Tropical Journal of Pharmaceutical Research June 2018; 17 (6): 1119-1125
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v17i6.19

Original Research Article

Assessment of antioxidant activity of citronellal extract and fractions of essential oils of *Citrus hystrix* DC

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Sent for review: 13 February 2017 *Revised accepted: 15 May 2018*

Abstract

Purpose: *To evaluate the antioxidant activities and chemical compositions of citronellal extract and fractions of essential oils of Citrus hystrix.*
Methods: *Antioxidant activity was examined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Essential oil was produced by steam distillation while isolated citronellal and fractional distillates were obtained on a PiloDist 104-VTU fractional column at 5 mBar pressure and a reflux ratio of 20:10. Gas chromatography-mass spectrometry was used to determine their chemical composition.*
Results: *All the essential oils produced from parts of C. hystrix, i.e., twigs oil (CHT), leaves-twigs oil (CHTL), leaves oil (CHL), and fruit peels oil (CHP), citronellal extract of CHL oil (CHLCE) and the distillation fractions of CHT oil (F1–F9), exhibited antioxidant activity. CHP exhibited the highest antioxidant activity. The chemical composition of CHT, CHTL and CHL was dominated by oxygenated monoterpenes (OMs), whereas that of CHP and F1 comprised mainly of a hydrocarbon monoterpene (HM).*
Conclusion: *The antioxidant activities of essential oils from parts of C. hystrix and the fractions produced by essential oil fractional distillation exhibited a combination of HMs and OMs. Essential oils of CHP and F3 produced from the essential oil fractional distillation of CHT with a nearly equal composition of HM and OM compounds had higher antioxidant activity than the other essential oils and fractions, with IC_{50} values of 6.43 and 2.40 µL/mL, respectively.*

Keywords: *Citrus hystrix, Essential oil, Hydrocarbon monoterpene, Oxygenated monoterpene, Antioxidant*

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INTRODUCTION

Herbs and essential oils (EOs) are sources of bioactive compounds. These ingredients have been used for medicinal purposes and also used as the source of new drugs [1,2]. The high content of phenolics and flavonoids in medicinal plants has associated with their antioxidant activities, which involve in the prevention of age-related disease, particularly those caused by oxidative stress [3]. According to previous studies, EOs exert biological activity to treat

infectious and cardiovascular diseases and against pathogenic bacteria [3,4]. EOs are also widely used as food preservatives, cosmetics, virucides, fungicides, antiparasitic, and insecticides, as well as anticancer, antioxidant, and antidiabetic agents [1,2].

EOs are volatile and are composed of complex compounds. As the secondary metabolites of aromatic plants, they can identify by their strong odor. These volatile molecules include monoterpenes (hydrocarbon monoterpenes [HMs] and oxygenated monoterpenes [OMs]) as terpenes biogenerated by the mevalonate pathway and sesquiterpenes (hydrocarbon sesquiterpenes and oxygenated sesquiterpenes). They also contain phenolic compounds, which are derived via the shikimate pathway [4]. Biosynthesis process diversity in different parts of the plant leads to different metabolite compositions. Therefore, the EO content in different parts of the plant varies, including the roots, stems, leaves, flowers, fruits, and seeds [5].

Previous researchers have noted that some EOs from the Malaysian citrus (Rustacea), including *Citrus aurantifolia, C. grandis, C. hystrix*, and *C. microcarpa*, have different chemical compositions and bioactivities, including antimicrobial and antifeedant activities [6]. The distribution of major and minor components of each kaffir lime oil fraction can be obtained using fractional distillation [7]. Furthermore, the use of different cooking methods causes various effects on the phenolic compounds and antioxidant properties of kaffir lime leaf [8]. This study aimed to test the antioxidant activity of EOs from parts of *C. hystrix* and citronellal extract and to identify changes in their antioxidant activity and chemical composition by fractional distillation.

EXPERIMENTAL

Plant material

The plant used in this study was *C. hystrix*. According to Backer and Van den Brink (1968), it is in the family Rutacea, the genus Citrus, and the species *C. hystrix* DC. *C. hystrix* twigs and *C. hystrix* twigs-leaves (nD^{20} 1.44 g/m and specific gravity of 0.85 g/mL) obtained from a local farmer in Ngunut, Tulungagung, Indonesia in April 2015. Oils from *C. hystrix* leaves and peels collected from steam distillation of leaves and peel were collected from *C. hystrix* plants grown in Ngunut, Indonesia in May 2015. Samples were stored at the Institute of Essential Oils, Brawijaya University.

Distillation of *C. hystrix* oil from leaves and peels

Fresh C. hystrix leaves (2 kg) and peels (2.7 kg) were separately distilled with water–steam for four h. EOs were dried over anhydrous sodium sulfate and stored at 4°C for further analysis.

Fractional distillation of *C. hystrix* leaves and twigs oil

C. *hystrix* twigs oil (2 L) distilled by steam distillation under reduced pressure using a PiloDist 104-VTU column with a column length of 2 m and 120 stages equipped with a heating jacket coat as the heat source. Fractional distillation was performed using a 20:10 reflux ratio and five mbar of pressure. Each fraction collected, and the volume was measured. The distillation process conducted at the LIPI Research Center for Chemistry, Serpong, Indonesia.

Citronellal extraction of *C. hystrix* leaves oil

Citronellal of *C. hystrix* leaves oil was isolated by adding 20 mL of twigs-leaves oil into 15 mL of saturated Na_2SO_3 while being stirred homogeneously. The pH adjusted to ~8 with the addition of 1 M H_2SO_4 drop-wise. The precipitation salt was filtered and washed with ethanol. The salt was then hydrolyzed using saturated Na_2CO_3 solution (1.424 g/4.07 mL in distilled water), and the organic layer was separated. The organic phase (citronellal) was collected in vials, dried under anhydrous sodium sulfate, and stored at 4 °C.

Evaluation of antioxidant activity

Antioxidant activities of the EOs were evaluated by measuring DPPH bleaching (purple-colored ethanol solution). All samples were prepared at various concentrations (1.25, 2.5, 5, 7.5, 10, 12.5, 15, and 20 µg/mL) in ethanol solvent; 1 mL of 0.4 mg/mL DPPH solution (in ethanol) was added to the samples. The solution was mixed using a vortex and incubated for 30 min at room temperature in the dark. The absorbance was measured at λ=517 nm. Butylated hydroxytoluene (BHT; Sigma-Aldrich, St. Louis, MO, USA) used as a positive control. The inhibition percentage of free DPPH radicals (I %) was calculated using Eq. (1):

$$I\ (\%) = \{1 - (As/Ab)\}100 \quad \ldots\ldots\ldots\ldots\ldots\ (1)$$

where Ab and As represent the absorbance of the blank and sample reaction, respectively.

Gas chromatography-mass spectrometry (GC–MS) analysis

GC–MS analysis performed using a Shimadzu QP 2010S equipped with a DB-1 capillary column (L 30 m, ID 0.25 mm). For GC–MS detection, an electron bombardment system was used with ionization energy of 70 eV (injector temperature, 300°C; detector temperature, 320°C; pressure, 12 Pa). The column temperature was initially set at 50°C and gradually increased to 260°C at a rate of 5°C/min. Helium was used as the carrier-gas at three mL/min flow rate. The components were identified based on the comparison of their relative retention time and mass spectra with those of NBS75K library data of the GC–MS system. The mass spectra fragmentation pattern with a similarity index of more than 95 was used to confirm the type of compound.

RESULTS

Antioxidant activities of the citronellal extract and fractions, and EOs of *C. hystrix*

An antioxidant defined as a substance that can compete with other oxidizable substrates at low concentrations, preventing their oxidation. The DPPH radical-scavenging activities of the citronellal extract and fractions and EOs of parts of *C*. *hystrix*, citronella, and the standard (BHT) were tested. The 50% inhibition concentrations (IC_{50} values) of each sample are presented in Figure 1.

Chemical compounds of citronellal extract and fractions and EOs of *C. hystrix*

The constituent chemical compounds of the EOs produced by steam distilled twigs, twigs-leaves and peels of *C. hystrix*, extracts of *C. hystrix* leaves oil and analyzed fractions produced by the fractional distillation of *C. hystrix* twigs oil were analyzed by GC-MS method. The chemical compositions (grouped in HMs and OMs) listed in Table 1, and the fractions produced by fractional distillation under reduced pressure and reflux ratio presented in Figure 2 and Figure 3 as HM and OM groups, respectively. Chemical composition of citronellal extract is presented in Figure 4.

The content of chemical compounds in CHT, CHTL, and CHL of the HM group was much less than that of the OM group. Therefore, the HM: OM ratio is small in CHP, and the chemical content of the HM group is slightly higher than that of the OM group (ratio HM: OM value 1.15).

The initial fractions (F1–F4) of the fractionated distillation product of CHT contained HM group compounds, i.e., sabinene, (β)-pinene, (β)-myrcene, limonene, (β)-ocimene, and (α)-terpinene (Fig. 2). In F1, the content of the sabinene compound exhibited the highest percent increase (from 5.91% to 19.83%). The three chemical components of the OM group distributed in F1–F9 were linalool, citronellal, and isopulegol (Figure 3).

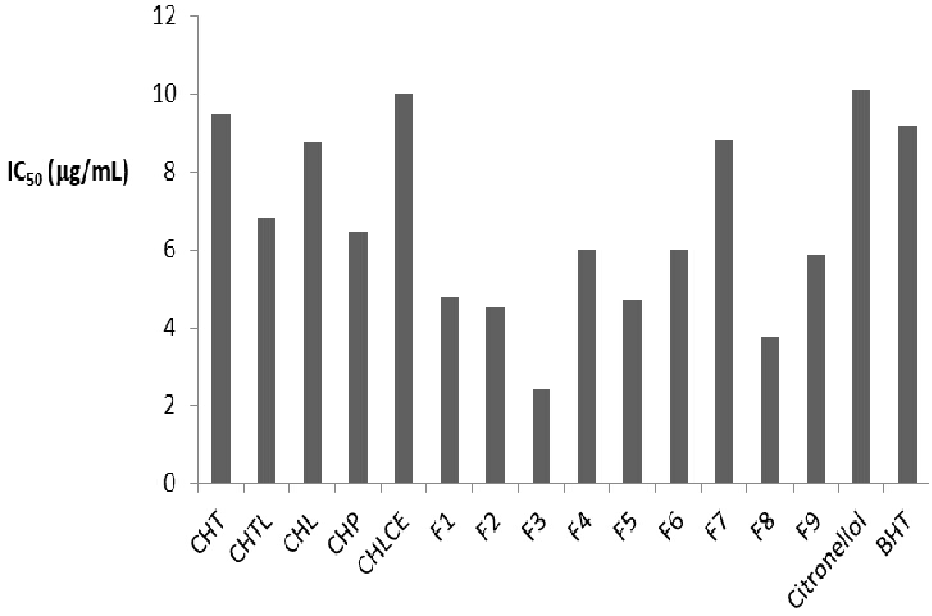

Figure 1: Antioxidant activity of each sample. CHT = *C.hystrix* twig oil, CHTL = *C. hystrix* twigs-leaves oil, CHL = *C.hystrix* leaves oil, CHP = *C. hystrix* peel oil, CHLCE = citronellal extracted of CHL, F1 to F9 = fractions were produced by CHLT fractional distillation, BHT = butylated hydroxytoluene

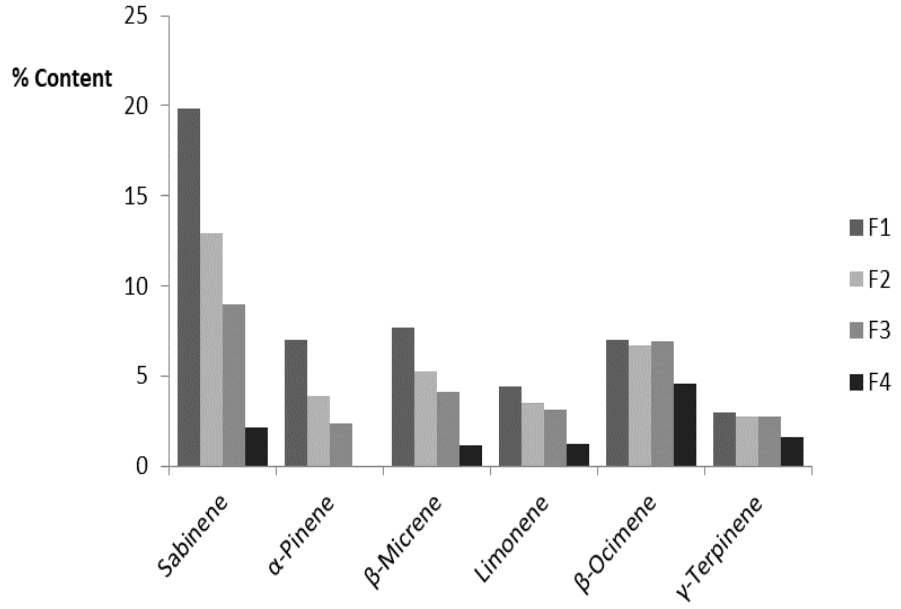

Figure 2: Composition of hydrocarbon monoterpene (HM) in F1 to F4 of CHT oil

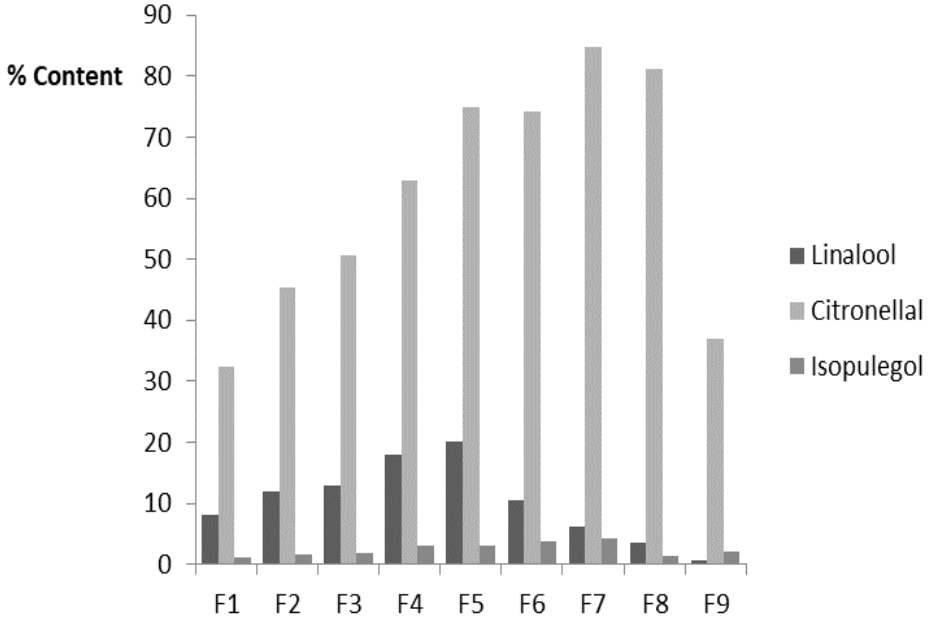

Figure 3: Composition of oxygenated monoterpenes in F1–F9 of CHT oil

Citronellal isolation performed by converting it into a salt using saturated Na_2SO_3 solution followed by hydrolysis in Na_2CO_3 solution, which increased the citronellal level from 81.52 to 88.58 %.

DISCUSSION

In this study, the EO antioxidant activities of parts of *C. hystrix*, i.e., CHT, CHTL, CHL, and CHP, were evaluated by the DPPH radical scavenging assay and expressed as IC_{50} values. The same tests were also carried out for the fractions produced by fractionation distillation of CHT. EOs with higher antioxidant activities exhibited lower IC_{50} values. The highest DPPH inhibition from the four types of *C. hystrix* oil was exerted by CHP, with an IC_{50} value of 6.43 µL/mL, followed by CHTL, CHT, and CHL. Except for F7, the antioxidant activities of other fractions produced by fractional distillation of CHT were higher than the EOs of all parts of *C. hystrix*, with IC_{50} values ranging from 2.40 to 6.01 µg/mL. This result suggests that CHT fractions exhibit a stronger

Table 1: Constituent hydrocarbon and oxygenated monoterpene group of the EOs of *C. hystrix*

Groups	Compound	Similarity Index (SI)	Content (%)			
			CHT	***CHTL***	***CHL***	***CHP***
Hydrocarbon Monoterpene (HM)	Phellandrene	97	–	–	–	0.10
	(α)-Pinene	97	–	–	–	1.26
	Sabinene	96	5.91	3.57	2.79	9.21
	(β)-Pinene	97	1.24	1.17	0.33	21.44
	(β)-Myrcene	97	1.27	0.97	1.04	1.98
	Cymene	97	0.80	–	–	–
	(γ)-Terpinene	97	–	–	–	1.23
	Limonene	95	0.90	0.26	0.13	12.59
	(β)-Ocimene	97	1.56	0.77	0.44	–
	(α)-Terpinene	96	0.51	0.22	–	2.29
	(α)-Terpinolene	97	–	–	–	0.62
	Cyclo-germacrene	96	–	–	0.3	–
	Copaene	97	–	–	–	0.18
	Caryophyllene	97	1.48	0.88	1.77	0.24
	Cadinene	96	–	–	0.22	0.23
Oxygenated Monoterpene (OM)	Linalool oxide	97	–	–	0.33	1.57
	Linalool	97	13.11	6.1	3.46	4.23
	Citronellal	97	46.40	81.52	85.07	20.91
	Isopulegol	95	1.57	–	–	–
	Terpinen-4-ol	96	1.52	0.5		11.93
	(α)-Terpineol	96	0.93	–	–	5.16
	Rhodinol	95	0.59	–	–	0.46
	Citronellol	98	11.03	–	–	–
	Citronellyl acetate	96	6.76	3.65	2.77	–
	Geranyl acetate	98	0.77	0.37	0.61	0.43
	Nerodinol	95	1.11	–	–	–
Rasio HM/OM			0.16	0.09	0.08	1.15

CHT : *C. hystrix* twigs oil, CHTL : *C. hystrix* twigs-leaves oil, CHL : *C. hystrix* leaves oil, CHP : *C. hystrix* peels oil

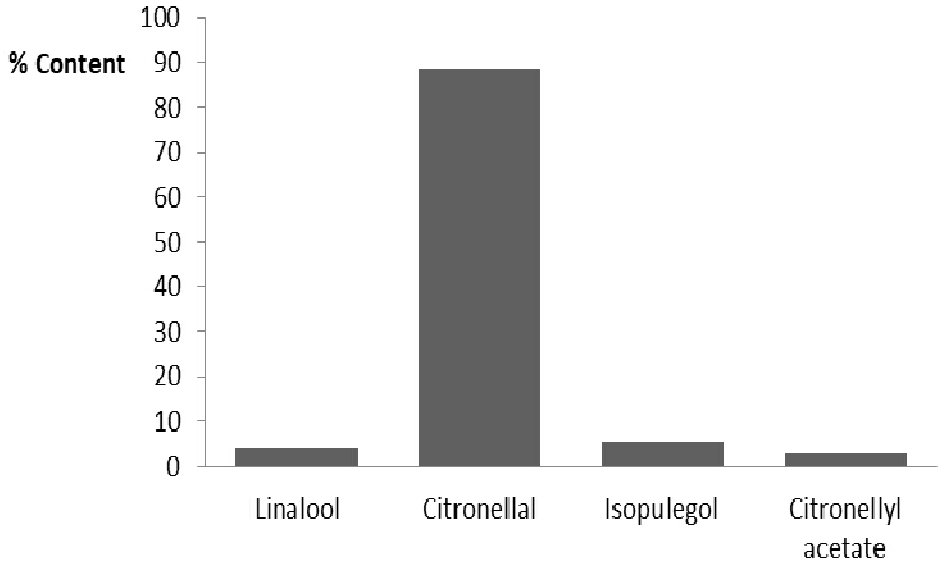

Figure 4: Composition of OMs in the citronellal extract of CHL

inhibitory effect against DPPH radicals, as demonstrated by F3, with an IC_{50} value of 2.40 µL/mL.

The antioxidant activities of CHT, CHTL, and CHL, as well as the CHT fractions (F1–F9), were stronger than those of citronellol and the BHT reference.

The difference in antioxidant activities of the EOs was due to the different chemical compounds containing HM and OM groups. A previous study reported that DPPH scavenging capacity was due to different HM groups, such as β-pinene,

sabinene, and γ-terpinene pomelo of the EOs of the peels [9,10], and the phi (π) bonds in the monoterpene compounds are responsible for the free radical-scavenging activity of DPPH [11,12]. These three components were higher in CHP than in CHT, CHTL, and CHL. Furthermore, the terpene compounds (F1–F4) produced by fractional distillation, thus causing antioxidant activities that were higher than CHT, CHTL, and CHL (including CHP).

Although HMs is an active compound to the free radical-scavenging activity of DPPH, none are stronger than OMs. The antioxidant activities of EOs may attribute to the synergistic effects of their different major and minor components. EOs from *Lavandula angustifolia* (*France*) with a linalool compound content of 23.49% and 37.31% exhibit robust antioxidant activity [13]. Previous studies have shown that OM compounds, such as thymol, carvacrol, α-terpineol [14], trans-citral, cis-citral, and geraniol [15], play an important role in antioxidant activity.

The results from the present study indicate that the antioxidant activities of F1–F9 (except F7) of CHT attributed to the combination of linalool and isopulegol compounds. The distribution patterns of these two compounds in the fractions increased from F1 to F5, with values ranging from 9.43%–23.21%, and decreased in F6–F9, with values ranging from 13.98%–2.74%. Citronellal acts as the primary component of citronellal extract) (Fig. 3), fractions of CHT (Fig. 2), and the EOs of part of *C. hystrix* (Table 1). However, their inhibitory effects against DPPH radicals were not related to the higher content of citronellal, including the citronellol compound.

CONCLUSION

The EOs of CHP and F3 produced from the EO fractional distillation of CHT, with nearly equal composition of HMs and OMs, exhibited higher antioxidant activity than the other EOs and fractions.

DECLARATIONS

Acknowledgement

The authors thank Institute of Essential Oils of Brawijaya University for facilitating this research.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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