum* Dyer) extract in soybean oil and emulsions. J Agric Food Chem 54: 2719–2725
14. Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG (2005) Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. J Food Chem 91:571–577
15. Molyneux P (2004) The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol 26:211–219