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ORIGINAL ARTICLE

GC-MS profiling and *in vitro* assessment of antioxidant and 5-lipoxygenase inhibitory activities of essential oils from five indigenous *Hedychium* species in Thailand

Orawan Theanphong[1*], Withawat Mingvanish[2] and Thaya Jenjittikul[3]

[1]Department of Pharmacognosy, College of Pharmacy, Rangsit University, Pathum Thani 12000, Thailand
[2]Organic Synthesis, Electrochemistry & Natural Product Research Unit, Department of Chemistry, Faculty of Science, King Mongkut's University of Technology Thonburi, Bangkok 10140, Thailand
[3]Department of Plant Science, Faculty of Science, Mahidol University, Bangkok 10400, Thailand

Abstract: The genus *Hedychium* (Zingiberaceae) consists of rhizomatous aromatic plants widely utilized in Thai traditional medicine and cosmetic formulations. Although recognized ethnopharmacological, the phytochemistry and bioactivity of Thai *Hedychium* species have great potential for drug development. This study investigated the chemical profiles, *in vitro* antioxidant capacities, and *in vitro* 5-lipoxygenase (5-LOX) inhibitory activities of essential oils hydrodistilled from the fresh rhizomes of five Thai species—*H. bousigonianum*, *H. coccineum*, *H. coronarium*, *H. ellipticum*, and *H. flavescens*. Gas chromatography–mass spectrometry (GC–MS) analysis revealed distinct, species-specific chemotypes, characterized by α-eudesmol (37.41%) in *H. bousigonianum*; β-pinene (27.42% and 35.38%, respectively) in *H. coccineum* and *H. flavescens*; linalool (26.40%) in *H. coronarium*; and 1,8-cineole (89.36%) in *H. ellipticum*. The essential oils from the fresh rhizome of *H. bousigonianum*, *H. coccineum*, *H. coronarium*, *H. ellipticum*, and *H. flavescens* demonstrated antioxidant activity across multiple assays. Their free radical scavenging capacity was confirmed by the DPPH assay (IC_{50} = 17.92 ± 0.62, 20.26 ± 0.53, 20.02 ± 0.89, 18.27 ± 0.60, and 22.12 ± 0.60 µg/mL, respectively) and the ABTS assay (IC_{50} = 13.16 ± 0.95, 19.02 ± 0.43, 18.09 ± 0.34, 11.81 ± 0.67, and 19.34 ± 0.27 µg/mL, respectively). Similar trends were observed in the superoxide anion (IC_{50} = 28.41 ± 0.64, 28.96 ± 0.51, 31.47 ± 0.58, 26.19 ± 0.61, and 29.12 ± 0.42 µg/mL, respectively) and hydroxyl radical assays (IC_{50} = 26.67 ± 0.73, 27.98 ± 0.37, 28.92 ± 0.31, 25.34 ± 0.81, and 32.45 ± 0.55 µg/mL, respectively). Furthermore, the essential oils showed 5-LOX inhibitory activity (IC_{50} = 57.32 ± 1.09, 67.50 ± 1.70, 60.31 ± 1.25, 55.84 ± 1.53, and 75.34 ± 2.28 µg/mL, respectively), supporting their potential anti-inflammatory properties. Notably, the essential oils from *H. bousigonianum* and *H. ellipticum* showed the most potent dual antioxidant and anti-inflammatory activities, highlighting their promise as natural sources of bioactive compounds for future pharmaceutical development.

Keywords: 5-Lipoxygenase inhibitory, antioxidant, essential oil, *Hedychium*, Zingiberaceae

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1 Introduction

Free radicals are molecules characterized by the presence of unpaired electrons, which confer high chemical reactivity and intrinsic instability. These unpaired electrons enable free radicals to abstract electrons from neighboring molecules, resulting in their oxidation and the subsequent generation of new free radicals. This continuous, self-propagating process initiates a chain reaction that ultimately contributes to oxidative damage within biological systems (Wafula et al., 2017; Ayoka et al., 2022). Free radicals are major contributors to the pathogenesis of various non-communicable diseases, including Alzheimer's disease, cardiovascular disorders, diabetes, and cancer. Furthermore, accumulating scientific evidence indicates that free radicals play a crucial role in inflammatory processes (Arulselvan et al., 2016; Biswas et al., 2017; Eiz, 2022). Free radicals induce inflammation by promoting cellular damage and stimulating the production of inflammatory mediators and regulatory enzymes, including cytokines and lipoxygenase enzymes (Loncaric et al., 2021). In chronic inflammatory conditions, excessive production of free radicals exacerbates inflammation, establishing a persistent cycle of oxidative stress and inflammatory responses that

*Corresponding Author: Orawan Theanphong.
Email: orawan.t@rsu.ac.th

accelerates tissue damage and disease progression (Biswas et al., 2017).

The enzyme 5-lipoxygenase (5-LOX) plays a pivotal role in the inflammatory process by catalyzing the conversion of arachidonic acid into leukotrienes-potent inflammatory mediators-within myeloid cells (Radmark & Samuelsson, 2009; Sa et al., 2013; Giménez-Bastida et al., 2021; Loncaric et al., 2021). Therefore, inhibition of 5-lipoxygenase (5-LOX) can attenuate leukotriene-mediated inflammation, serving as a reliable indicator of anti-inflammatory potential in natural product screening.

Antioxidants are compounds that mitigate or prevent oxidation by inhibiting the formation of free radicals or by interrupting the initiation and propagation of oxidative chain reactions. They exert their protective effects through multiple mechanisms, including free radical scavenging, metal ion chelation, and termination of oxidative chain reactions (Wafula et al., 2017; Ayoka et al., 2022).

Plants of the genus *Hedychium* are rhizomatous herbs with aromatic rhizomes belonging to the family Zingiberaceae. They thrive in both terrestrial and epiphytic habitats and are distributed throughout China, India, Myanmar, Thailand, Laos, Vietnam, and Malaysia (Leong-Skornickova & Newman, 2015). The rhizomes of several Hedychium species have been widely employed in traditional medicine to treat fever, indigestion, inflammation, rheumatism, diabetes mellitus, and diarrhea (Chuakul, 2005; Tushar et al., 2010; Rawat et al., 2018; Tavares et al., 2020; Inta et al., 2023).

Previous studies have investigated the chemical composition and biological activities of essential oils derived from the rhizomes of *Hedychium* species (George et al., 2007; Joshi et al., 2008; Suksathan et al., 2013; Noriega et al., 2019; Lima et al., 2021; Thomas & Mani, 2023). The present study aims to examine the chemical constituents, antioxidant activity, and 5-lipoxygenase inhibitory potential of essential oils obtained from the fresh rhizomes of five *Hedychium* species indigenous to Thailand.

2 Materials and Methods

2.1 Plant Sample

Fresh rhizomes of *H. bousigonianum* Pierre ex Gagnep. (Hb), *H. ellipticum* Buch.-Ham. ex Sm. (He), and *H. flavescens* Carey ex Roscoe (Hf) were collected from Tak Province, whereas rhizomes of *H. coccineum* Buch.-Ham. ex Sm. (Hc) and *H. coronarium* J. Koenig (Ho) were collected from Chiang Rai and Prachinburi Provinces, respectively. All plant samples were collected between February-March 2019 and were identified by Assoc. Prof. Dr. Thaya Jenjittikul. Voucher specimens (RSU 0102-RSU 0106) were deposited at the College of Pharmacy, Rangsit University, Thailand.

2.2 Essential Oil Extraction

The fresh rhizomes of all plant samples were thoroughly washed with tap water and cut into small pieces and subsequently ground using a blender. Each ground rhizome sample (300 g) was subjected to hydrodistillation using a Clevenger apparatus for 4 hours. The essential oils were collected, dried over anhydrous sodium sulfate, and stored at 4°C until GC-MS analysis.

2.3 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The qualitative analysis of essential oil constituents was conducted using gas chromatography-mass spectrometry (GC-MS) at the RSU Scientific and Technological Research Equipment Center (RSU-STREC). An Agilent Technologies 7890A gas chromatography system coupled with a 5975C inert XL electron ionization/chemical ionization mass spectrometry (EI/CI-MS) detector was employed. Separations were performed using a DB-5 MS capillary column (30 m × 0.25 mm i.d., 0.25 μM film). Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The GC oven temperature was programmed as follows: an initial isothermal hold at 60°C for 1 minute, followed by a linear ramp of 3°C/min to a final temperature of 240°C, maintained for 5 minutes. The injector and GC-MS interface temperatures were set at 180°C and 290°C, respectively. Samples were prepared by diluting the essential oil 1:100 (v/v) in methanol, and 1 μL was injected in splitless mode. Electron impact ionization (EI) was conducted at 70 eV, with mass spectra acquired in positive ion mode over a mass range of 40–650 m/z at a scan rate of 2.42 amu/s. The total chromatographic run time was 70 minutes.

2.4 Identification of Essential Oil Components

The essential oil composition was identified by comparing their mass spectra mass spectra with the mass fragmentation patterns in the Adams Essential Oil Mass Spectral Library and the NIST05 Mass Spectral Library.

2.5 Antioxidant Activity Assays

2.5.1 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical Scavenging Activity Assay

The DPPH radical scavenging activity of the essential oils was evaluated using the method described by Sudha et al. (2011), based on the procedure first described by Blois (1958), with slight modifications. Briefly, 100 μL of each essential oil in methanol, at final concentrations ranging from 0.5 to 500 μg/mL, was added to a 96-well plate. Subsequently, 100 μL of 0.2 mM DPPH solution in methanol was added to each well. The reaction mixture was allowed to stand in the dark at room temperature for 30 minutes. The absorbance was then measured using a microplate reader (ThermoFisher) at 517 nM. *L*-Ascorbic acid was used as a positive control. The percentage scavenging activity was calculated using the following equation:

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

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

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2.5.2 2,2′-Azino-bis-3-Ethylbenzothiazoline-6-Sulfonic Acid (ABTS) Radical Scavenging Activity Assay

The ABTS radical scavenging activity of the essential oils was determined using the protocol described by Biskup et al. (2013), based on the method originally reported by Miller et al. (1993), with slight modifications. Briefly, the ABTS radical was generated by combining 7 mM ABTS solution and 2.45 mM potassium persulfate (1:1), and the mixture was kept in the dark at room temperature for 16 hours. It was then diluted to an absorbance of 1.00 at 734 nM (Reagent A). A 100 µL of each essential oil (final concentrations ranging from 0.5 to 500 µg/mL) was mixed with 100 µL of Reagent A in a 96-well plate and allowed to stand at room temperature for 6 minutes. Absorbance at 734 nM was measured using a microplate reader (ThermoFisher). Trolox® was used as a positive control. ABTS scavenging was calculated using Equation (1).

2.5.3 Superoxide Anion Radical Scavenging Activity Assay

The superoxide anion radical scavenging capacity of the essential oils was assessed using a modified protocol based on the method described by Hussein (2011), following the original procedure of Liu et al. (1997). Briefly, 50 µL of each essential oil in methanol, at final concentrations ranging from 0.5 to 500 µg/mL, was added into a 96-well plate. Subsequently, 50 µL of 50 µM nitroblue tetrazolium, 78 µM nicotinamide adenine dinucleotide (NADH), and 10 µM phenazine methosulfate, all prepared in 16 mM Tris-HCl buffer (pH 8.0), were sequentially added. After that 50 µL of 16 mM Tris-HCl buffer (pH 8.0) were added. The reaction mixture was incubated at 25°C for 5 minutes. The absorbance was measured at 560 nM using a microplate reader (ThermoFisher). *L*-Ascorbic acid was used as a positive control. The percentage scavenging activity was determined using Equation (1).

2.5.4 Hydroxyl Radical Scavenging Activity Assay

The hydroxyl radical scavenging activity of the essential oils was assessed using the method described by Sudha et al. (2011), originally developed by Halliwell et al. (1987), with minor modifications. Briefly, 50 µL of each essential oil in methanol, at final concentrations ranging from 0.5 to 500 µg/mL, was successively mixed with 50 µL of 1.5 mM ferric sulfate in deionized water, 15 µL of 20 mM sodium salicylate, and 35 µL of 6 mM hydrogen peroxide in a 96-well plate. The reaction mixture was then incubated at 37°C for 1 hour, after which the absorbance was recorded at 562 nM using a microplate reader (ThermoFisher). *L*-Ascorbic acid was used as a positive control. The percentage scavenging activity was determined using the following equation:

$$\%\text{Scavenging} = \left[(A_1 - A_2)/A_0\right] \times 100$$

where A_0 is the absorbance of the control, A_1 is the absorbance of the sample and A_2 is the absorbance of the sample without sodium salicylate.

2.6 5-Lipoxygenase Inhibitory Activity Assay

The 5-lipoxygenase inhibitory activity of the essential oils was determined using a protocol based on Ashour et al. (2009). The assay was carried out following the procedure outlined by Pinto et al. (2007), with minor modifications as reported by Dzoyem and Eloff (2015). A 1:10 dilution of 7.9 units/mL soybean lipoxygenase in 0.1 M phosphate buffer (pH 9.0) was prepared by mixing 5 µL of the enzyme solution with 45 µL of phosphate buffer (pH 9.0). After that 20 µL of each essential oil, dissolved in methanol at final concentrations ranging from 0.5 to 500 µg/mL, were added to individual wells of a 96-well plate containing the diluted enzyme and allowed to stand at room temperature for 10 minutes. The reaction was initiated by the addition of 25 µL of 62.5 mM sodium linoleate. The absorbance was measured at 234 nM using a microplate reader (ThermoFisher). Indomethacin was used as a positive control. The percentage inhibition of 5-lipoxygenase activity was calculated using Equation (1).

2.7 Statistical Analysis

All experimental assays were performed in triplicate, and the results are expressed as the mean ± standard deviation (SD). Half-maximal inhibitory concentration (IC_{50}) values were calculated from linear regression equations derived from plots of percentage scavenging or percentage inhibition versus concentration. Statistical analysis was conducted using SPSS version 22 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was employed, followed by Duncan's multiple range test for post-hoc comparisons. Statistical significance was determined at a threshold of $p < 0.05$.

3 Results and Discussion

3.1 Chemical Composition of Essential Oils

The hydrodistilled essential oils from the fresh rhizomes of five *Hedychium* species were clear, bright yellow oils, with yields ranging from 0.21% to 0.25% v/w. Table 1 lists the chemical compositions identified in each essential oil through GC-MS, along with their respective percentages and Kovats Indices (KIs) and %yield. Across all samples, 80 compounds were identified, representing 96.42 to 99.63% of the total oil composition. Individual oil samples contained between 9 and 38 identified compounds.

The essential oil from the rhizome of *H. bousigonianum* was rich in an oxygenated sesquiterpenes (80.40%), represented by α-eudesmol (31.41%), elemol (18.93%), and γ-eudesmol (14.55%).

For *H. coccineum* and *H. flavescens*, the rhizome essential oils contained a high percentage of monoterpene hydrocarbons (37.28 and 56.27%, respectively). β-Pinene (27.42%), nerolidol (9.52%) and τ-muurolol (7.28%) were the three dominant components in rhizome essential oil from *H. coccineum*, whereas β-pinene (35.38%), linalool (20.87%), and 1,8-cineole (14.38%) were the three major components in the rhizome essential oil from *H. flavescens*.

Monoterpenes, including monoterpene hydrocarbons (43.00%) and oxygenated monoterpenes (47.93%), were the

Table 1. Chemical constituents of essential oil from the fresh rhizomes of five *Hedychium* species

No.	Compound name	KI*	Content (%)				
			Hb	Hc	Ho	He	Hf
	Monoterpene hydrocarbons						
1	Tricyclene	926	0.03	–	–	–	–
2	α-Thujene	930	–	–	0.03	–	–
3	α-Pinene	939	1.56	2.90	11.31	1.66	11.59
4	Camphene	954	0.43	2.60	1.30	–	0.43
5	Sabinene	975	0.06	0.23	–	–	–
6	β-Pinene	979	3.40	**27.42**	21.78	3.16	**35.38**
7	Myrcene	990	0.08	0.79	0.88	–	0.77
8	2-Carene	1002	–	–	0.04	–	–
9	α-Phellandrene	1002	2.3	–	2.55	–	1.36
10	3-Carene	1011	0.05	–	–	–	–
11	α-Terpinene	1017	–	–	0.29	–	–
12	*p*-Cymene	1024	0.11	0.25	1.44	–	3.68
13	Limonene	1029	0.49	1.64	1.89	–	1.47
14	β-Ocimene	1050	–	1.45	0.06	–	–
15	γ-Terpinene	1059	0.06	–	1.07	0.20	1.59
16	α-Terpinolene	1088	0.02	–	0.36	–	–
	Oxygenated monoterpenes						
17	1,8-Cineole	1031	0.39	4.41	14.89	**89.36**	14.38
18	Linalool oxide	1072	–	–	0.09	–	–
19	Linalool	1096	0.64	6.60	**26.40**	–	20.87
20	*cis*-*p*-Menth-2-en-1-ol	1121	–	–	0.09	–	–
21	exo Fenchol	1121	–	–	0.06	–	–
22	Camphor	1146	–	0.96	–	–	–
23	Camphene hydrate	1149	–	–	0.04	–	–
24	Citronellal	1153	0.02	–	–	–	–
25	Pinocarvone	1164	–	–	0.09	–	–
26	Borneol	1169	1.74	0.42	1.48	0.32	0.48
27	Terpinen-4-ol	1177	0.13	1.21	2.57	1.43	1.02
28	α-Terpineol	1188	–	1.16	1.55	3.26	0.99
29	Citronellol	1225	0.08	–	0.07	–	–
30	Geraniol	1252	–	–	0.05	–	–
31	Linalyl acetate	1257	–	–	0.17	–	–
32	Bornyl acetate	1288	–	2.28	0.05	0.21	–
33	α-Terpinyl acetate	1349	–	–	0.30	–	–
34	Geranyl acetate	1381	–	–	0.03	–	–
	Sesquiterpene hydrocarbons						
35	α-Longipinene	1352	–	1.41	–	–	–
36	β-Elemene	1390	0.15	0.34	–	–	–
37	Caryophyllene	1419	1.35	0.33	0.29	0.04	
38	*trans*-α-Bergamotene	1434	–	0.60	–	–	–
39	γ-Elemene	1435	–	–	–	–	0.46
40	*cis*-β-Farnesene	1442	–	0.31	–	–	–
41	Humulene	1454	0.78	0.46	–	–	–
42	(*E*)-β-Farnesene	1456	–	0.32	–	–	–
43	Alloaromadendrene	1461	0.1	–	0.08	–	–
44	2-Isopropyl-5-methyl-9-methylene-bicyclo[4.4.0]dec-1-ene	1464	–	1.49	–	–	–
45	γ-Muurolene	1479	1.34	–	–	–	0.24
46	*Ar*-Curcumene	1480	–	6.29	–	–	–
47	Germacrene D	1485	0.29	–	–	–	–
48	β-Selinene	1490	–	–	0.19	–	–

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Table 1. (Continued)

No.	Compound name	KI*	Content (%)				
			Hb	Hc	Ho	He	Hf
49	Valencene	1496	–	–	0.09	–	–
50	α-Selinene	1498	0.13	–	–	–	–
51	Bicyclogermacrene	1500	0.74	–	–	–	–
52	β-Himachalene	1500	–	1.87	–	–	–
53	α-Muurolene	1500	–	–	0.16	–	–
54	*E*,*E*-α-Farnesene	1505	–	2.00	–	–	–
55	β-Bisabolene	1505	–	–	0.18	–	–
56	γ-Cadinene	1513	0.05	–	–	–	–
57	β-Curcumene	1515	–	1.94	–	–	–
58	δ-Cadinene	1523	0.31	1.67	–	–	–
		Oxygenated sesquiterpenes					
59	Elemol	1549	18.93	0.62	–	–	–
60	Nerolidol	1563	–	9.52	6.30	–	–
61	Spathulenol	1578	1.08	–	–	–	–
62	Caryophyllene oxide	1583	0.89	0.58	–	–	–
63	Guaiol	1600	2.54	–	–	–	–
64	Ledol	1602	0.08	–	–	–	–
65	γ-Eudesmol	1632	14.55	–	0.66	–	–
66	Hinesol	1641	1.86	–	–	–	–
67	τ-Muurolol	1642	–	7.28	–	–	–
68	α-Eudesmol	1653	**37.41**	–	–	–	–
69	α-Cadinol	1654	–	3.46	–	–	–
70	7-epi-α-eudesmol	1663	3.06	–	–	–	–
71	β-Bisabolol	1675	–	1.61	–	–	–
		Diterpene hydrocarbons					
72	β-Springene	1918	–	–	0.06	–	–
		Hydrocarbons					
73	Heptadecane	1700	–	–	–	–	0.83
74	Octadecane	1800	–	–	–	–	0.46
75	Nonadecane	1900	–	–	–	–	0.58
76	Eicosane	2000	–	–	–	–	0.53
77	Heneicosane	2100	–	–	–	–	0.51
78	Docosane	2200	–	–	–	–	0.42
79	Tricosane	2300	–	–	–	–	0.39
80	Tetracosane	2400	–	–	–	–	0.22
	Total identify (%)		**97.23**	**96.42**	**98.94**	**99.63**	**98.66**
	Yield (%volume/dry weight)		**2.75**	**2.47**	**2.04**	**2.29**	**2.43**

Note: *The Kovats index is determined relative to n-alkanes (C6-C24) on a DB-5 MS column. - Not detected. Hb: *H. bousigonianum*; Hc: *H. coccineum*; Ho: *H. coronarium*; He: *H. ellipticum*; Hf: *H. flavescens*.

main component in the essential oil from the rhizome of *H. coronarium*.

Linalool (26.40%), β-pinene (21.78%), and 1,8-cineole (14.89%) were the three major components. 1,8-Cineole (89.36%), an oxygenated monoterpene, was found to be the only significant compound in rhizome essential oil from *H. ellipticum*.

Previous studies have reported that β-pinene was the main component in the rhizome essential oil from *H. flavescens*, while linalool and 1,8-cineole were the main components in the rhizome essential oils of *H. coronarium* and *H. ellipticum*, respectively (George et al., 2007; Joshi et al., 2008; Suksathan et al., 2013; Thomas & Mani, 2023).

In contrast, the results differed from other previously reported findings. Several reports showed that 1,8-cineole was the main component in rhizome essential oil from *H. coronarium* (George et al., 2007; Joy et al., 2007; Ray et al., 2018; Noriega et al., 2019; Lima et al., 2021).

However, the variations in essential oil composition are likely influenced by several factors, such as environmental conditions, harvesting season, extraction method, and plant adaptive metabolism (Theanphong et al., 2016; Theanphong et al., 2017; Assaeed et al., 2020; Barut et al., 2022; Labhar

et al., 2024; Qian et al., 2024). For example, Ray et al. (2018) collected the rhizomes of *H. coronarium* from 10 different locations in India. The results showed that the essential oil obtained from *H. coronarium* collected in a tropical wet and dry region at high altitude contained β-pinene as the major component whereas 1,8-cineole predominated in the essential oil obtained from plants growing in tropical wet and dry regions at low altitude. In addition, linalool and coronarin E were identified as the major constituents in the essential oil of *H. coronarium* collected from alpine and humid subtropical regions, respectively.

3.2 *In Vitro* Activities of Essential Oils

3.2.1 Antioxidant Activities

The antioxidant activities of the essential oils were evaluated using DPPH, ABTS, superoxide anion, and hydroxyl radical scavenging assays. These assays, each targeting a different radical species, collectively confirmed the antioxidant potential of the essential oils.

The essential oils from the rhizomes of *H. bousigonianum* displayed the highest antioxidant activity against DPPH radicals, significantly higher than that of *L*-ascorbic acid whereas the essential oils from the rhizomes of *H. ellipticum*, *H. coronarium*, and *H. coccineum* showed non-significant antioxidant activity against DPPH radicals when compared with *L*-ascorbic acid. The essential oils from the rhizomes of *H. flavescens* displayed weak antioxidant activity against DPPH radicals.

For ABTS and hydroxyl radical scavenging activity, the rhizome essential oil of *H. ellipticum* exhibited significantly higher radical scavenging activity than the positive control, while the rhizome essential oil from *H. bousigonianum* showed no significant difference compared with the positive control. The rhizome essential oils of *H. coronarium*, *H. coccineum*, and *H. flavescens* showed weak activity.

The rhizome essential oil of *H. ellipticum* exhibited significantly higher superoxide anion radical scavenging activity when compared with *L*-ascorbic acid. The rhizome essential oils from *H. bousigonianum*, *H. coccineum*, and *H. flavescens* exhibited no significant difference in scavenging superoxide anion radicals compared with *L*-ascorbic acid. The rhizome essential oil of *H. coronarium* showed significantly lower superoxide anion radical scavenging activity than *L*-ascorbic acid.

The EC_{50} values of the essential oils and positive controls are shown in Table 2.

The experimental results indicated that the essential oils from the rhizomes of *H. ellipticum* and *H. bousigonianum* exhibit strong antioxidant activity against DPPH, ABTS, superoxide anion, and hydroxyl radicals. The strong antioxidant activities of essential oils from the rhizomes of *H. ellipticum* and *H. bousigonianum* are likely associated with their respective high 1,8-cineole and α-eudesmol content. Hoch et al. (2023) and Tan et al. (2024) reported that 1,8-cineole neutralizes reactive oxygen species through direct scavenging, whereas Hao et al. (2024) suggested that eudesmol may exert antioxidant effects. In addition, Assaeed et al. (2020) suggested that the antioxidant activity of oxygenated sesquiterpenes may be due to their ability to donate electrons, resulting from their high degree of oxygenation.

In agreement with previous reports by George et al. (2007), Joshi et al. (2008) and Noriega et al. (2019) the rhizome essential oils from *H. coronarium*, *H. ellipticum*, *H. spicatum*, and *H. venustum*, containing 1,8-cineole as the major constituent, exhibit potent antioxidant activity.

3.2.2 5-Lipoxygenase Inhibitory Activity Assay

Table 2 showed that the rhizome essential oils of *H. bousigonianum*, *H. ellipticum*, and *H. coronarium* demonstrated strong 5-lipoxygenase inhibition, comparable to that of indomethacin. The strong anti-inflammatory activity of *H. ellipticum* and *H. coronarium* might be due to the presence of oxygenated monoterpene, i.e. 1,8-cineol and linalool as the main components at high concentrations. These compounds have been reported for anti-inflammatory activity (Peana et al., 2002; Kamatou & Viljoen, 2008; Sa et al., 2013, 2015; Kim et al., 2019; Quintans et al., 2019; Demirci et al., 2022; Hoch et al., 2023). In addition, Sa et al. (2013) also suggested that monoterpene compounds could be used as novel anti-inflammatory agents.

Table 2. The IC_{50} values of essential oils from the fresh rhizomes of five *Hedychium* species

Biological	IC_{50} (μg/mL)					
Activity test	Hb	Hc	Ho	He	Hf	Positive control
DPPH	17.92 ± 0.62^{a}	20.26 ± 0.53^{c}	20.02 ± 0.89^{c}	18.27 ± 0.60^{a,b}	22.12 ± 0.60^{d}	19.12 ± 0.36^{b,c}
ABTS	13.16 ± 0.95^{b}	19.02 ± 0.43^{c}	18.09 ± 0.34^{c}	11.81 ± 0.67^{a}	19.34 ± 0.27^{d}	13.08 ± 0.36^{b}
Superoxide anion	28.41 ± 0.64^{b}	28.96 ± 0.51^{b,c}	31.47 ± 0.58^{d}	26.19 ± 0.61^{a}	29.12 ± 0.42^{b,c}	29.91 ± 0.89^{c}
OH	26.67 ± 0.73^{b}	27.98 ± 0.37^{c}	28.92 ± 0.31^{c}	25.34 ± 0.81^{a}	32.45 ± 0.55^{d}	26.82 ± 0.48^{b}
5-LOX	57.32 ± 1.09^{a,b}	67.50 ± 1.70^{c}	60.31 ± 1.25^{b}	55.84 ± 1.53^{a}	75.34 ± 2.28^{d}	58.53 ± 1.74^{a,b}

Note: Mean ± SD in each row superscripted with different lowercase letters are significantly different ($p < 0.05$). IC_{50}: Half-maximal inhibitory concentration; DPPH: DPPH radical scavenging activity assay; ABTS: ABTS radical scavenging activity assay; Superoxide anion: Superoxide anion radical scavenging activity assay; OH: Hydroxyl radical scavenging activity assay; 5-LOX: 5-Lipoxygenase inhibitory activity assay; *L*-Ascorbic acid was used as positive control for DPPH, OH and superoxide anion radical scavenging activity assay, Trolox® was used as positive control for ABTS radical scavenging activity assay, indomethacin was used as positive control for 5-LOX inhibitory activity assay; Hb: *H. bousigonianum*; Hc: *H. coccineum*; Ho: *H. coronarium*; He: *H. ellipticum*; Hf: *H. flavescens*.

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Rec. Nat. Prod. 2026, 20(2):e25103689

For *H. bousigonianum*, the anti-inflammatory activity might be associated with its strong antioxidant activity, as supported by the results from Theanphong et al. The essential oils from the fresh roots and rhizomes of *B. longiflora* exhibit strong antioxidant and anti-5-lipoxygenase activities (Theanphong et al., 2022).

Earlier studies indicated that essential oils derived from various Zingiberaceous plants, such as *A. danielli*, *A. murdochi*, *C. longa*, and *Z. officinale*, possess significant 5-lipoxygenase inhibitory effects (Odukoya et al., 1999; Miguel, 2010; Priya et al., 2012; Bayala et al., 2014).

Furthermore, prior research suggested that essential oils can alleviate inflammation through multiple pathways, such as enhancing cytokine release, reducing the production of pro-inflammatory mediators, and demonstrating antioxidant capabilities (Kim et al., 2019; Quintans et al., 2019; Biltekin et al., 2022; Hoch et al., 2023). This study investigated the chemical composition, *in vitro* antioxidant, and *in vitro* 5-lipoxygenase (5-LOX) inhibitory activities of essential oils obtained from the fresh rhizomes of five Thai *Hedychium* species. It provides the first report on the chemical constituents and antioxidant activities of *H. bousigonianum* and *H. coccineum*, as well as the first evidence demonstrating 5-LOX inhibitory activity across all five species.

4 Conclusion

The strong antioxidant and 5-LOX inhibitory activities demonstrated in this study suggest that these essential oils have considerable potential to protect against oxidative stress and inflammation-related disorders. Their dual action highlights the capacity of the essential oils to modulate key biochemical pathways linked to chronic inflammation and oxidative tissue damage. In particular, the essential oils from *H. bousigonianum* and *H. coccineum* appear to be valuable natural sources of potent bioactive compounds and promising candidates for pharmaceutical and cosmeceutical development. These findings not only broaden current phytochemical and biological knowledge of these underexplored Thai *Hedychium* species but also emphasize their translational potential. Further research should explore additional anti-inflammatory mechanisms such as nitric oxide (NO) synthase inhibition, cyclooxygenase (COX) modulation, and prostaglandin E_2 (PGE_2) suppression alongside *in vivo* anti-inflammatory, toxicity, and safety evaluations to confirm their therapeutic applicability. In summary, the bioactivities identified here support the potential of *H. bousigonianum* and *H. coccineum* as promising natural resources for future development into effective and safe medicinal and cosmeceutical products.

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Authors Contributions

Concept and design: all authors. Analysis and interpretation: Orawan Theanphong and Withawat Mingvanish. Plant authentication: Thaya Jenjittikul. Data collection: all authors. Writing the article: Orawan Theanphong. Critical revision of the article: all authors. Final approval of the article: all authors. Statistical analysis: Orawan Theanphong. Obtained funding: Orawan Theanphong and Thaya Jenjittikul. Overall responsibility: Orawan Theanphong.

Availability of Data and Materials

The authors declare that should any raw data files be needed about the further data of the study, they are available from the corresponding author upon reasonable request. Source data are provided with this paper.

Conflict of Interest

The authors declare that they have no competing financial interests.

ORCID

Orawan Theanphong: 0000-0002-4411-5366
Withawat Mingvanish: 0000-0002-3448-482X
Thaya Jenjittikul: 0000-0003-3974-9492

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