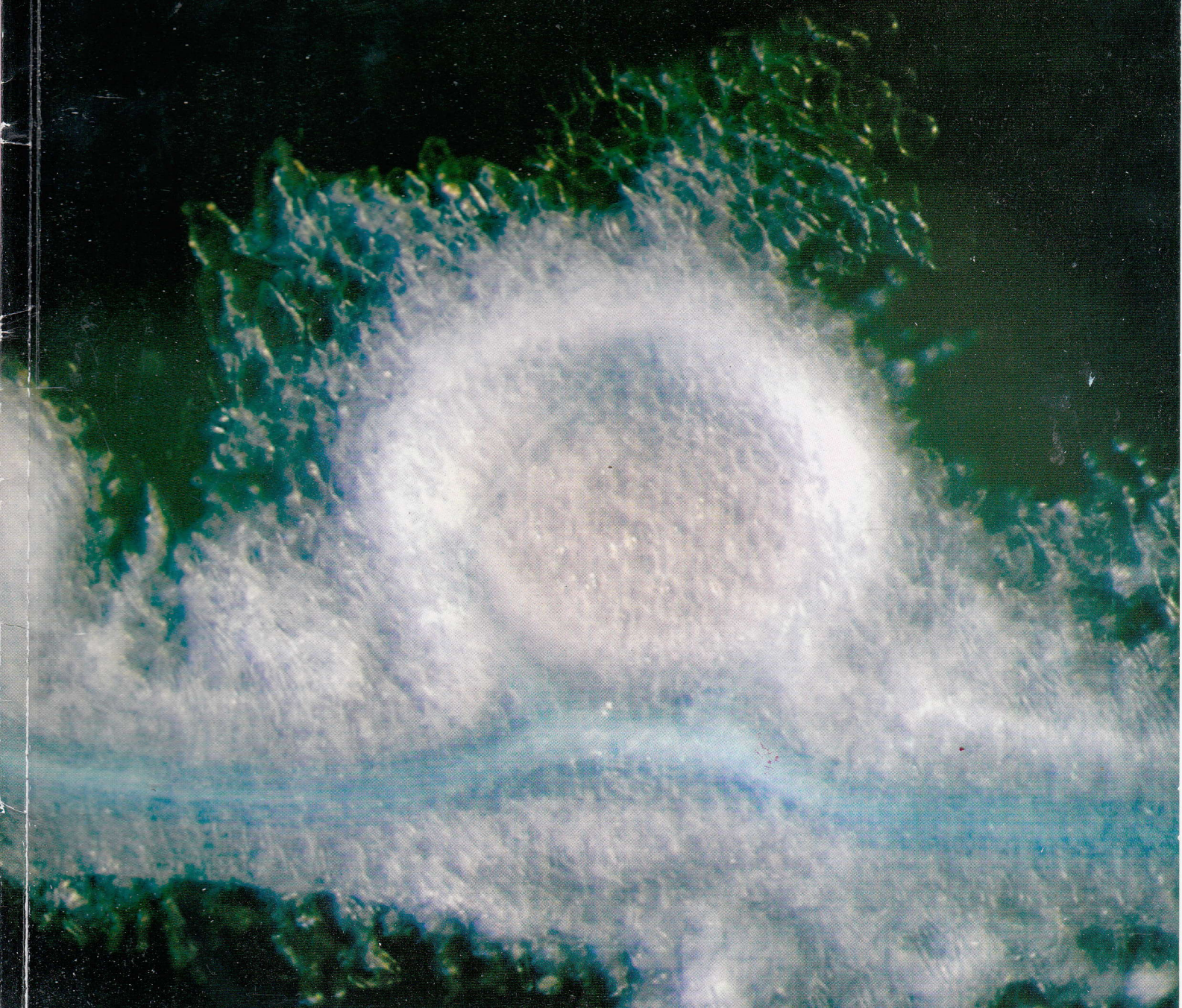


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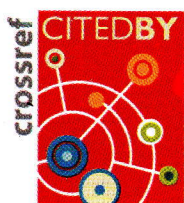
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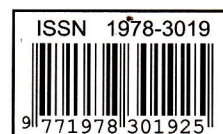
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## Volatile Compounds of *Pluchea indica* Less and *Ocimum basilicum* Linn Essential Oil and Potency as Antioxidant

PAINI SRI WIDYAWATI<sup>1\*</sup>, CHRISTOFORA HANNY WIJAYA<sup>2</sup>,  
PENI SUPRPTI HARDJOSWORO<sup>3</sup>, DONDIN SAJUTHI<sup>4</sup>

<sup>1</sup>Food Technology Study Programme, Faculty of Agricultural Technology, Widya Mandala Catholic University,  
Dinoyo Street No. 42-44, Surabaya 60265, Indonesia

<sup>2</sup>Department of Food Science and Technology, Faculty of Agricultural Technology, Bogor Agricultural University,  
Darmaga Campus, Bogor 16680, Indonesia

<sup>3</sup>Department of Animal Production and Technology, Faculty of Animal Science, Bogor Agricultural University,  
Darmaga Campus, Bogor 16680, Indonesia

<sup>4</sup>Primate Research Center (PSSP), Bogor Agricultural University, Darmaga Campus, Bogor 16680, Indonesia

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This research was conducted to identify volatile compounds of *pluchea* and *basil* essential oils and their antioxidant capacity to scavenge a DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical and inhibit lipid peroxidation. Essential oil of *pluchea* and *basil* leaves was prepared by hydrodistillation method and then their volatile compounds were identified by GC-MS. The volatile compounds in the essential oil of *pluchea* leaves consist of 66 components with (10S,11S)-Himachala-3-(12)-4-diene (17.13%) made up the highest proportion of volatile compounds. *Basil* leaves had 70 volatile components in which the major components were (E)-3,7-dimethyl-2,6-octadienal (23.98%) and (Z)-3,7-dimethyl-2,6-octadienal (17.35%). Total phenol levels in *pluchea* and *basil* essential oils were 275 and 209 ppm, respectively. DPPH scavenging activity of the essential oil of *pluchea* leaves was lower than that of *basil* leaves, conversely inhibition activity of lipid peroxidation in palm oil of *pluchea* essential oil was higher than that of *basil* leaves.

Keywords: essential oil, *pluchea* leaves, *basil* leaves, and antioxidant capacity

### INTRODUCTION

*Pluchea* and *basil* are plants that are used as fresh food and traditional medicine (Dalimarta 2003). *Pluchea indica* Less is taxonomically classified in family *Compositae* (*Asteraceae*). *Pluchea* leaves contain phytochemicals such as lignan, terpene, phenylpropanoid, benzenoid, alkanes (Luger *et al.* 2000), sterol, 2-(prop-1-ynyl)-5-(5,6-dihydroxy-hexa-1,3-dienyl)-thiophene, (-)-catechin (Biswas *et al.* 2005), phenol hydroquinone, saponin, tannin, alkaloid (Widyawati *et al.* 2011), flavonol (quercetin, kaempferol, myricetin) (Andarwulan *et al.* 2010). Traithip (2005) has informed that *pluchea* leaves comprises of volatile compounds of terpene groups such as boehmeryl acetate, HOP-17-(21)-en-3 $\beta$ -acetate, linaloyl glucoside, linaloyl apiosyl glucoside, linaloyl hydroxy glucoside, plucheoside C, 3'-(2',3'-diacetoxy-2'-methyl-butyl)-cuaahthemone, plucheol A, plucheol B, plucheoside A, plucheoside B, plucheoside E, and pterocarpritol. Widyawati *et al.* (2011) has informed that these phytochemicals in *pluchea* leaves cause having antioxidant activity.

*Basil* (*Ocimum basilicum* Linn) is categorized into *Lamiaceae* family that contains essential oils rich in phenolic compounds such as flavonoid and antocyanin (Juliani & Simon 2002). Bunrathep *et al.* (2007) has informed that essential oils of *Ocimum* genus contain monoterpene, oxygenated monoterpene, sequiterpene, oxygenated sequiterpene, and phenylpropanoid (eugenol, methyl eugenol, and methyl chavicol). Essential oil of *basil* is widely used as herbs culinary and traditional medicine.

Polyphenols are a major component of plants that have therapeutic effect and healthy (Tsai *et al.* 2011). These compounds show antioxidative activity in food products, such as species oxygen reactive and free radical scavenging activities, iron chelating and reducing activity,  $\beta$ -carotene linoleic acid bleaching assay (Subhasree *et al.* 2009).

Until now the comparison of volatile compounds contained and antioxidant activity between *pluchea* and *basil* essential oils had not been studied. Therefore this research was conducted to identify volatile compounds of *pluchea* and *basil* essential oils and to compare their antioxidant capacity to scavenge a DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical and inhibit lipid peroxidation.

\*Corresponding author. Phone: +62-31-5678478,  
Fax: +62-31-5610818, E-mail: wiwiedt@gmail.com

## MATERIALS AND METHODS

**Plant Material.** Leaves of *Pluchea indica* Less and *basil* (*Ocimum basilicum* Linn) were collected from home garden at Sidoarjo and Surabaya.

**Preparation of the Essential Oils by Hydrodistillation Method.** The leaves of the two plants were collected and washed thoroughly. The fresh leaves were cut and hydrodistilled by a Clevenger-type apparatus for 6 hours (Bunrathep *et al.* 2007). The distillate was dried with anhydrous sodium sulfate salt. This distillate was called essential oil further. And then the essential oils were stored in freezer before their volatile compounds and antioxidant activity were analysed.

**GC-MS Analysis.** The Volatile compounds of *pluchea* and *basil* essential oils were analysed by GC-MS (Agilent GC-MS 5975C, Palo Alto, CA, USA) equipped with a mass selective detector (MSD). The column used was a DB-10 (25 m x 0.25 mm x 0.25 mm film thickness), the carrier gas was helium (3 ml/min) and the column pressure was 70 kPa. The column temperature was set up at 50-250 °C with two increasing steps. Initial temperature was set up at 50 °C for 5 min, and then increased to 80 °C at a rate of 2 °C/min, and then the temperature was kept constant at 80 °C for 1 min. Then the temperature of the column was increased up to 250 °C at a rate of 4 °C/min and kept constant at 4.5 min. Injector volume was 1 µl and total running time was approximately 40 min. Injector and detector temperatures were held at 225 and 270 °C, respectively. Mass spectra obtained were compared with spectra from National Institute of Standard Technology/NIST library.

**Determination of Total Phenol Content.** The total phenolic contents of each essential oil were determined using the Folin–Ciocalteu reagent (modified by Sahreen *et al.* 2010). The reaction mixture contained 50-100 µl of samples, 1 ml of the Folin–Ciocalteu reagent, 5 ml of 20% sodium carbonate and then the mixture was added with distilled water until 10 ml. Absorbance of the sample was measured at 750 nm after 20 min reaction (Shimadzu UV-Vis 20D) and the reading was referring to gallic acid as a standard. The total phenolic contents were expressed as gallic acid equivalents (GAE), in mg/l essential oil.

**1,1-Diphenyl-2-Picrylhydrazyl Radical (DPPH) Scavenging Assay.** The ability of the essential oil to scavenge DPPH radicals was determined by the method of Sahreen *et al.* (2010). Essential oil in methanol at many concentrations was added to 3 ml of a 60 µM DPPH in methanol. And then methanol

was added until a total of 10 ml was reached. Absorbance at 517 nm was determined after 30 min and the percentages of inhibition was calculated as  $[(A_o - A_t)/A_o] \times 100\%$  ( $A_o$  = absorbance without essential oil;  $A_t$  = absorbance with essential oil).  $IC_{50}$  (concentration of sample required to scavenge 50% free radical) was determined by relationship linear curve between DPPH free radical scavenging activity and essential oil concentration.

**Peroxide Value (PV) Assay.** Essential oil at various concentrations was added in 100 ml fresh palm oil, and then heated for 1 hour in oven (oven test) at 180 °C. Peroxide value was measured using Capman and McKay (1949) method modification. The mixture of 0.2 ml essential oil, 5 ml ethanol 75%, and 0.2 ml  $FeCl_2$  20 mM (1 ml  $H_2SO_4$  2N in 100 ml  $FeCl_2$  solution) was mixed for 2 min, and then added by 0.2 ml of 30% ammonium thiocyanate. Into in mixture, 75% ethanol was added until a total of volume 10 ml. Stability time, maximum wave length, and absorbance of Fe-thiocyanate complex compounds were measured by a UV-Vis Spectrometer.

**Anisidine Value (p-AnV) Assay.** Anisidine value (AV) of the sample was determined by Shahidi and Zhong (2005) method. 0.2 ml sample was added with isooctane until a total volume reached 10 ml. Then 5 ml sample was mixed with 1 ml of p-anisidine for 10 min. Absorbance of the sample was measured using a UV-Vis spectrometer at 350 nm. The Totox value (TV) was a measure of the total oxidation, including primary and secondary oxidation products. It was a combination of PV and p-AnV. Totox value (TV) was calculated according to the following equation:  $2PV + p-AnV$ , PV means peroxide value, p-AnV means anisidine value.

**Statistic Analysis.** Statistical analysis was done using the SPSS (software package used for statistical analysis) version 17.0 for Windows. All the values were expressed as mean  $\pm$  Deviation standard (SD). The results were analyzed for statistical significance by using one way ANOVA, followed by the Duncan's test. P values  $< 0.05$  were considered as significant.  $IC_{50}$  (concentration of sample required to scavenge 50% free radical) was determined by linear regression curve.

## RESULTS

**Chemical Composition of the Essential Oil.** GC-MS analysis of *pluchea* essential oil based on more similarity than 80% with NIST library (National Institute of Standard Technology) (Table 1 & Figure 1) was detected 66 components representing 46.3% of total essential oil. *Pluchea* contained alcohols,

Table 1. Volatile compounds with aroma description of *pluchea* essential oil

Volatile compounds	TR (Min)	Area (%)
1-Propanol, 2-methyl	7.185	0.02
Butanal, 3-methyl	7.507	0.05
Butanal,2-methyl	7.616	0.63
Furan,2-ethyl	8.085	0.01
1-Butanol,2-methyl	8.560	0.47
Hexanal	9.738	0.06
Dimethyl sulfoxide	10.687	0.04
Dimethyl sulfoxide	10.884	0.02
3-Hexen-1-ol (Z)	11.020	1.18
1-Hexenol	11.249	0.58
Santolina triene	12.362	0.04
Bicyclo[3.1.0]hex-2-ene,2-methyl	13.099	0.04
1S-alpha-pinene	13.399	0.05
Benzaldehyde	14.059	0.03
1-Octen-3-ol	14.326	0.07
Bicyclo[3.1.0]hexane, 4-methylene	14.506	0.96
5-Hepten-2-one,6-methyl	14.577	0.24
Bicyclo[3.1.1]heptane,6,6-dimethyl	14.724	0.06
Furan,2-pentyl	14.822	0.04
3-Hexen-1-ol,acetate	15.144	0.04
1,3-Cyclohexadiene,1-methyl-4-(1-methylethyl)	15.859	0.06
Benzene,1-methyl-2-(1-methylethyl)-	16.104	0.13
Limonene	16.295	0.04
Bicyclo[3.1.0]hex-2-ene,4-methyl-1-(1-methylethyl)-	16.377	0.01
1,3,6-Octatriene,3,7-dimethyl	16.715	0.02
1,4-Cyclohexadiene,1-methyl-4-(1-methylethyl)-	17.343	0.17
Bicyclo[4.1.0]hept-2-ene,3,7,7-trimethyl-	18.603	0.04
Nonanal	18.925	0.04
3-Cyclohexen-1-ol,4 methyl-1-(1-methylethyl)-	23.196	0.49
p-Menth-1-en-8-ol	23.791	0.16
Benzoic acid,2-hydroxy-methyl ester	24.031	0.04
2,6-Octadien-1-ol,3,7 dimethyl-(Z)-	25.406	0.41
3,6-Octadien-1-ol,3,7-dimethyl-(Z)-	25.575	0.03
Bicyclo[4.1.0]hept-2-ene,3,7,7-trimethyl-	25.875	0.03
2,6-Octadenal,3,7-dimethyl,(Z)	26.120	0.38
2,6-Octadien-1-ol,3,7-dimethyl-(E)-	26.671	0.55
2,6-Octadienal,3,7-dimethyl-(E)	27.702	0.60
Napthalene,1,2-dihydro-1,1,6-trimethyl-	33.556	0.03
Ylangene	34.380	0.22
Tricyclo[5.4.0.0(2,8)]undec-9-ene,2,6,6,9-tetramethyl-	34.560	0.07
2-Buten-1-one,1-(2,6,6-trimethyl-1,3-cyclohexandien-1-yl)-(E)	34.631	0.19
Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-,[1S-(1.alpha,2beta,4beta	35.013	0.05
Bicyclo[7.2.0]undec-4-ene,4,11,11,trimethyl-8-methylene-	35.634	0.05
Azulene,1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-[1S-(1.alpha,7.alpha,8a.beta)]	35.782	0.11

Table 1. Continue

Volatile compounds	TR (Min)	Area (%)
Caryophyllene	36.153	11.88
Bicyclo[3.1.1]hept-2-ene,2,6-dimethyl-6-(4-methyl-3-pentenyl)-	36.267	0.23
1,6,10-Dodecatriene,7,11-dimethyl-3-methylene,(E)	36.638	0.11
1H-Benzocycloheptene,2,4a,5,6,7,8,9,9a,octahydro-3-5-5-trimethyl-9-methylene-	36.867	0.27
Alpha-Caryophyllene	36.933	0.62
Caryophyllene	37.287	0.04
Napthalene,1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7(1-methylethenyl)-[1S-(1.apha,7.alpha,8a.alpha)]	37.369	0.10
Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl	37.446	0.09
Cis(-)-2,4a,5,6,9a-hexahydro-3,5,5,9-tetramethyl(1H)benzocycloheptene	37.533	0.28
10s,11s-Himachala-3-(12),4-diene	37.746	17.13
Napthalene,1,2,3,4,4a,5,6,8a-octahydro-4a,8-imethyl-2(1-methylethenyl)-[2R-(2.apha,4a.alpha,8a.beta)]	37.915	0.28
1H-Benzocycloheptene,2,4a,5,6,7,8-hexahydro-3-5-5,9-tetramethyl-(R)-	38.035	0.39
1H-Cycloprop[e]azulene,decahydro-1,1,7-trimethyl-4-methylene-[1aR-(1,1,7-trimethyl-4-methylene-[1aR-(1.apha,4a.beta,7.alpha,7a.beta,7b.alpha)])-	38.204	0.08
1,6,10-Dodecatrien-3-ol,3,7,11-trimethyl-	39.055	0.12
Benzene,1-methyl-3-(1-methylethyl)-	39.502	0.07
Caryophyllene Oxide	39.939	3.06
Tricyclo[6.3.0.0(1,5)]undec-2-en-4-one,2,3,5,9-tetramethyl]-	40.708	0.63
10,10-Dimethyl-2,6-dimethylenebicyclo[7.2.0]undeca-5-beta-ol	40.970	2.08
1H-Cycloprop[e]azulene,1a,2,3,5,6,7,7a,7b-octahydro-1,14,7-tetramethyl-[1aR-(1.alpha,7.alpha,7a.alpha,7a.beta,7b.alpha)]	41.553	0.11
Anthracene	44.396	0.04
Nonadecane	46.316	0.07
Napthalene,1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-[2R-(2.alpha,4a.alpha,8a.beta)]	46.485	0.07

Volatile compounds of essential oil were determined based on more similarity than 80% with NIST library.



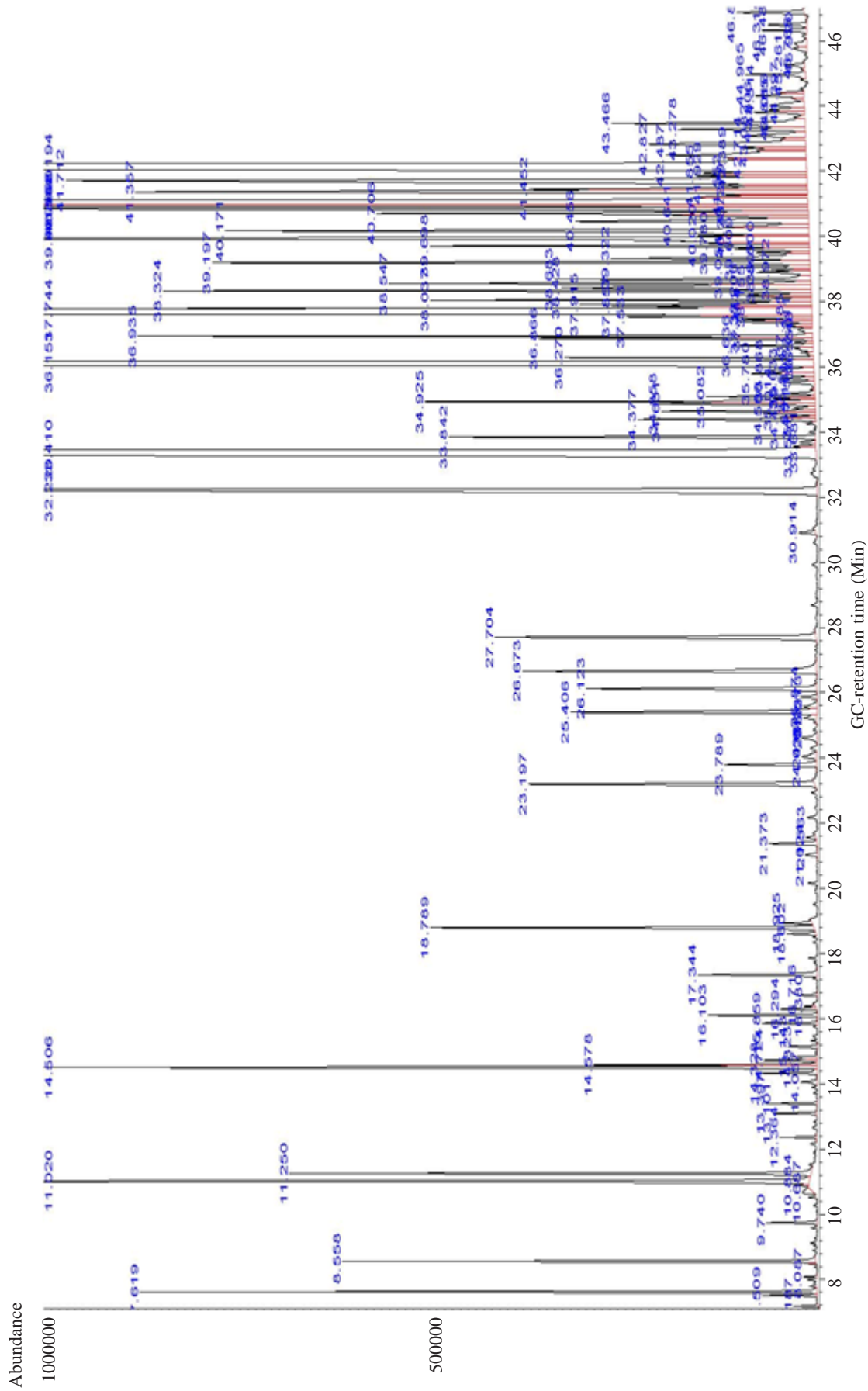


Figure 1. Chromatogram of volatile compounds in *Pluche indica* Less essential oil.

Table 2. Volatile compounds with aroma description of *basil* essential oil

Volatile compounds	TR (Min)	Area (%)
Butanal,3-methyl-	7.507	0.04
Butanal,2-methyl-	7.616	0.02
Furan,2-ethyl	8.085	0.02
1-Butanol,2-methyl-(+/-)	8.554	0.04
3-Hexen-1-ol, (Z)	11.020	1.37
Furan,2,5-diethyltetrahydro-	12.144	0.04
1S-Alpha-pinene	13.399	0.15
1-Octen-3-ol	14.402	0.65
Bicyclo[3.1.0]hex-2-ene,4-methyl-1(1-methylethyl)-	14.501	0.05
Beta-myrcene	14.822	0.19
3-Octanol	14.855	0.15
3-Hexen-1-ol, acetate	15.161	0.09
Benzene,1-methyl-3-(1-methylethyl)-	16.110	0.05
D-limonene	16.301	0.32
Eucalyptol	16.465	0.20
1,3,6-Octatriene,3,7-dimethyl,(Z)-	16.743	1.38
1,4-Cyclohexadiene,1-methyl-4-(1-methylethyl)-	17.354	0.01
alpha,methyl,apha,-[4-methyl-3-pentenyl]oxiramethanol	17.877	0.03
Linalool oxide trans	18.516	0.04
Benzenemethanol,4-(1-methylethyl)	18.652	0.09
1,6-Octadien-3-ol,3,7-dimethyl	18.919	8.30
Bicyclo[2.2.1]heptan-2-ol,1,3,3-trimethyl	19.912	0.18
6-Octenal,3,7-dimethyl, ®	21.385	0.17
p-menth-1-en-8-ol	23.840	1.32
Estragole	24.080	0.37
6-Octen-1-ol,7-methyl-3-methylene	24.997	0.09
6-Octen-1-ol,3,7-dimethyl, ( R)	25.318	0.12
2,6-Octadien-1-ol,3,7-dimethyl (Z)	25.651	6.50
3,6-Octadien-1-ol, 3,7-dimethyl, (Z)	25.799	0.51
2,6-Octadienal,3,7-dimethyl, (Z)	26.453	17.35
2-Cyclohexene-1-one,3-methyl-6-(1-methylethyl)-	25.293	0.08
2,6-Octadienal,3,7-dimethyl, (E)-	28.155	23.98
2,6-Octadienoic acid, 3,7-dimethyl, methyl ester	31.052	0.12
Geranic acid	33.000	0.24
Syn-Tricyclo[5.1.0.0(2,4)]oct-5-ene, 3,3,5,6,8,8-hexamethyl,	33.288	0.28
Eugenol	33.414	0.12
2,6-Octadien-1-ol,3,7-dimethyl-,propanoate, (Z)-	33.474	0.18
2,6-Octadien-1-ol,3,7-dimethyl-, acetate, (Z)	34.265	0.21
Copaene	34.560	0.22
Bicyclo[3.1.1]hept-2-ene,2,6-dimethyl-6-(4-methyl-3-pentenyl)	34.827	0.05
Cyclobuta[1,2,3,4]dicyclopentene	34.931	0.05
Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-,[1S-(1alpha,2 beta,4 beta)	35.023	0.23
Bicyclo[3.1.1]hept-2-en-6-one, 2,7,7-trimethyl-	35.651	0.07
1H-cycloprop[e]azulene,1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-,[1aR-(1a apha, 4 alpha, 4a beta, 7b alpha)]	35.733	0.21

Table 2. Continue

Volatile compounds	TR (Min)	Area (%)
Caryophyllene	36.087	3.06
Bicyclo[3.1.1]hept-2-ene,2,6-dimethyl-6-(4-methyl-3-pentenyl)-	36.289	1.17
1,6,10-Dodecatriene,7,11-dimethyl-3-methylene-	36.436	0.15
1,6,10-Dodecatriene,7,11-dimethyl-3-methylene-, (Z)	36.627	0.25
Alpha caryophyllene	36.949	1.38
1H-Cycloprop[e]azulene,decahydro-1,1,7-trimethyl-4-methylene-, [1aR-(1a alpha, 4a beta, 7 alpha, 7a beta, 7b alpha)	37.118	0.05
1,6-Octadien-3-ol,3,7-dimethyl-,formate	37.200	0.05
Naphtalene, 1,2,3,4,4a,5,6,8a,octahydro-7-methyl-4-methylene-1-(1-methylethyl)-,(1 apha, 4a alpha, 8a alpha)-	37.369	0.06
1H-Cyclopenta[1,3]cyclopropa[1,2]benzene,octahydro-7-methyl-3-methylene-4-(1-methylethyl)-,[3as-(3a alpha, 3b beta, 4 beta, 7 alpha, 7aS*)]-	37.571	1.38
10S,11S-Himachala-3(12),4-diene	37.626	0.06
Eudesma-4(14), 11-diene	37.713	0.12
1H-Cyclopro[e]azulene, decahydro-1,1,7-trimethyl-4-methylene, [1aR-(1a apha, 4a beta, 7 alpha, 7a beta, 7b alpha)]-	37.778	0.11
Seychellene	37.898	0.30
Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-, (S)-	37.964	0.17
Naphtalene, 1,2,4a, 5, 6, 8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	38.253	0.05
Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	38.329	0.04
Naphtalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)- (1S,cis)	38.400	0.29
Cis, apha-Bisabolene	38.717	3.43
1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene	39.644	0.03
Caryophyllene oxide	39.901	0.21
Cycloheptane,4-methylene-1-methyl	40.288	0.03
Tau cardinal	40.975	0.36
Bicyclo[4.4.0]dec-1-ene,2-isopropyl-5-methyl-9-methylene	41.052	0.07
Alpha Cardinol	41.275	0.30
Alpha bisabol	41.690	0.13
1,6-Octadien-3-ol, 3,7-dimethyl-, formate	42.972	0.08

Volatile compounds of essential oil were determined based on more similarity than 80% with NIST library.

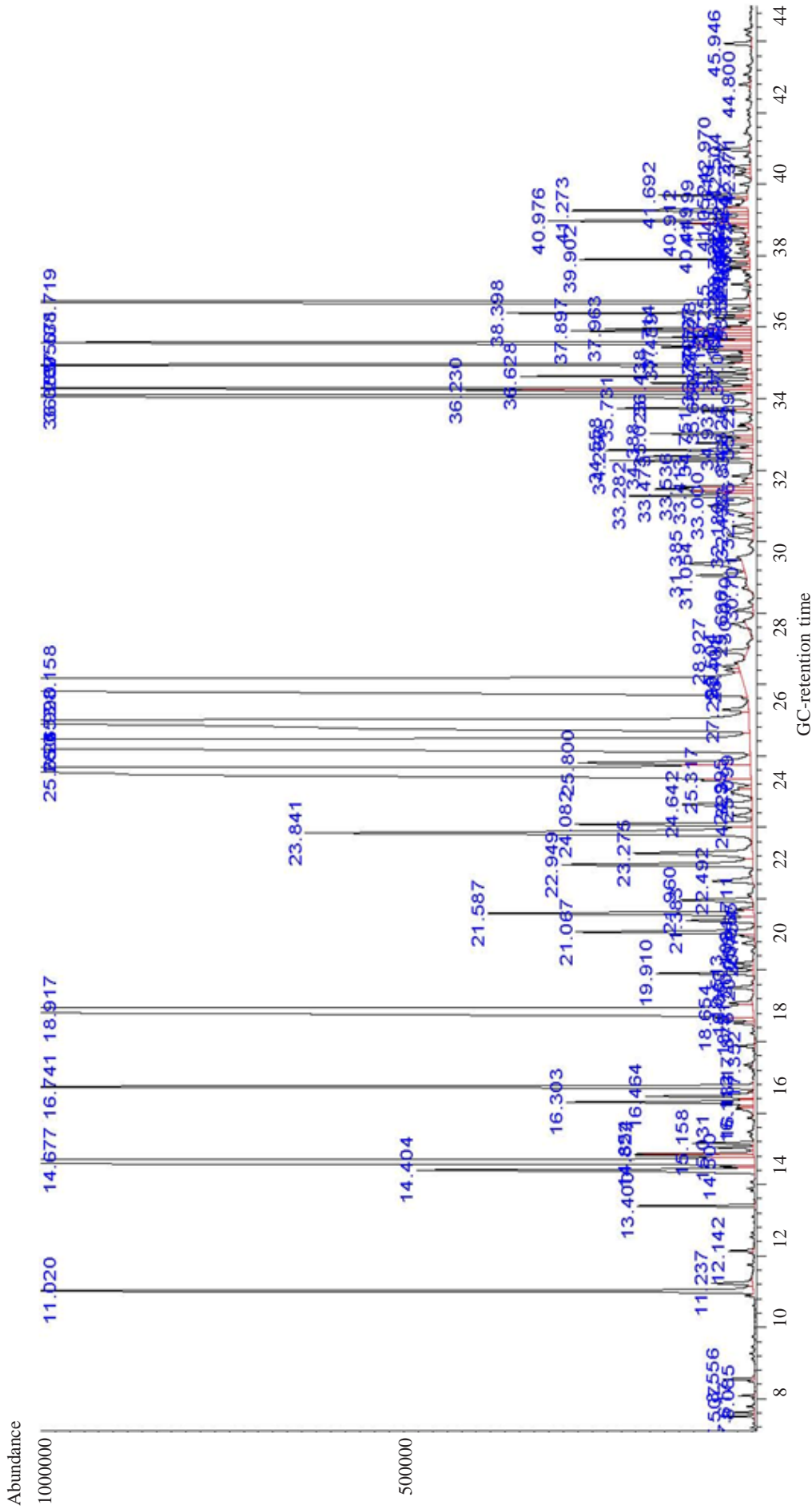


Figure 2. Chromatogram of essential oil of *Ocimum basillicum* Linn.



aldehydes, aliphatic unsaturated hydrocarbons (aliphatic, cyclic, aromatic, heterocyclic), esters, ketones, ethers, and sulfoxides. Cyclic unsaturated hydrocarbon compounds were the most numerous, followed by alcohols (6.16%), ketones (3.49%), aromatic hydrocarbons (2.05%), aldehydes (1.79%), aliphatic unsaturated hydrocarbons (1.35%), esters (0.08%), sulfoxides (0.06%), and heterocyclic hydrocarbons (0.05%). (10*S*,11*S*)-Himachala-3-(12)-4-diene (17.13%) made up the highest proportion of volatile compounds.

Seventy volatiles were identified in essential oil of *Basil* and listed in Table 2 and Figure 2. All of volatile compounds detected were represented 79.21% of oil. These basil volatiles were classified as aldehyde (41.56%), alcohol (19.85%), heterocyclic compounds (0.06%), aliphatic unsaturated hydrocarbons (2.42%), cyclic unsaturated hydrocarbons (7.45%), esters (0.22%), aromatic compounds (6.96%), ethers (0.04%), acids (0.36%), and ketones (0.29%). Among the compounds identified, (E)-3,7-dimethyl-2,6-octadienal, and (Z)-3,7-dimethyl-2,6-octadienal were found to be the major abundant compound in essential oil.

**Total Phenol Content.** The amount of total phenolics in the *basil* and *pluchea* essential oils was determined spectrophotometrically using Folin-Ciocalteu reagent and calculated as garlic acid equivalents. The *pluchea* essential oil exhibited higher total phenolics content (275.21 mg GAE/l oil) than that of *basil* essential oil (209.30 mg GAE/l oil) (Figure 3).

**Scavenging Effect on DPPH Radical.** The DPPH free radical is a stable free radical, which has been widely used to estimate free radical-scavenging activity of antioxidants. The compounds can donate or transfer electron/hydrogen atoms to DPPH, so that the radical is neutralized to form non radical compound. The color of the reaction mixture

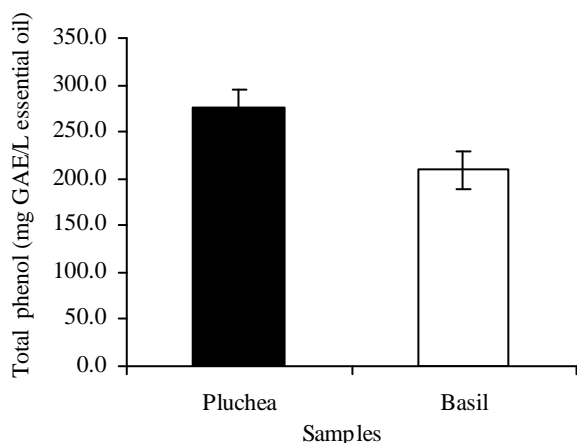


Figure 3. Total phenol of *basil* and *pluchea* leaves essential oils.

changes from purple to yellow, and a decrease in absorbance. The essential oil obtained from *basil* was more effective in scavenging of DPPH than that of *pluchea* (Figure 4). This was indicated by  $IC_{50}$  (concentration of sample required to scavenge 50% free radical) of *basil* (53.78 ppm) lower than that of *pluchea* (74.38 ppm).

**Lipid Peroxidation Inhibition Activity.** Lipid peroxidation of palm oil can be exhibited by primary and secondary oxidation products. Peroxide value is used to measure primary oxidation and anisidine value is used to determine secondary oxidation. Totox value is a parameter of total oxidation. Data showed that *Pluchea* essential oil could reduce higher peroxide, anisidine, and totox values than *basil* essential oil (Figure 5, 6, and 7).

## DISCUSSION

Volatile compounds contained in two kinds of essential oils were quantitative and qualitative differences. Differences composition between the

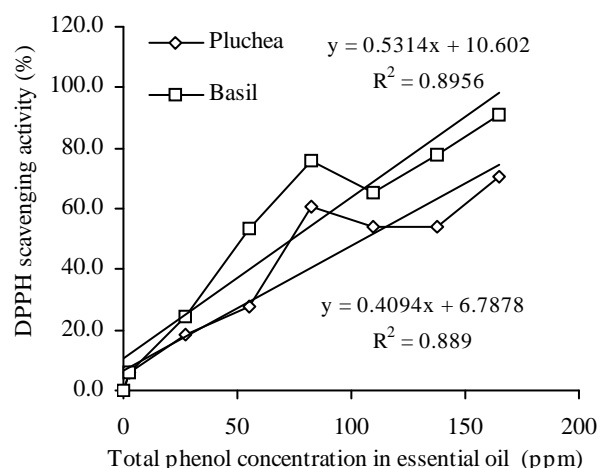


Figure 4. DPPH free radical scavenging activity of *basil* and *pluchea* essential oils.

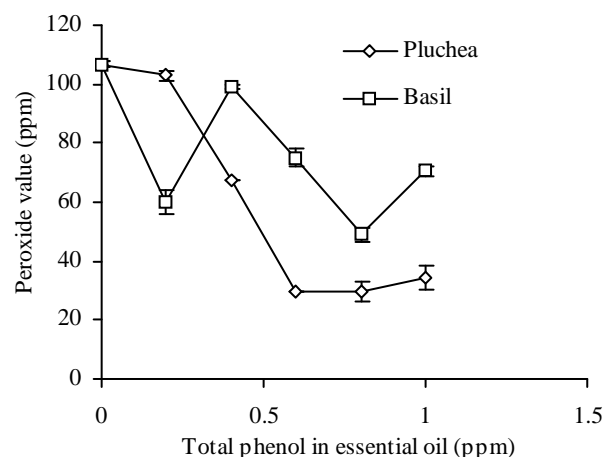


Figure 5. Peroxide value in palm oil with essential oil addition.

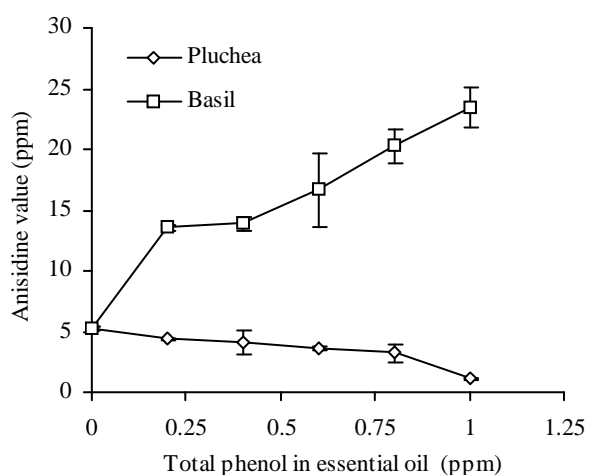


Figure 6. Anisidine value in palm oil with essential oil addition.

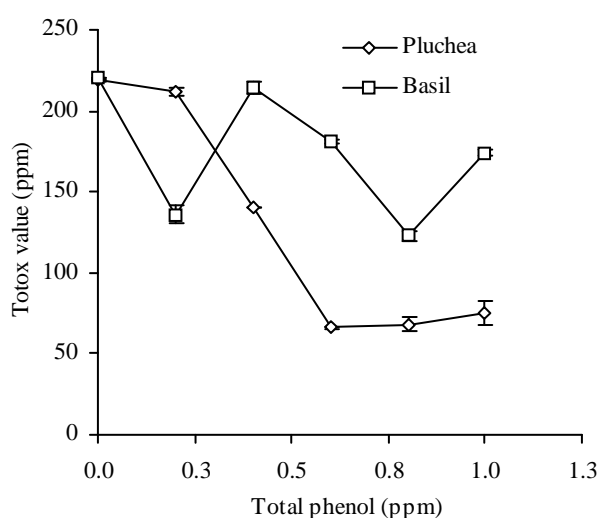


Figure 7. Totox value in palm oil with essential oil addition.

varieties determined total phenol content and antioxidant activity. Data showed that the essential oil of *plucheia* had higher total phenol than that of *basil*. It caused chemical components in *plucheia* essential oil could donor electron/hydrogen atom stronger than that in *basil* essential oil. Total phenolic contents were determined by capacity of chemical compounds in oil to involve in redox reaction with molybdenum atom in Folin Ciocalteus's phenol reagent. Type, number and position of hydroxyl group substitution in benzene ring and stability structure form after electron donating were effective to determine total phenol measurement.

The antioxidant activity assay showed that DPPH radical scavenging activity of *plucheia* essential oil was lower than that of *basil*, whereas lipid peroxidation inhibition of *plucheia* essential oil was stronger than that of *basil*. Phenolic antioxidants are products of secondary metabolism in plants, and the antioxidant activity is mainly due to their redox properties and chemical structure, which can play

an important role in chelating transitional metals, inhibiting lipoxygenase and scavenging free radicals (Al-Reza *et al.* 2010). The radical-scavenging activity is one of various mechanisms contributing to overall activity, thereby creating synergistic effects. Polar and non polar compounds in essential oil can be contributed to antioxidant activity. Antioxidant activities of essential oils from aromatic plants are mainly attributed to the active compounds present in them. This can be due to the high percentage of main constituents, but also to the presence of other constituents in small quantities or to synergy among them (Politeo *et al.* 2006).

In this study, the concentration and composition differences of volatile compounds between *plucheia* and *basil* essential oils caused different antioxidant activity. This research is also informed by Tachakittirungrad *et al.* (2007). Its means that the essential oil obtained from *basil* was effectively donating electrons/hydrogen atoms to convert picrylhydrazyl radical into their non-radical form picrylhydrazine. It was predicted that polar compounds in *basil* trended more potential to scavenge DPPH free radical than that in *plucheia*, while non polar compounds in *plucheia* essential oil were potentially to inhibit lipid peroxidation.

Volatile compounds detected in *basil* essential oil were higher qualitative and quantitative than those in *plucheia* essential oil (Table 1 & 2). Cyclic unsaturated hydrocarbons were dominant volatile compounds in *plucheia* oil but aldehyde and alcohol compounds were major volatile compounds in *basil* oil. Several volatile compounds found in *basil* essential oil were not detected in *plucheia* essential oil. These compounds included eugenol, estragole, eucalyptol,  $\alpha$ -cardinal,  $\alpha$ -bisabol,  $\beta$ -myrcene, and copaena. The chemical composition shows a close relationship between the relative percentage of eugenol and the antioxidant activity (Juliani & Simon 2002). Type, concentration, and position of hydroxyl group substitution of aromatic ring determined antioxidant activity (Conforti *et al.* 2009). Demirci *et al.* (2007) reported that the essential oil containing mainly monoterpene hydrocarbons such as  $\beta$ -phellandrene, limonene,  $\beta$ -pinene, caryophyllene, (+)-4-carene, and 1S- $\alpha$ -pinene, does not exhibit strong antioxidant activity. Bunrathep *et al.* (2007) stated that antioxidant effect can be caused by non phenolic compounds, such as monoterpene aldehyde and ketones (citronelal, eugenol,  $\beta$ -caryophyllene, and linalool).

The antioxidant activity of volatile components involved phenolic and non phenolic compounds (Hayes *et al.* 2011), hydrophilic and hydrophobic



compounds (Manian *et al.* 2008), and many antioxidant mechanisms, such as iron chelating and reducing activities, donating hydrogen atoms, and peroxidative decomposition (Zhu *et al.* 2010). Sun *et al.* (2010) reported that strong antioxidant activity was supported by ferric reducing power. Bloknina *et al.* (2003) stated that lipid peroxidation inhibition activity, one of antioxidant mechanism, is free radical scavenging activity. Kubola and Siriamornpun (2008) presented that ferric reducing power is related to radical chain breakdown activity with donating hydrogen atoms.

The difference of antioxidant activity between *pluchea* and *basil* essential oil was caused polarity difference in system assay. The polar compounds dominant in *basil* essential oil were effective in polar system, conversely the non polar compounds dominant in *pluchea* essential oil were effective in non polar system. It is based on like dissolve like (Houghton & Raman 1998).

The types and amounts of volatile compounds detected in *pluchea* essential oil differed in *basil* essential oil. The different compositions caused different total phenolic content and antioxidant capacity. The total phenolic content of *pluchea* essential oil was higher than that of *basil*. There was a negative correlation between total phenolic content and DPPH free radical scavenging test. Conversely, *pluchea* essential oil was stronger lipid peroxidation inhibition in palm oil than that of *basil*.

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