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Research Article

Exploring the Composition and Bioactivity of *Hedychium flavum* Leaf and Stem Essential Oil: *In Vitro* Assessment of Antibacterial, Antioxidant, Cytotoxicity, and Enzyme Inhibitory Activities

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Hedychium flavum is an edible, medicinal, and aromatic plant widely cultivated for its essential oil. So far, little studies have been done on its phytoconstituents and bioactivities. Hence, the research aimed to determine the chemical constituents of leaf essential oil (L-EO) and stem essential oil (S-EO) of *H. flavum* and first estimate their antibacterial, antioxidant, cytotoxic, and enzyme inhibitory activities. According to gas chromatography-flame ionization detector/mass spectrometer (GC-FID/MS) assay, L-EO was mainly composed of *β*-pinene (33.4%), *α*-pinene (10.4%), humulene (6.8%), *β*-caryophyllene (6.0%), eucalyptol (6.0%), caryophyllene oxide (5.5%), *endo*-borneol (3.8%), humulene epoxide II (3.7%), and *D*-limonene (3.2%). The predominant components of S-EO were *β*-pinene (17.2%), eucalyptol (9.7%), nerolidol (7.8%), *α*-phellandrene (6.7%), *α*-pinene (5.8%), *β*-caryophyllene (5.4%), terpinen-4-ol (4.5%), *D*-limonene (4.4%), *p*-cymene (3.7%), *endo*-borneol (3.5%), and *α*-terpineol (3.5%). For the bioactivities, L-EO and S-EO showed strong antibacterial activity against *Escherichia coli*, *Enterococcus faecalis*, and *Bacillus subtilis* with the minimum inhibitory concentration (MIC) of 9.77–625.00 *μ*g/mL and the minimum bactericidal concentration (MBC) of 9.77–1250.00 *μ*g/mL. Both L-EO and S-EO exhibited moderate antioxidant activity in the DPPH (14.52 ± 0.93 mg/mL and 4.73 ± 0.15 mg/mL, respectively) and ABTS (4.05 ± 0.75 mg/mL and 2.38 ± 0.14 mg/mL, respectively) free radical scavenging capacity. S-EO showed selective cytotoxicity on human leukemic K562 cell line (IC_{50} = 93.94 ± 0.91 *μ*g/mL), which was nearly three times that of noncancer L929 cell line (IC_{50} = 294.49 ± 9.40 *μ*g/mL). In enzyme inhibitory properties, L-EO exerted a potent inhibition on *α*-glucosidase (IC_{50} = 1.03 ± 0.02 mg/mL), and both essential oils had weak inhibition against cholinesterase and tyrosinase. Thus, *H. flavum* L-EO and S-EO possess antibacterial, antioxidant, cytotoxic, and enzyme inhibitory activities with potential for exploitation in the food, cosmetic, and pharmaceutical industries.

1. Introduction

Essential oils are fluid blends of volatile components extracted from aromatic plants [1]. Essential oils are commonly used in several industries, including foods, medicines, cosmetics, agriculture, and sanitation, due to their aromatic odor and multiple biological activities [2–4]. *Hedychium* genus (Zingiberaceae) contains about 104 species, most of which are enriched with essential oils and extensively utilized to treat stomach problems, influenza, bronchitis, diarrhea, leishmaniasis, snake bites, nausea, and asthma in traditional medicine [5–7]. Its essential oils have been confirmed to possess anthelmintic, pediculicidal, antifungal, antibacterial, antiviral, anti-inflammatory, anticancer, and antidiabetic activities [8–12].

Hedychium flavum Roxb. has edible and medicinal values and is a perennial and aromatic plant distributed primarily throughout Southwest China, Myanmar, Thailand,

and India [13, 14]. *H. flavum* is rich in essential oil, which is utilized in traditional medicines and perfumes [15]. As a traditional Chinese medicine, *H. flavum* rhizome, commonly known as "*Yehansu*," is utilized to treat many diseases like bruise, abdominal pain, cold, headache, rheumatism, and cough [16, 17]. *H. flavum* rhizome essential oil mainly consisted of coronarin E (20.3%), β-pinene (16.8%), and *E*-nerolidol (11.8%) and exhibited a variety of bioactivities, including antifungal, antibacterial, anti-inflammatory, anticancer, and insecticidal activities [14, 18, 19]. Its flower is a spice used for food seasoning; it is a traditional Chinese medicine used to treat stomach pain, diarrhea, and indigestion [20]. *H. flavum* flower essential oil exhibited notable anti-inflammatory effects, primarily consisting of β-pinene (20.2%), α-pinene (9.3%), and α-phellandrene (8.3%), and exerted significant anti-inflammatory activity [21]. *H. flavum* leaf and stem are utilized as condiments and vegetables [22]. According to previous studies, the main components of *H. flavum* leaf and stem essential oils were β-caryophyllene (10.4% and 11.8%, respectively), β-pinene (22.5% and 11.2%, respectively), and α-humulene (15.7% and 18.9%, respectively) [13]. Besides, its aboveground part essential oil is composed mainly of β-pinene (49.6%) and β-caryophyllene (26.9%) [23].

H. flavum is an edible, medicinal, and aromatic plant widely cultivated for its essential oil. Normally, *H. flavum* relies on the asexual reproduction of rhizome to expand the population, and its flower is only present during specific periods. Only harvesting the leaf and stem of *H. flavum* can minimize its destruction and contribute to its sustainable utilization. However, there is little research on its phytoconstituents and biological activities, which may limit its exploitation. Therefore, we analyzed the chemical composition of *H. flavum* L-EO and S-EO by GC-FID/MS and first tested their antibacterial, antioxidant, cytotoxic, and enzyme inhibitory properties.

2. Materials and Methods

2.1. Plant Material. Plant samples were collected from Nayong, Bijie, Guizhou, China, on August 2019. Prof. Guoxiong Hu of Guizhou University identified the species as *Hedychium flavum* of the *Hedychium* genus (Zingiberaceae). The voucher specimen was kept in the Guizhou Engineering Center for Innovative Traditional Chinese Medicine and Ethnic Medicine, Guizhou University (Voucher No: HF-20190821).

2.2. Essential Oil Preparation. Fresh leaf and stem of *H. flavum* (2 kg) were separately washed, chopped, and then used to obtain essential oil with hydrodistillation in a Clevenger apparatus for 4 h. Essential oil was dried with anhydrous disodium sulfate (Na_2SO_4) and kept in a refrigerator at 4°C until further analysis.

2.3. Analysis of EO by GC-FID/MS. The L-EO and S-EO were quantitatively analyzed by an Agilent 6890 GC-FID equipped with a flexible quartz capillary column (HP-5MS, 60 m × 0.25 mm, 0.25 μm film thickness). The carrier gas was high-purity helium (99.999%), and the flow rate was 1.0 mL/min. A split injection (split ratio 10 : 1) was used, and the injection volume was 2 μL. The following GC oven temperature was used: initial temperature 70°C (2 min), ramped up to 180°C at 2°C/min (55 min), then to 290°C at 10°C/min (11 min), run time (68 min). The qualitative analysis was performed using an Agilent 6890/5975C GC-MS with the same oven operating conditions as those set in GC-FID. The mass spectrometer was operated as follows: ion source (EI source, temperature 230°C); quadrupole temperature (150°C); interface temperature (280°C); mass range (29–500 amu); multiplier voltage (1847 V); emission current (34.6 μA); electron energy (70 eV). The retention index (RI) was defined with reference to n-alkanes (C_8–C_{22}). The components of L-EO and S-EO were determined by comparison of their RI and mass spectrum with those listed in Wiley 275 and NIST 2020 databases [24].

2.4. Antibacterial Activity. We measured the antibacterial activities of L-EO and S-EO on *Escherichia coli* CICC 10389, *Bacillus subtilis* ATCC 6633, and *Enterococcus faecalis* ATCC 19433. The MIC and MBC were assessed employing the microplate dilution method [25]. In a 96-well plate, the bacterial suspension (100 μL, 10^5 CFU/mL) was mixed with the two-fold serial dilution of sample solution (100 μL). After incubation for 24 h at 37°C, resazurin solution (20 μL, 0.1 mg/mL) was pipetted into each well and allowed to stand for 2 h at 37°C in the absence of light. The minimum sample dose at which the blue color did not change was determined as the MIC. Samples without color change (10 μL) were incubated in Mueller–Hinton agar for 24 h. The minimum dose with no visible bacterial growth was considered the MBC.

2.5. Antioxidant Activity

2.5.1. DPPH Assay. The DPPH method was performed with reference to previous literature [26]. The test was divided into three groups: experimental group (2 mL DPPH solution and 2 mL sample solution), control group (2 mL sample solution and 2 mL solvent), and blank group (2 mL solvent and 2 mL DPPH solution). The optical density (OD) values of the above three groups were determined after allowing to stand for 30 min in the absence of light (wavelength 517 nm). Positive controls were ascorbic acid and BHT (butylated hydroxytoluene). The final results were expressed as IC_{50} (fifty percent inhibitory concentration) and ascorbic acid equivalent values (mg AEs/g sample). The DPPH scavenging rate was calculated as follows:

$$\text{DPPH scavenging rate} = \left[1 - \frac{\left(\text{OD}_{\text{experimental}} - \text{OD}_{\text{control}}\right)}{\text{OD}_{\text{blank}}}\right] \times 100\%. \quad (1)$$

2.5.2. ABTS Assay. The ABTS method was derived from past literature [26]. Three groups, including experimental group (0.4 mL sample solution and 4 mL ABTS•$^+$ solution), control group (0.4 mL sample solution and 4 mL solvent), and blank group (0.4 mL solvent and 4 mL ABTS•$^+$ solution), were incubated in the dark for 10 min. Then, the OD values of the above three groups were determined (wavelength 734 nm). The ABTS free radical scavenging rate was obtained from the above equation. The final results were expressed as IC_{50} and ascorbic acid equivalent values (mg AEs/g sample).

2.6. Cytotoxicity. Mouse fibroblast cell line (L929), lung adenocarcinoma (A549), leukemia (K562), non-small-cell lung cancer (NCI-H1299), and prostate cancer (PC-3) cell lines were selected for cytotoxicity evaluation using MTT test [14]. In a 96-well plate, cell suspension (80 μL, 5×10^3 cells/well) was cultivated for 24 h. Afterward, sample solutions (20 μL) were added and allowed to stand for 48 h. Next, the MTT solution (10 μL, 5 mg/mL) was added and allowed to stand for 4 h. Finally, DMSO (150 μL) was added for the purpose of dissolving formazan crystal. Absorbance was measured at 490 nm using an iMark microplate reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Cisplatin was selected as the positive control, and the results were indicated by IC_{50} values.

2.7. Enzyme Inhibition Activity

2.7.1. Cholinesterase Inhibitory Activity. The acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activity assays were performed with reference to previous literature [27]. Galanthamine was chosen as the positive control. The experiment was divided into the following four groups. In the experimental group (A1), the AChE or BChE solution (10 μL, 0.5 U/mL) was added to the sample solution (50 μL) and reacted at 4°C for 15 min. Then, the 5,5-dithiobis-(2-nitrobenzoic acid) solution (20 μL, 2 mM) and acetylthiocholine or butyrylthiocholine solution (20 μL, 2 mM) were added and reacted at 37°C for 30 min. In the sample blank group (A2), PBS (pH 8) was used to replace the enzyme. In the negative group (A3), the sample solution was replaced with PBS (pH 8). In the blank group (A4), both the enzyme and the sample solution were replaced with PBS (pH 8). Finally, the absorbance value was measured at 405 nm. The results were expressed as IC_{50} values. The calculation formula for the inhibition rate of AChE or BChE was as follows:

$$\text{AChE or BChE inhibition rate} = \left[1 - \frac{(A1 - A2)}{(A3 - A4)}\right] \times 100\%. \quad (2)$$

2.7.2. α-Glucosidase Inhibitory Activity. According to the literature [28], the test was divided into the following four groups. In the experimental group (A1), sample solution (30 μL) and α-glucosidase (10 μL, 0.8 U/mL) were added to phosphate buffer (60 μL, pH 6.8) and reacted for 15 min at 37°C. Then, *p*-nitrobenzene-α-*D*-glucopyranoside was added and incubated at room temperature for 15 min. Next, the reaction was terminated by adding sodium carbonate solution (80 μL, 0.2 mM). In the sample blank group (A2), PBS (pH 6.8) was used to replace the enzyme. In the negative group (A3), the sample solution was replaced with PBS (pH 6.8). In the blank group (A4), both the enzyme and the sample solution were replaced with PBS (pH 6.8). Finally, the absorbance values were determined at 405 nm. The results were expressed IC_{50} values. The α-glucosidase inhibition rate was calculated using the above equation.

2.7.3. Tyrosinase Inhibitory Activity. Referring to the related literature [29], the experiment was divided into the following four groups. In the experimental group (A1), tyrosinase (100 μL, 100 U/mL) was added to the sample solution (70 μL) and reacted at 37°C for 5 min. L-Tyrosine (80 μL, 5.5 mM) was added and incubated at 37°C for 30 min. The remaining three groups needed to change as shown above. Finally, the absorption value (wavelength 492 nm) was determined. Arbutin was selected as the positive control, and the results were shown as IC_{50} values. The tyrosinase inhibition rate was obtained from the above equation.

2.8. Statistical Analysis. The results from three replicate experiments were expressed as mean ± standard deviation (SD). Results were analyzed using SPSS 25.0 software. Statistical analysis was performed using a two-tailed unpaired *t*-test or one-way analysis of variance (ANOVA). Differences were considered significant when $p < 0.05$.

3. Results and Discussion

3.1. Chemical Composition. Based on fresh weight, the extraction yield of the hydrodistilled essential oils from leaf and stem was 0.64% (w/w) and 1.37% (w/w), respectively. GC-FID/MS analysis showed that 43 and 56 compounds were identified, accounting for 98.4% and 98.0% of the total oil content of L-EO and S-EO, respectively (Table 1). L-EO was mainly composed of β-pinene (33.4%), α-pinene (10.4%), humulene (6.8%), β-caryophyllene (6.0%), eucalyptol (6.0%), caryophyllene oxide (5.5%), *endo*-borneol (3.8%), humulene epoxide II (3.7%), and *D*-limonene (3.2%) (Figure 1). The predominant components of S-EO were β-pinene (17.2%), eucalyptol (9.7%), nerolidol (7.8%), α-phellandrene (6.7%), α-pinene (5.8%), β-caryophyllene

Table 1: Chemical composition of *H. flavum* L-EO and S-EO.

RT (min)	Compounds[a]	RI[b]	RI[c]	CAS	% area	
					L-EO	S-EO
7.08	Octane	800	800	000111-65-9	0.3	0.3
9.15	*o*-Xylene	872	888	000095-47-6	—	0.2
9.99	Nonane	900	900	000111-84-2	—	0.2
11.14	Tricyclene	927	925	000508-32-7	0.1	tr[d]
11.24	α-Thujene	929	929	002867-05-2	0.5	0.4
11.60	α-Pinene	937	937	000080-56-8	10.4	5.8
12.30	Camphene	953	952	000079-92-5	2.1	1.4
13.36	Sabinene	977	974	003387-41-5	1.6	2.5
13.60	β-Pinene	982	979	000127-91-3	33.4	17.2
14.02	β-Myrcene	992	991	000123-35-3	0.7	2.1
14.88	α-Phellandrene	1009	1005	000099-83-2	0.4	6.7
15.20	3-Carene	1015	1011	013466-78-9	tr[d]	1.2
15.51	α-Terpinen	1020	1017	000099-86-5	0.2	0.5
15.94	*p*-Cymene	1028	1025	000099-87-6	0.5	3.7
16.17	*D*-Limonene	1032	1031	005989-27-5	3.2	4.4
16.35	Eucalyptol	1035	1032	000470-82-6	6.0	9.7
17.09	β-Ocimene	1048	1037	013877-91-3	—	0.1
17.83	γ-Terpinen	1061	1060	000099-85-4	0.5	2.0
18.34	4-Thujanol	1070	1075	000546-79-2	0.1	0.1
19.60	α-Terpinolene	1093	1088	000586-62-9	0.1	1.0
20.12	Linalool	1102	1099	000078-70-6	0.7	0.5
21.63	(*Z*)-*p*-Menth-2-en-1-ol	1125	1122	029803-82-5	—	0.2
21.92	α-Campholenal	1130	1125	004501-58-0	tr[d]	—
22.72	Nopinone	1143	1139	038651-65-9	0.1	—
22.82	*L*-Pinocarveol	1144	1139	000547-61-5	0.2	—
23.18	*L*-Camphor	1150	1145	000464-48-2	0.1	0.9
24.30	Pinocarvone	1168	1164	030460-92-5	0.1	tr[d]
24.49	*endo*-Borneol	1171	1167	000507-70-0	3.8	3.5
25.20	Terpinen-4-ol	1182	1177	000562-74-3	1.2	4.5
26.02	α-Terpineol	1195	1189	000098-55-5	0.9	3.5
26.47	Myrtenal	1202	1193	000564-94-3	0.5	0.1
29.20	Neral	1244	1240	000106-26-3	—	tr[d]
31.09	Citral	1273	1273	005392-40-5	—	tr[d]
32.21	Bornyl acetate	1290	1285	000076-49-3	0.7	0.4
32.32	Isobornyl acetate	1292	1286	000125-12-2	—	tr[d]
32.50	Dihydroedulan I	1295	1293	072746-44-2	—	tr[d]
32.85	Dihydroedulan II	1300	1318	041678-32-4	0.5	0.2
33.11	Thymol	1305	1291	000089-83-8	—	tr[d]
36.24	α-Terpinyl acetate	1354	1350	000080-26-2	0.3	1.8
38.11	α-Cubebene	1383	1351	017699-14-8	—	tr[d]
39.08	Elemene	1398	1391	000515-13-9	—	0.1
40.11	Isocaryophyllene	1415	1406	000118-65-0	—	0.1
40.95	β-Caryophyllene	1429	1419	000087-44-5	6.0	5.4
41.64	γ-Elemene	1440	1433	029873-99-2	—	0.1
42.66	Alloaromadendrene	1457	1440	000489-39-4	0.1	0.2
43.03	Humulene	1463	1454	006753-98-6	6.8	2.1
44.40	γ-Curcumene	1485	1482	000451-55-8	—	0.2
44.59	α-Curcumene	1488	1483	000644-30-4	1.1	0.4
45.03	Selinene	1495	1494	000473-13-2	tr[d]	0.1
46.00	α-Farnesene	1511	1508	000502-61-4	0.2	0.2
46.15	β-Bisabolene	1514	1509	000495-61-4	—	0.5
46.33	β-Curcumene	1517	1514	028976-67-2	0.9	0.4
47.13	δ-Cadinene	1531	1524	000483-76-1	—	0.3
49.32	Nerolidol	1568	1544	000142-50-7	0.9	7.8
50.80	Caryophyllene oxide	1593	1581	001139-30-6	5.5	1.5
52.30	Humulene epoxide II	1620	1606	019888-34-7	3.7	0.6
52.80	Selin-6-en-4α-ol	1629	1636	118173-08-3	1.8	1.2
53.61	Humulenol-II	1643	1650	019888-00-7	1.8	—
54.76	α-Cadinol	1664	1653	000481-34-5	—	0.8

Table 1: Continued.

RT (min)	Compounds[a]	RI[b]	RI[c]	CAS	% area	
					L-EO	S-EO
61.01	Ambrial	1817	1809	003243-36-5	tr[d]	—
66.88	Coronarin E	2162	2136	117591-81-8	—	0.4
	Total				98.4	98.0
	Yield (*w/w*) (%)				0.64	1.37

[a]Compounds were listed based on the elution order on the HP-5MS column. [b]Retention index (RI) calculated using n-alkanes (C_8–C_{22}) as a reference. [c]RI from NIST 2020 database. —: not detected. [d]tr: trace (trace <0.1%).

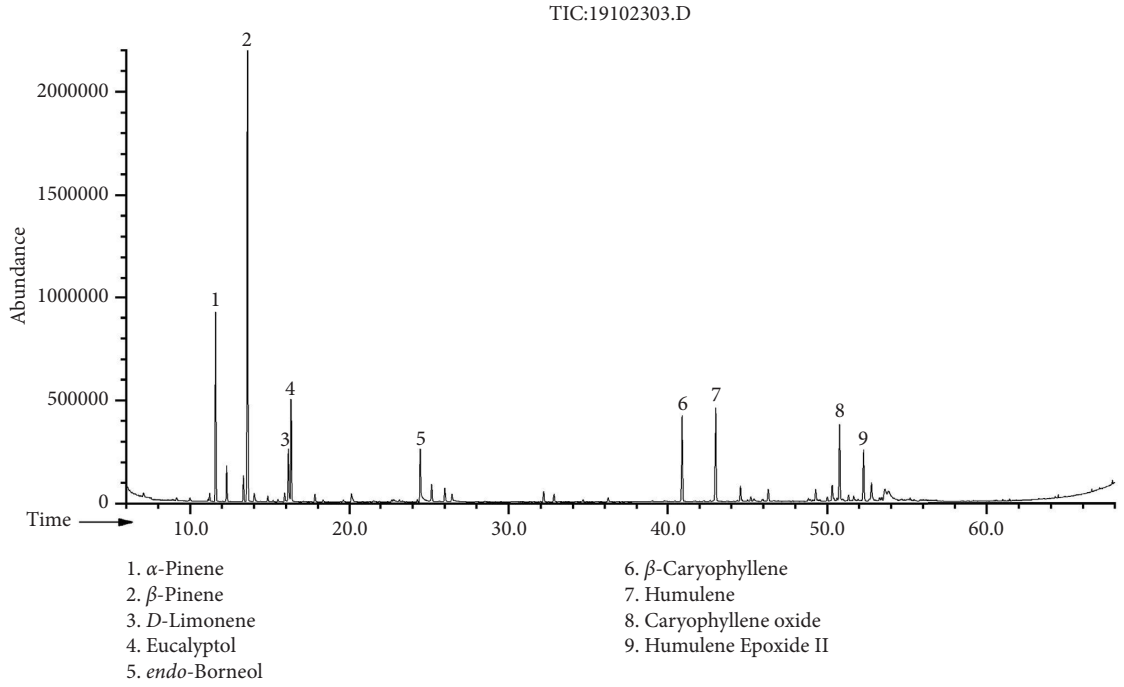

Figure 1: GC-MS chromatogram of *H. flavum* L-EO.

(5.4%), terpinen-4-ol (4.5%), *D*-limonene (4.4%), *p*-cymene (3.7%), *endo*-borneol (3.5%), and *α*-terpineol (3.5%) (Figure 2). According to a previous study, the main components of *H. flavum* L-EO from Vietnam were *β*-pinene (22.5%), *α*-humulene (15.7%), and *β*-caryophyllene (10.4%); besides, the main components of S-EO were *α*-humulene (18.9%), *β*-caryophyllene (11.8%), and *β*-pinene (11.2%) [13]. The chemical composition of *H. flavum* L-EO and S-EO in the present study was very diverse, which may be due to a variety of factors, like genetic factors, climatic conditions, growth conditions, and developmental stages [30]. In our earlier research, the yield of *H. flavum* rhizome essential oil was 0.56% (*w/w*), and the hydrodistillation of *H. flavum* flower yielded essential oil at 0.31% (*w/w*) [14, 21]. *H. flavum* leaf and stem produced a larger amount of essential oil compared to the rhizome and flower, especially the stem having the highest yield. *β*-Pinene possesses diverse biological activities, including antibacterial, antioxidant, antitumor, antimalarial, anti-inflammatory, and analgesic effects, and is widely utilized in food, cosmetics, and pharmaceuticals [31]. Hence, *H. flavum* L-EO and S-EO can be used as a new source of *β*-pinene.

3.2. Antibacterial Activity. The antibacterial activities of *H. flavum* L-EO and S-EO were tested using the MIC and MBC values (Table 2). According to a past study, essential oils have a strong antibacterial effect when the MIC value is less than 5000 *μ*g/mL [32]. Hence, L-EO and S-EO showed potent antibacterial effect on *Escherichia coli* (MIC: 9.77–156.25 *μ*g/mL, MBC: 9.77–312.50 *μ*g/mL), *Enterococcus faecalis* (MIC: 78.13–312.50 *μ*g/mL, MBC: 78.13–625.00 *μ*g/mL), and *Bacillus subtilis* (MIC: 625.00 *μ*g/mL, MBC: 625.00–1250.00 *μ*g/mL). Based on previous studies, *β*-pinene demonstrated marked antibacterial activity on *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Staphylococcus pyogenes*, and *Streptococcus pneumoniae*, with MIC values ranging from 20 *μ*g to 40 *μ*g/mL [33]. *α*-Humulene, *β*-caryophyllene, and *α*-pinene displayed significant inhibitory activities against *Staphylococcus aureus* with MIC values of 2.6 *μ*g/mL, 5.1 *μ*g/mL, and 13.6 *μ*g/mL, respectively [34]. In addition, other chemical compositions such as nerolidol [35], eucalyptol [36], terpinen-4-ol [37], *D*-limonene [38], caryophyllene oxide [39], and *α*-terpineol [40] have obvious antibacterial activity. Consequently, the existence of these main components may interpret the

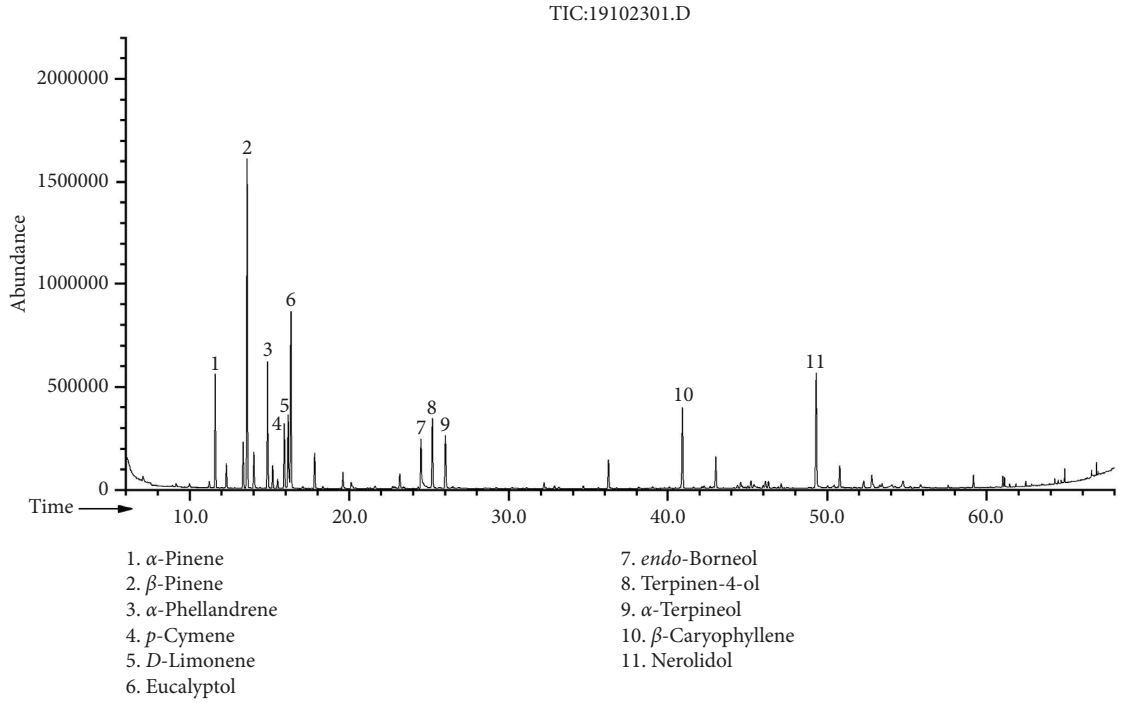

Figure 2: GC-MS chromatogram of *H. flavum* S-EO.

Table 2: The MIC and MBC values of *H. flavum* L-EO and S-EO.

Bacterial strains	MIC and MBC (μg/mL)[1]				Streptomycin	
	L-EO		S-EO			
	MIC (μg/mL)	MBC (μg/mL)	MIC (μg/mL)	MBC (μg/mL)	MIC (μg/mL)	MBC (μg/mL)
E. coli	156.25	312.50	9.77	9.77	0.31	0.63
E. faecalis	312.50	625.00	78.13	78.13	0.63	1.25
B. subtilis	625.00	625.00	625.00	1250.00	0.08	0.16

[1]Minimal inhibitory concentration (MIC); minimal bactericidal concentration (MBC); streptomycin as a positive control.

remarkable antibacterial activity of L-EO and S-EO. Based on these findings, *H. flavum* L-EO and S-EO could be utilized as a natural source of antibacterial agents.

3.3. Antioxidant Activity. Antioxidant activities of *H. flavum* L-EO and S-EO were determined using the DPPH and ABTS radical scavenging tests (Table 3). In contrast to the positive control (ascorbic acid and BHT), both L-EO (14.52 ± 0.93 mg/mL and 4.05 ± 0.75 mg/mL, respectively) and S-EO (4.73 ± 0.15 mg/mL and 2.38 ± 0.14 mg/mL, respectively) showed moderate DPPH and ABTS free radical scavenging capacity. The antioxidant activity of S-EO (0.69 ± 0.02 and 1.28 ± 0.01 mg AEs/g sample, respectively) was higher than that of L-EO (0.22 ± 0.01 and 0.79 ± 0.15 mg AEs/g sample, respectively) in the DPPH and ABTS assays, as expressed in milligram ascorbic acid equivalents per gram of sample. *H. flavum* rhizome essential oil rich in β-pinene, α-pinene, nerolidol, and α-terpineol was proven to exhibit antioxidant activity [14]. According to past research, essential oils from various *Hedychium* plants rich in α-pinene and β-pinene (e.g., *H. coronarium*, *H. greenii*, and *H. forrestii*) possess antioxidant activity [11, 41, 42]. β-Pinene demonstrated significant antioxidant activity in the DPPH assay [43]. Eucalyptol is a terpene oxide isolated from the eucalyptus tree and has been shown to have strong DPPH free radical scavenging properties [44, 45]. *D*-Limonene is a monocyclic monoterpene with remarkable antioxidant activity in ABTS ($IC_{50} = 603.23\,\mu$M) and DPPH ($IC_{50} = 384.73\,\mu$M) [46]. According to past reports, β-caryophyllene [47], nerolidol [48], α-pinene [43], caryophyllene oxide [40], α-phellandrene [49], *p*-cymene [50], terpinen-4-ol [51], and α-terpineol [52] exhibited antioxidant effects. The moderate antioxidant ability of *H. flavum* L-EO and S-EO may be related to these major components.

3.4. Cytotoxic Activity. The cytotoxic activities of *H. flavum* L-EO and S-EO were investigated against human tumor cell lines (K562, A549, PC-3, and NCI-H1299) and murine fibroblast cell line (L929) using the MTT assay with cisplatin as a positive control (Table 4). Compared with noncancerous L929 cells ($IC_{50} = 182.22 \pm 5.19$ μg/mL), L-EO exhibited stronger cytotoxicity on four human tumor cell lines with IC_{50} values ranging from $115.42 \pm 0.98\,\mu$g/mL to $154.41 \pm 7.58\,\mu$g/mL. In addition, S-EO was significantly

Table 3: The DPPH and ABTS free radical scavenging capacity of *H. flavum* L-EO and S-EO.

Treatment	DPPH		ABTS	
	IC_{50} (mg/mL)[1]	mg AEs/g sample[2]	IC_{50} (mg/mL)[1]	mg AEs/g sample[2]
L-EO	14.52 ± 0.93^{a}	0.22 ± 0.01^{a}	4.05 ± 0.75^{a}	0.79 ± 0.15^{a}
S-EO	4.73 ± 0.15^{b}	0.69 ± 0.02^{b}	2.38 ± 0.14^{b}	1.28 ± 0.01^{b}
BHT[3]	337.87 ± 1.94^{c}		16.99 ± 4.87^{c}	
Ascorbic acid[3]	3.25 ± 0.06^{d}		3.14 ± 0.04^{d}	

[a–d]Different letters in the same column indicate significant differences ($p < 0.05$). [1]IC_{50}: sample concentration with 50% scavenging effect. [2]mg AEs/g sample: milligram ascorbic acid equivalents per gram of sample. [3]Positive controls were ascorbic acid and BHT (IC_{50}, μg/mL).

Table 4: The cytotoxic effects of *H. flavum* L-EO and S-EO.

Treatment	Cell line (IC_{50}, μg/mL)[1]				
	K562	A549	PC-3	NCI-H1299	L929
L-EO	117.85 ± 3.33^{aA}	115.42 ± 0.98^{aA}	124.81 ± 4.61^{aA}	154.41 ± 7.58^{aB}	182.22 ± 5.19^{aC}
S-EO	93.94 ± 0.91^{bA}	157.58 ± 6.49^{bB}	103.09 ± 3.32^{bA}	114.76 ± 5.40^{bC}	294.49 ± 9.40^{bD}
Cisplatin	4.28 ± 0.27^{cA}	8.21 ± 0.26^{cB}	16.72 ± 1.20^{cC}	8.53 ± 0.69^{cB}	5.71 ± 0.09^{cA}

[1]IC_{50}: sample concentration that inhibits cell viability by 50%. Cell lines: human leukemic K562 cell line, human lung adenocarcinoma A549 cell line, human prostatic carcinoma PC-3 cell line, human non-small-cell lung cancer NCI-H1299 cell line, and murine fibroblast L929 cell line. Different letters in the same column (a–c) or same row (A–D) represent significant differences ($p < 0.05$).

Table 5: Enzyme inhibitory activities of *H. flavum* L-EO and S-EO.

Samples	Enzyme inhibitory activity (IC_{50}, mg/mL)[1]			
	Acetylcholinesterase	Butyrylcholinesterase	α-Glucosidase	Tyrosinase
L-EO	7.72 ± 0.30^{a}	33.42 ± 1.53^{a}	1.03 ± 0.02^{a}	8.34 ± 0.99^{a}
S-EO	$>50^{b}$	18.27 ± 0.86^{b}	22.39 ± 2.76^{b}	6.02 ± 0.14^{b}
Acarbose			0.15 ± 0.01^{c}	
Arbutin				0.18 ± 0.05^{c}
Galanthamine *	0.26 ± 0.05^{c}	4.77 ± 0.21^{c}		

[1]IC_{50}: sample concentration with 50% enzyme inhibition. [a–c]Different letters in the same column indicate significant differences ($p < 0.05$). *Galanthamine: IC_{50} (μg/mL).

more cytotoxic to human tumor K562 ($IC_{50} = 93.94 \pm 0.91\,\mu$g/mL), A549 ($IC_{50} = 157.58 \pm 6.49\,\mu$g/mL), PC-3 ($IC_{50} = 103.09 \pm 3.32\,\mu$g/mL), and NCI-H1299 ($IC_{50} = 114.76 \pm 5.40\,\mu$g/mL) cell lines than to noncancerous L929 cells ($IC_{50} = 294.49 \pm 9.40\,\mu$g/mL) ($p < 0.05$). Interestingly, S-EO had considerable selective cytotoxicity against K562 cells, which was almost three times that of L929 cells. Based on previous studies, *H. flavum* rhizome essential oil enriched with β-pinene, α-pinene, nerolidol, and α-terpineol showed higher cytotoxicity against cancer cells A549 ($72.86 \pm 6.39\,\mu$g/mL), PC-3 ($63.16 \pm 9.20\,\mu$g/mL), K562 ($27.16 \pm 2.18\,\mu$g/mL), and NCI-H1299 ($70.74 \pm 9.56\,\mu$g/mL) than noncancer L929 cells ($129.91 \pm 5.27\,\mu$g/mL) [14]. Moreover, essential oils extracted from *Hedychium* plants, such as *H. spicatum* and *H. gardnerianum*, are rich in α-pinene and β-pinene, which have been shown to have anticancer effects [53, 54]. β-Pinene possessed potent cytotoxicity against a variety of cancer cells, including SCC25 ($IC_{50} = 67\,\mu$g/mL), HCT-8 ($IC_{50} = 24.1\,\mu$g/mL), and SF-295 ($IC_{50} = 26.3\,\mu$g/mL) cell lines [55, 56]. Eucalyptol was cytotoxic to colorectal carcinoma HCT 116 and HT-29 cells with IC_{50} values of 4 mM and 7.5 mM, respectively [57]. The cytotoxic activities of caryophyllene oxide against SNU-16, HeLa, AGS, SNU-1, and HepG2 cells were potent, with IC_{50} values ranging from 3.95 to 27.39 μM [58]. In addition, the antitumor effects of α-pinene, caryophyllene [56], *D*-limonene [59], humulene [60], nerolidol [61], terpinen-4-ol [62], and α-terpineol [63] have been demonstrated. Thus, the existence of these major constituents could interpret the antitumor effects of *H. flavum* L-EO and S-EO.

3.5. Enzyme Inhibitory Activity. The inhibitory effects of *H. flavum* L-EO and S-EO on cholinesterases (AChE and BChE), α-glucosidase, and tyrosinase were investigated, and the results are presented in Table 5.

Cholinesterase inhibitors enhance cholinergic neurotransmission and positively impact cognition, mood, and behavior in Alzheimer's disease (AD) patients and have become the most effective treatment strategy for AD [64]. Compared with S-EO ($IC_{50} > 50$ mg/mL), L-EO ($IC_{50} = 7.72 \pm 0.30$ mg/mL) had more potent inhibition on AChE. On the contrary, S-EO ($IC_{50} = 18.27 \pm 0.86$ mg/mL) had a greater inhibitory effect on BChE than L-EO ($IC_{50} = 33.42 \pm 1.53$ mg/mL). The cholinesterase inhibitory activities of β-pinene, α-pinene, caryophyllene oxide, caryophyllene, α-terpineol, terpinen-4-ol, and

nerolidol have been demonstrated [65–67]. Therefore, the weak cholinesterase inhibition of *H. flavum* L-EO and S-EO may be related to these major components.

Inhibition of α-glucosidase, a key enzyme in the small intestinal absorption of carbohydrates, reduces postprandial blood glucose and insulin levels and is an important therapy for type 2 diabetes [68]. The current study showed that L-EO ($IC_{50} = 1.03 \pm 0.02$ mg/mL) had a potent inhibition of α-glucosidase. In contrast, S-EO ($IC_{50} = 22.39 \pm 2.76$ mg/mL) showed weaker inhibition on α-glucosidase. α-Pinene, eucalyptol, and *p*-cymene showed promising inhibition of α-glucosidase with IC_{50} values of 1.42 μL/mL, 1.12 μL/mL, and 1.31 μL/mL, respectively [69]. Besides, previous studies have reported that terpinen-4-ol and α-terpineol exhibited excellent inhibition of α-glucosidase [70]. Thus, the α-glucosidase inhibitory effects of *H. flavum* L-EO and S-EO may be related to these major components, and L-EO can be a novel source of natural α-glucosidase inhibitors.

Tyrosinase plays a key role in the enzymatic browning of fruits and mammalian melanogenesis [71, 72]. As shown in Table 5, S-EO ($IC_{50} = 6.02 \pm 0.14$ mg/mL) exhibited more potent tyrosinase inhibition compared to L-EO ($IC_{50} = 8.34 \pm 0.99$ mg/mL). Nevertheless, L-EO and S-EO showed weaker inhibition of tyrosinase compared to the positive control arbutin ($IC_{50} = 0.18 \pm 0.05$ mg/mL).

4. Conclusion

To our knowledge, the antibacterial, antioxidant, cytotoxic, and enzyme inhibitory activities of *H. flavum* L-EO and S-EO are reported for the first time. Based on GC-FID/MS analysis, forty-three and fifty-six compounds were identified in L-EO and S-EO, respectively. L-EO and S-EO showed strong antibacterial activity against *E. coli*, *E. faecalis*, and *B. subtilis* and exhibited moderate antioxidant activities in DPPH and ABTS assays. In addition, S-EO had considerable selective cytotoxicity against human leukemic K562 cells and low cytotoxicity against noncancerous L929 cells. L-EO and S-EO had weak inhibitory effects on cholinesterase and tyrosinase, whereas L-EO showed a potent inhibition on α-glucosidase. Hence, the leaf and stem of *H. flavum* can be considered a potential source of bioactive compounds with potential for exploitation in the food, pharmaceutical, and cosmetic industries.

Data Availability

The data used to support the findings of this study are available from the corresponding authors upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

FD and XW performed the material preparation and experiments. NY and JN analyzed the data. FD drafted the manuscript. YH and MT supervised the work, revised the manuscript, and provided funding. All authors have read and approved the final manuscript.

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