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Research article

Chemical composition and antioxidant, anti-tyrosinase and anti-inflammatory activities of essential oil from *Boesenbergia longiflora* (Wall.) Kuntze

Orawan Theanphong[a,*], Withawat Mingvanish[b], Thaya Jenjittikul[c]

[a] *Department of Pharmacognosy, College of Pharmacy, Rangsit University, Pathum Thani 12000, Thailand*

[b] *Organic Synthesis, Electrochemistry & Natural Product Research Unit, Department of Chemistry, Faculty of Science, King Mongkut's University of Technology Thonburi, Bangkok 10140, Thailand*

[c] *Department of Plant Science, Faculty of Science, Mahidol University, Bangkok 10400, Thailand*

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Abstract

Importance of the work: *Boesenbergia longiflora* (Wall.) Kuntze is a medicinal plant in the family Zingiberaceae. Currently, there are no known published reports on the chemical composition and biological activities of essential oils from the roots and rhizomes of *B. longiflora*.

Objectives: To investigate the chemical composition of the essential oils from the fresh roots and rhizomes of *B. longiflora* and to evaluate their antioxidant, anti-inflammatory and anti-tyrosinase activities.

Materials & Methods: The chemical composition was investigated of hydrodistilled essential oils from the fresh roots and rhizomes of *B. longiflora* using gas chromatography-mass spectrometry. The scavenging potential of the essential oils was evaluated based on 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radicals, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals, superoxide anion radicals, hydroxyl radicals and copper chelating, as well as anti-tyrosinase and anti-inflammatory activities.

Results: β-Phellandrene and γ-terpinene was the main component in the essential oils from the roots and rhizomes, respectively.

Main finding: Both these essential oils had strong antioxidant and anti-inflammatory activities compared to the positive controls.

* Corresponding author.
E-mail address: orawan.t@rsu.ac.th (O. Theanphong)

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Introduction

Boesenbergia longiflora, a member of the family Zingiberaceae, is a perennial herb with aromatic roots and rhizomes. Its roots and rhizomes have been used in ethnomedicine to treat dysentery, diarrhea, eye infections, neurological disorders and respiratory problems (Rai and Lalramnghinglova, 2010; Daimei and Kumar, 2013; Haque et al., 2014).

Essential oils, a complex mixture of low molecular weight organic compounds, have been reported to have several types of biological activity, such as antimicrobial (Thambi and Shafi, 2016), anti-inflammatory (Da Silva et al., 2015), antioxidant (Al-Mamary et al., 2011; George et al., 2015) and anti-tyrosinase (Al-Mamary et al., 2011; Fiocco et al., 2011).

Free radicals (molecules or molecular fragments containing one or more unpaired electrons) are inherently unstable and can react strongly with various cells and tissues in the body over a short period (Biswas et al., 2017). The damage caused by free radicals to cells and tissues within the body is the major cause of several chronic and degenerative diseases, such as cancer, diabetes and cardiovascular disease (Pham-Huy et al., 2008; Ray et al., 2012; Sisein, 2014). In addition, free radicals are associated with the inflammation process caused by the activation of inflammatory mediators (Conner and Grisham, 1996; Falcão et al., 2005).

Antioxidants are substances that can prevent diseases caused by free radicals by inhibiting or delaying free radical reactions using different mechanisms, such as scavenging, chelating and breaking chain reactions (Lobo et al., 2010; Brewer, 2011; Nimse and Pal, 2015).

Tyrosinase, a copper-containing enzyme, is the key enzyme involved in melanin biosynthesis (Zolghadri et al., 2019). Melanin is one of the main causes of hyperpigmentation in human skin (Zolghadri et al., 2019). At present, many natural substances, including essential oils, have been evaluated for their anti-tyrosinase activity and often serve as whitening agents in cosmetic products (Zolghadri et al., 2019).

Based on the current available literature, there have been no reported studies of the chemical composition and biological activities of essential oils from the fresh roots and rhizomes of *B. longiflora*. Therefore, the current study aimed to investigate the chemical compositions of the essential oils from the fresh roots and rhizomes of *B. longiflora* and to evaluate their antioxidant, anti-inflammatory and anti-tyrosinase activities.

Materials and Methods

Plant materials

Fresh roots and rhizomes of *B. longiflora* were collected in October, 2019 from Chiang Mai province, Thailand. The plant sample was identified according to Flora of China (Wu and Raven, 2000). The voucher specimen (no. 9321) of the plant sample was deposited at Suanluang Rama IX Royal Botanic Garden, Bangkok, Thailand.

Isolation of essential oils

Fresh roots and rhizomes of *B. longiflora* were washed with tap water, air dried, cut into small pieces and ground in a blender. Each sample of ground material (300 g) was hydrodistilled for 3 hr using a Clevenger apparatus. The essential oils were collected and stored at 4°C in an air-tight container before analysis using gas chromatography-mass spectrometry.

Gas chromatography-mass spectrometry analysis

The GC-MS analysis was performed using an Agilent 7890A gas chromatography unit equipped with a 5975C inert XL EI/CI MAD and Triple-Axis detector. The GC column was a DB-5 MS capillary column (30 m × 0.25 mm; film thickness 0.25 µm). The GC settings were: initial oven temperature held at 60°C for 1 min and heated at 240°C at a rate of 3°C/min, then held for 5 min. The GC injector and GC-MSD interface temperatures were set at 180°C and 290°C, respectively. Each sample (1 µL, diluted 100:1 in ethanol) was injected; the splitless mode was used. The carrier gas was helium at a flow rate of 1 mL/min. For electron ionization, mass spectra were used with ionization energy of 70 eV and ionization voltages over the mass range of 40–650 m/z at a scanning rate of 2.42 amu/sec.

Identification of oil components

Identification of essential oil components was made by comparison of their mass spectra with those stored in the Adams Essential Oil Mass Spectral Library and NIST 05 Mass Spectral Library. Component relative percentages were determined based on peak area measurement. Kovats index (KI) values were determined using the method of Adams (2007).

Sample preparation for biological activities assay

The root and rhizome essential oils were diluted in methanol to obtain concentrations in the range 0.5–300 µg/mL.

Antioxidant activity assays

2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical scavenging activity assay

The ABTS radical scavenging activity of the essential oils was evaluated according to the method of Biskup et al. (2013) with a few modifications. Briefly, a mixture of 10 mL of 7 mM ABTS and 10 mL of 2.45 mM potassium persulfate was kept in the dark at room temperature for 16 hr to generate the $ABTS^+$ reaction solution. After that, the mixture was diluted with distilled water to obtain an absorbance of 1.00 at 734 nm. Next, 1 mL of sample was mixed with 1 mL of freshly prepared $ABTS^+$ solution. The reaction mixture was left at room temperature for 6 min. The absorbance of the reaction mixture was measured against a blank at 734 nm. Trolox® was used as a positive control. The experiment was performed in triplicate. The percentage of ABTS radical scavenging was calculated using Equation 1:

$$\text{Percentage scavenging} = [(A_0\text{-}A_1)/A_0] \times 100\% \quad (1)$$

where A_0 is the absorbance of the control (without the sample) and A_1 is the absorbance of the sample.

2,2-Diphenyl-1-picrylhydrazyl radical scavenging assay

The DPPH radical scavenging activity of the essential oils was evaluated according to the method of Sudha et al. (2011) with a few modifications. Briefly, 1 mL of samples was mixed with 1 mL of 0.2 mM DPPH. The mixture was shaken vigorously and allowed to incubate in the dark at room temperature for 30 min. The absorbance of the reaction mixture was measured against a blank at 517 nm. L-ascorbic acid was used as a positive control. The experiment was performed in triplicate. The percentage of DPPH radical scavenging was calculated using Equation 1.

Superoxide anion radical scavenging assay

The superoxide anion radical scavenging activity of the essential oils was evaluated according to the method of Hussein (2011) with a few modifications. Briefly, 0.5 mL of each sample was mixed with 0.5 mL of 50 µM nitroblue tetrazolium, 0.5 mL of 78 µM nicotinamide adenine dinucleotide + hydrogen (NADH), 0.5 mL of 16 mM Tris-HCl buffer pH 8.0 and 0.5 mL of 10 µM phenazine methosulfate. Then, the reaction mixture was left at room temperature for 5 min. The absorbance of the reaction mixture was measured against a blank at 560 nm. L-ascorbic acid was used as a positive control. The experiment was performed in triplicate. The percentage of superoxide anion radical scavenging was calculated using Equation 1.

Hydroxyl radical scavenging assay

The hydroxyl radical scavenging activity of the essential oils was evaluated according to the method of Sudha et al. (2011) with a few modifications. Briefly, 1 mL of sample was mixed with 1 mL of 1.5 mM ferrous sulfate, 0.7 mL of 6 mM hydrogen peroxide and 0.3 mL of 20 mM sodium salicylate. The reaction mixture was incubated at 37°C for 1 hr. The absorbance of the reaction mixture was measured against a blank at 562 nm. L-ascorbic acid was used as a positive control. The experiment was performed in triplicate. The percentage of $OH^{\bullet}$ radical scavenging was calculated using Equation 1.

Copper chelating assay

The copper chelating activity of the essential oils was evaluated according to the method of Sellal et al. (2019) with a few modifications. Briefly, 0.5 mL of sample was mixed with 1.6 mL of deionized water and 0.1 mL of 8 mM copper sulfate in 0.1 M HCl buffer. After 30 sec, 0.1 mL of 5 mM ammonium purpurate in 0.1 M HCl buffer was added. The reaction mixture was allowed to react in the dark at room temperature for 10 min. The absorbance of the reaction mixture was measured against a blank at 462 nm. Ethylenediaminetetraacetic acid (EDTA) was used as a positive control. The experiment was performed in triplicate. The percentage of copper chelating was calculated using Equation 1.

Tyrosinase inhibitory activity assay

The tyrosinase inhibitory activity of the essential oils was evaluated according to the method of Matsuura et al. (2006) with a few modifications. Briefly, 1 mL of 2.5 mM L-3,4-dihydroxyphenylalanine (L-DOPA) was mixed with 1.8 mL of 0.1 M phosphate buffer (pH 6.8). The reaction mixture was incubated at room temperature for 10 min. After that, 0.1 mL of each sample and 138 units of tyrosinase in 0.1 M phosphate buffer (pH 6.8) was added. The absorbance of the reaction mixture was measured against a blank at 475 nm. Kojic acid was used as a positive control. The experiment was performed in triplicate. The percentage of tyrosinase inhibitor was calculated using Equation 1.

5-Lipoxygenase inhibitory activity assay

The 5-lipoxygenase inhibitory activity of the essential oils was evaluated according to the method of Ashour et al. (2009) with a few modifications. Briefly, 10 µL of 7.9 units/mL soybean lipoxygenase in 0.1 M phosphate buffer pH 9.0 was diluted 1:10 by volume. The diluted enzyme solution was mixed with 20 µL of sample. The reaction mixture was shaken vigorously and left to react at room temperature for 10 min. Next, 25 µL of 62.5 µM sodium linoleate was added to initiate the reaction. The absorbance of the reaction mixture was measured against a blank at 234 nm. Indomethacin was used as a positive control. The experiment was performed in triplicate. The percentage of 5-lipoxygenase inhibition was calculated using Equation 1.

Statistical analysis

The experimental results were reported as mean ± SD. Data analyses were performed using the SPSS software version 18 (SPSS Inc.; Chicago, IL, USA) whereby one-way analysis of variance and Duncan's multiple range test were employed. The tests were considered significance at $p < 0.05$.

Results and Discussion

Chemical compositions of essential oils

The hydrodistillation of the fresh roots and rhizomes of *B. longiflora* gave clear and bright yellow essential oils with yields of 0.30% and 0.36% volume per weight, respectively. The GC chromatograms of the root and rhizome essential oils of *B. longiflora* are shown in Figs 1 and 2, respectively. Table 1 shows that the chemical compositions of the essential oils and their relative peak area percentages and KIs in order of their increasing elution time on the DB-5 MS column; 53 (97.74%) and 52 compounds (97.37%) were identified in the root and rhizome essential oils, respectively. The root essential oil was rich in monoterpene hydrocarbons (64.77%), followed by oxygenated monoterpenes (12.33%) and oxygenated sesquiterpenes (10.75%). The three major compounds of the root essential oil were *β*-phellandrene, *α*-phellandrene and 4-carene. Monoterpene hydrocarbons (51.10%), sesquiterpene hydrocarbons (25.04%) and oxygenated monoterpenes (8.58%) were the major classes in the rhizome essential oil and *γ*-terpinene, *γ*-elemene and *α*-terpinene were three major components in the rhizomes. The experimental results were different from those reported by Kar et al. (2015), who identified longipinocarvone, oxygenated sesquiterpenes, as the major components in the rhizome essential oil of *B. longiflora* from India. However, it was possible that the chemical compositions of the essential oils may have been affected by the agricultural location, harvesting stage and extraction method (Al-Rezaa et al., 2010; Saeb and Gholamrezaee, 2012; Khalid and El-Gohary, 2014; Theanphong et al., 2017).

Biological activities of essential oils

Antioxidant activity

The antioxidant activities of the fresh root and rhizome essential oils obtained using hydrodistillation were investigated using five different methods (ABTS radical scavenging assay, DPPH radical scavenging assay, superoxide anion radical scavenging assay, hydroxyl radical scavenging assay and copper chelating assay).

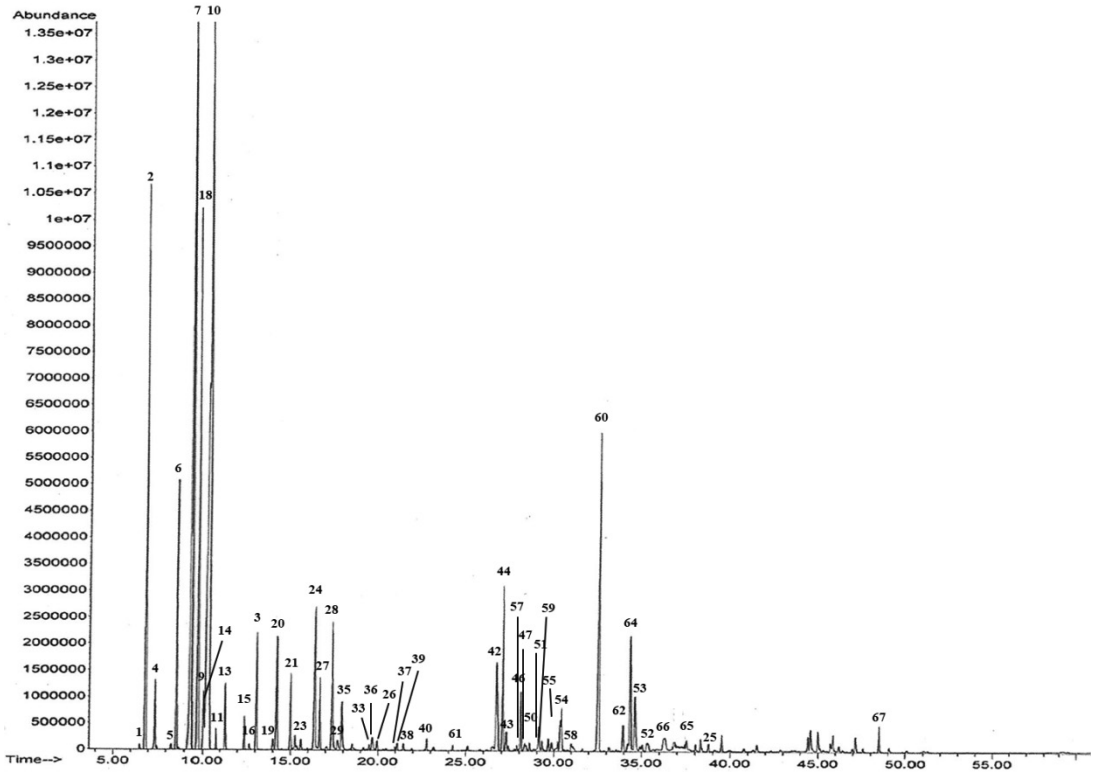

Fig 1 Gas chromatogram of root essential oil of *Boesenbergia longiflora*

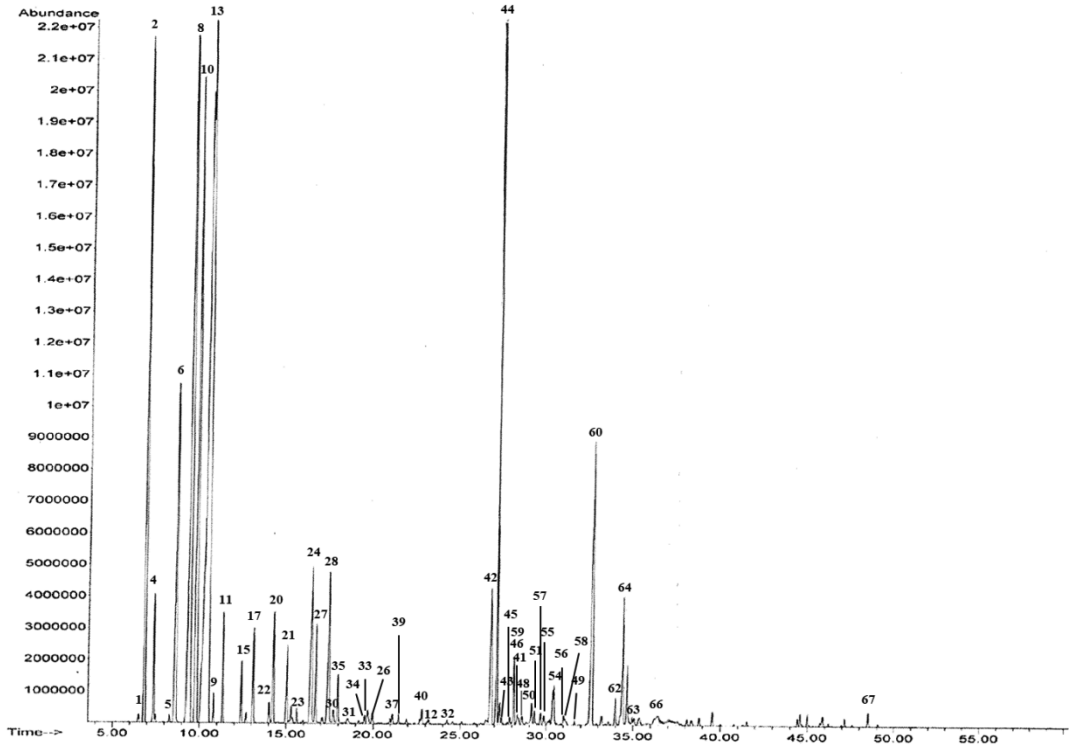

Fig 2 Gas chromatogram of rhizome essential oil of *Boesenbergia longiflora*

Table 1 Essential oil compositions of fresh roots and rhizomes of *Boesenbergia longiflora*

No.	Chemical composition	KI	Class	Content (%)	
				Root	Rhizome
1	Tricyclene	926	MH	0.08	0.07
2	*α*-Pinine	939	MH	7.75	7.37
3	*β*-Citronellene	950	MH	1.80	-
4	Camphene	954	MH	0.97	1.03
5	*β*-Pinene	979	MH	0.07	0.08
6	Myrcene	990	MH	3.92	3.89
7	*α*-Phellandrene	1002	MH	14.79	-
8	*α*-Terpinene	1017	MH	-	9.59
9	*p*-Cymene	1024	MH	1.22	0.08
10	*β*-Phellandrene	1029	MH	15.27	7.57
11	*β*-Ocimene	1050	MH	0.27	0.20
12	*p*-Mentha-2,4(8)-diene	1050	MH	-	0.05
13	*γ*-Terpinene	1059	MH	0.90	26.78
14	*β*-Terpinene	1071	MH	8.54	-
15	*α*-Terpinolene	1088	MH	0.42	0.48
16	*p*-Cymenene	1091	MH	0.09	-
17	Linalool	1096	OM	-	0.86
18	4-Carene	1128	MH	8.67	-
19	exo Fenchol	1121	OM	0.18	-
20	(*Z*)-*p*-Mentha-2,8-dien-1-ol	1140	OM	3.11	1.09
21	Camphor	1146	OM	0.24	0.16
22	Camphene hydrate	1149	OM	-	0.19
23	Isoborneol	1160	OM	0.04	0.03
24	Borneol	1169	OM	2.86	2.08
25	1,11-Dodecadiene	1169	Other	0.14	-
26	Phellandral	1175	OM	0.06	0.06
27	Terpinen-4-ol	1177	OM	1.18	0.95
28	*α*-Terpiniol	1188	OM	2.59	2.11
29	*cis*-Piperitol	1196	OM	0.83	-
30	*trans*-Piperitol	1208	OM	-	0.46
31	*cis*-Carveol	1229	OM	-	0.02
32	Nerol	1229	OM	-	0.06
33	Carvone	1243	OM	0.10	0.08
34	Isopiperitone	1246	OM	-	0.10
35	Geraniol	1252	OM	0.50	0.10
36	Piperitone	1252	OM	0.17	-
37	Bornyl acetate	1288	OM	0.12	0.09
38	Thymol	1290	OM	0.04	-
39	2-Undecanone	1294	Other	0.11	0.10
40	Myrtenyl acetate	1326	OM	0.19	0.13
41	*α*-Longipinene	1352	SH	-	0.09
42	*α*-Santalene	1417	SH	2.13	1.89

Table 1 Continued

No.	Chemical composition	KI	Class	Content (%)	
				Root	Rhizome
43	*trans*-α-Bergamotene	1434	SH	1.01	0.29
44	γ-Elemene	1435	SH	2.81	20.80
45	*cis*-β-Farnesene	1442	SH	-	0.61
46	*Epi*-β-Santalene	1449	SH	0.09	0.07
47	(*E*)-β-Farnesene	1456	SH	0.92	-
48	β-santalene	1459	SH	-	0.05
49	γ-Muurolene	1479	SH	-	0.07
50	*Ar*-Curcumene	1480	SH	0.19	0.15
51	α-Zingiberene	1493	SH	0.17	0.09
52	Valencene	1496	SH	0.28	-
53	Epizonarene	1501	SH	0.12	-
54	β-Bisabolene	1505	SH	0.47	0.32
55	*E*,*E*-α-Farnesene	1505	SH	0.33	0.05
56	*cis*-α-Bisabolene	1507	SH	-	0.04
57	β-Curcumene	1515	SH	0.49	0.31
58	β-Sesquiphellandrene	1522	SH	0.07	0.07
59	*cis*-Calamenene	1529	SH	0.17	0.13
60	Nerolidol	1563	OS	6.97	4.09
61	Geranyl butyrate	1564	OM	0.09	-
62	Guaiol	1600	OS	0.48	0.30
63	γ-Eudesmol	1632	OS	-	0.15
64	α-Eudesmol	1653	OS	2.70	1.73
65	α-Bisabolol	1675	OS	0.16	-
66	*trans* Farnesol	1715	OS	0.43	0.07
67	*trans*-Geranylgeraniol	2192	OD	0.41	0.12
Total identified (%)				97.74	97.37
% Yield (% volume per weight)				0.32	0.36

KI = Kovats index determined relative to n-alkanes (C6–C24) on a DB-5 mass spectroscopy column; MH = monoterpene hydrocarbons; OM = oxygenated monoterpenes; SH = sesquiterpene hydrocarbons; OS = oxygenated sesquiterpenes; OD = oxygenated diterpenes

As seen in Table 2, the rhizome essential oil exhibited significantly higher antioxidant activity against the DPPH radicals, superoxide anion radicals and hydroxyl radicals than the root essential oil and L-ascorbic acid whereas the ABTS radical scavenging activity for the root and rhizome essential oils were not significantly different to Trolox®. In addition, the rhizome essential oil had strong copper chelating activity that was not significantly different to that of EDTA. The EC_{50} values of the essential oils and positive control are shown in Table 2.

These results were similar to the research reported by Byahatti and Thangadurai (2019), who found that the essential oil from *B. pulcherrima* had high antioxidant properties.

The strong antioxidant activities of the root and rhizome essential oils might have been due to the synergistic effect of their chemical components in the oils and the high content of monoterpenes (Graßmann, 2005; Gonzalez-Burgos and Gomez-Serranillos, 2012; Andrade et al., 2013). In addition, Asghari et al. (2018) reported that the hydroxyl group existing in some chemical constituents of essential oils, such as linalool, borneol and α-terpiniol, is essential for providing metal chelating activity.

Table 2 Half maximal effective concentration (EC_{50}) values of essential oils from fresh roots and rhizomes of *Boesenbergia longiflora*

Test	EC_{50} (µg/mL)		
	Root essential oil	Rhizome essential oil	Positive control
ABTS radical scavenging activity	13.04±0.42[a]	12.58±0.44[a]	13.08±0.36[a]
DPPH radical scavenging assay	18.86±0.50[a]	17.03±0.50[b]	19.12±0.36[a]
Superoxide anion radical scavenging assay	29.37±0.57[a]	24.24±0.37[b]	29.91±0.89[a]
OH· radical scavenging assay	27.00±0.66[a]	23.08±0.73[b]	26.82±0.48[a]
Copper chelating assay	175.16±2.77[a]	168.37±1.95[b]	167.45±2.83[b]
Tyrosinase inhibitory activity assay	21.77±0.52[a]	19.34±0.70[b]	20.81±0.93[a,b]
5-Lipoxygenase inhibitor activity assay	63.01±0.47[a]	62.43±1.21[a]	62.21±1.08[a]

ABTS = 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid; DPPH = 2,2-diphenyl-1-picrylhydrazyl
Mean±SD in each row superscripted with different lowercase letters are significantly ($p < 0.05$) different

Tyrosinase inhibitory activity

The tyrosinase inhibitory activity of the root and rhizome essential oils was evaluated using the dopachrome method with L-DOPA as the substrate. Table 2 shows there was no significant different in the effectiveness of the rhizome essential oil and kojic acid at tyrosinase activity inhibition. However, the root essential oil had much lower tyrosinase inhibitory activity than kojic acid. Kojic acid inhibits the oxidation of tyrosine substrate that is catalyzed by tyrosinase for producing the melanin pigments with the chelation of the copper-ion coenzyme of the multifunctional copper-containing tyrosinase enzyme (Hashemi and Emami, 2015; Zolghadri et al., 2019). As with kojic acid, some chemical components in the rhizome essential oil might perform the same mechanism of tyrosinase inhibition action but with weak potential.

5-Lipoxygenase inhibitory activity

The root and rhizome essential oils had strong 5-lipoxygenase inhibitory activity with EC_{50} values that were not significantly different to that of indomethacin (Table 2).

Several essential oils from Zingiberaceous plants such as *A. murdochi, C. longa* and *Z. officinale* have shown strong 5-lipoxygenase inhibition activity (Miguel, 2010; Priya et al., 2012; Bayala et al., 2014). In addition, Sá et al. (2013) suggested that monoterpene compounds could be applied as a new anti-inflammatory agent.

The results showed that the essential oils from the fresh roots and rhizomes of *B. longiflora* could inhibit or retard oxidative reactions via various mechanisms of action and could also inhibit the 5-lipoxygenase catalyzed inflammatory process. Therefore, it could be that both essential oils could be used for the treatment of several degenerative diseases. In addition, the essential oils might be applicable as an alternative natural ingredient for spa, cosmetic and pharmaceutical products.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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