

Chemical composition and enzymatic inhibitory activities related to diabetes of *Hedychium malayanum* rhizome and leaf essential oils

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Abstract

In this study, the composition of essential oils (EOs) from *H. malayanum* rhizomes and leaves, collected from Bidoup-Núi Bà National Park in Southern Vietnam, and their α -amylase and α -glucosidase activity are demonstrated. Gas chromatography-mass spectrometry (GC-MS) analysis identified 34 compounds (99.54%) in rhizome EO (RHM-EO) and 40 compounds (99.13%) in leaf EO (LHM-EO). The major components of rhizome EO included 1,8-cineole (39.33 $\pm$ 0.34), β -pinene (23.36 $\pm$ 0.13), α -pinene (9.65 $\pm$ 0.03), and α -limonene (5.57 $\pm$ 0.06), while the leaf EO was rich in β -pinene (57.8 $\pm$ 0.25), α -pinene (14.93 $\pm$ 0.13), and β -(*E*)-caryophyllene (9.43 $\pm$ 0.17). Both oils exhibited α -amylase inhibitory activity, with IC₅₀ values of 499.10 $\pm$ 6.20 μ g/mL (LHM-EO) and 609.04 $\pm$ 14.72 μ g/mL (RHM-EO), respectively, which were lower than the reference compound, acarbose (IC₅₀ = 271.51 $\pm$ 13.51 μ g/mL). Unfortunately, both *H. malayanum* EOs were found to be inactive against α -glucosidase under the tested experimental conditions. To our best knowledge, this is the first report on the composition of rhizome EO and bioactivities of *H. malayanum* from Vietnam.

Keywords: *H. malayanum*., essential oil, chemical composition, enzyme inhibition, α -amylase.

1. Introduction

The genus *Hedychium* J. Koenig is one of the largest members in the Zingiberaceae, comprising more than 100 species.¹ These herbs are widely distributed throughout the tropical and subtropical regions of Asia, particularly in China, Nepal, Indonesia, and the Himalayan region.¹ Several species, such as *H. coronarium* and *H. spicatum*, have been used in traditional medicine in China, India, and Vietnam for centuries to treat various ailments, including inflammation, asthma, skin diseases, and digestive ailments.¹ Studies have demonstrated that the essential oils of

the genus *Hedychium* possess diverse biopharmacological properties, such as antimicrobial, antioxidant, anti-inflammatory, and cytotoxic activities.^{2–9} Furthermore, the essential oils of *Hedychium* species are also capable of inhibiting some key enzymes that are involved in the management and pathogenesis of various metabolic and physiological disorders.^{2,10} α -Amylase and α -glucosidase inhibitory activities of *Hedychium* species were also documented.^{11,12} In the context of modern healthcare, the search for natural enzyme inhibitors has gained momentum. α -Amylase and α -glucosidase inhibitors play a crucial role in managing postprandial hyperglycemia in type 2

diabetes by slowing down carbohydrate digestion.^{10,13} While some synthetic inhibitors exist, their use is often limited by side effects, prompting a shift toward safer, plant-derived alternatives.^{13,14}

Vietnam is recognized as a biodiversity hotspot for the Zingiberaceae family, harboring numerous endemic and rare species.^{15,16} Among them, *H. malayanum* Ridl. ex Burkill & Holttum (Figure 1) (syn. *Hedychium poilanei*,

Vietnamese name: Ngải tiên) remains a relatively understudied species. To date, most studies on this species have focused on its botanical description or crude solvent extracts, leaving the chemical composition of its essential oils and their specific enzymatic inhibitory potentials largely unexplored. In this work, we aim to investigate the chemical composition of the rhizome and leaf essential oils of *H. malayanum* collected in Bidoup-Núi Bà National



Figure 1. Photos of *Hedychium malayanum* Ridl. ex Burkill & Holttum

Park in Southern Vietnam as well as to evaluate their *in vitro* α -amylase and α -glucosidase inhibitory activities. The results are expected to lay a foundation for further studies on this herbal plant.

2. Experimental

2.1. Plant material

The fresh rhizomes and leaves of *Hedychium malayanum* were collected in Bidoup-Núi Bà National Park, Lam Dong province, Vietnam, in September 2024. The scientific name of this species was identified by MSc. Truong Quang Cuong, a botanist from in Bidoup-Núi Bà National Park. A voucher specimen (No. HC160-2024) was preserved in the Herbarium of Department of Chemistry, College of Education, Vinh University, Vietnam.

2.2. Extraction of the EOs

Fresh rhizomes and leaves of *H. malayanum* (500 g each) were separately hydrodistilled using a Clevenger-type apparatus for 4.5 h (until oil extraction was completed). The resulting essential oils were collected, dried over anhydrous sodium sulfate to remove residual moisture, and preserved in sealed vials at 4 °C prior to use. The oil yield was calculated as a percentage of the fresh plant mass using the formula: Yield (% v/w) = (V/m) $\times$ 100%, where 'V' is the volume of the extracted oil (mL) and 'm' is the weight of the fresh material (g). All extractions were performed in triplicate.

2.3. Gas chromatography-mass spectrometry (GC-MS) analysis

The chemical profile of the *H. malayanum* essential oils was characterized using an Agilent GC-MSD system equipped with an HP-5MS UI capillary column (30 m $\times$ 0.25 mm $\times$ 0.25 μ m film thickness). Helium served as the carrier gas at a constant flow of 1.0 mL/min. The oven temperature was initially held at 60 °C for 3 min, increased to 145 °C at a rate of 5 °C/min, and then increased to 250 °C at a rate of 5 °C/min, then maintained for a final 5 min. Injection (1.5 μ L, split ratio 40:1) was performed at 300 °C, while the MS source and quadrupole were set to 230 °C and 150 °C, respectively. Mass spectra were acquired in the m/z range of 45–500 at 70 eV. Compounds were identified by comparing their retention indices (RIs), determined via a C₇–C₃₀ n-alkane series, and mass spectra with the NIST 17 and Adams libraries.¹⁷ Relative concentrations were determined by peak area normalization.

2.4. α -Amylase inhibitory activity

The α -amylase inhibitory activity of the *H. malayanum* essential oils was determined following a modified

version of a previously reported method.¹⁸ Briefly, 10 μ L of α -amylase solution (0.14 U/mL) in the phosphate buffer was mixed with the diluted essential oil and incubated at 37 °C for 15 min. The enzymatic reaction was initiated by adding 0.25% starch solution and allowed to proceed for an additional 15 min at 37 °C. A blank was prepared following the same procedure but omitting the enzyme. The reaction was stopped by adding 50 μ L of 1 M HCl, after which 100 μ L of KI₃ solution was added to develop the color. The absorbance was recorded at 595 nm.

2.5. α -Glucosidase inhibitory activity

The α -glucosidase inhibitory potential was evaluated by mixing 50 μ L of the essential oil (diluted in DMSO) with 40 μ L of α -glucosidase (0.05 U) in phosphate buffer. After an initial 20-min incubation at 37 °C, the reaction was started by adding 40 μ L of 5 mM 4-nitrophenyl- β -D-glucopyranoside (p-NPG) and incubated for another 20 min at 37 °C. The reaction was quenched with 130 μ L of 0.2 M sodium carbonate (Na₂CO₃) solution, and the absorbance was measured at 405 nm. Acarbose was used as the positive control. The α -amylase inhibitory activity of the *H. malayanum* essential oils was determined following a modified version of a previously reported method.¹⁸

2.6. Molecular docking methodology

The three-dimensional structure of α -amylase was retrieved from the RCSB Protein Data Bank (PDB) under the accession code 5E0F. The protein structure was prepared for docking using AutoDock Tools (ADT) software by removing all water molecules and co-crystallized ligands. Polar hydrogen atoms were added, and Kollman charges were assigned. The prepared protein was then saved in PDBQT format for subsequent docking analysis. The major compounds identified in the essential oils of rhizome and leaf of *Hedychium poilane*y were selected based on GC–MS analysis. The chemical structures of these compounds were drawn using ChemSketch software and subsequently subjected to geometric optimization using Avogadro software with MMFF94s force field.¹⁹ The optimized ligand structures were converted to PDBQT format, with Gasteiger charges assigned and rotatable bonds defined. Molecular docking simulations were carried out using AutoDock Vina v1.2.7 to evaluate the binding affinity between the essential oil compounds and α -amylase enzyme.²⁰ The grid box was defined to cover the active site of the enzyme, with the grid center determined based on the position of the co-crystallized ligand in the 5E0F structure (x = –8, y = 6.1, z = –23 Å). The grid dimensions were set sufficiently large to allow flexible ligand movement within the binding pocket (X $\times$ Y $\times$ Z of 24 $\times$ 24 $\times$ 24). Docking calculations were performed using the default parameters of AutoDock Vina program. Docking results were ranked according to the binding free energy

(kcal/mol), and the ligand conformation with the lowest binding energy was selected for further analysis. Key interactions between the ligands and α -amylase, including hydrogen bonding, hydrophobic interactions, and π - π interactions, were analyzed and visualized using Discovery Studio Visualizer software.

3. Results and discussion

3.1. The yield and chemical compositions

The essential oils obtained from the hydrodistillation of fresh rhizomes and leaves of *H. malayanum* were pale yellow in color, providing yields of $0.08 \pm 0.02\%$ and

$0.05 \pm 0.01\%$ (v/w, calculated on a fresh weight basis), respectively.

GC-MS analysis was used to determine chemical compositions of the two essential oil samples (Figure S1, supporting information). The rhizome EO consisted of 34 compounds, representing 99.67 ± 0.90 of its total composition (Table 1), while the leaf EO contained 40 compounds, accounting for 99.09 ± 1.05 of its total content (Table 2). In the rhizome EO, the major constituents were 1,8-cineole (39.33 ± 0.34), β -pinene (23.36 ± 0.13), α -pinene (9.65 ± 0.03), and α -limonene (5.57 ± 0.06), while the leaf EO was dominated by β -pinene (57.8 ± 0.25), α -pinene (14.93 ± 0.13), and β -(E)-caryophyllene (9.43 ± 0.17). 1,8-cineole was rich in the rhizome EO but it was a minor component in the leaf

Table 1. Chemical constituents of *H. malayanum* rhizome EO from Vietnam

No.	RT (min)	RI (exp.)	RI (lit.)	Compounds	%
1	6.872	924	925	Tricyclene	0.27±0.00
2	7.055	929	929	α -Thujene	0.31±0.01
3	7.281	936	937	α -Pinene	9.65±0.03
4	7.794	951	952	Camphene	4.33±0.01
5	8.743	975	974	Sabinene	1.95±0.02
6	8.853	978	979	β -Pinene	23.36±0.13
7	9.451	992	991	β -Myrcene	0.78±0.01
8	9.949	1004	1005	α -Phellandrene	0.06±0.01
9	10.185	1010	1011	3-Carene	0.68±0.00
10	10.463	1017	1017	α -Terpinene	0.28±0.01
11	10.798	1026	1025	p-Cymene	1.16±0.01
12	10.974	1030	1030	α -Limonene	5.57±0.06
13	11.092	1033	1032	1,8-Cineole	39.33±0.34
14	12.282	1061	1060	γ -Terpinene	1.28±0.00
15	13.576	1088	1088	α -Terpinolene	0.27±0.01
16	13.818	1093	1092	2-Nonanone	0.06±0.00
17	14.148	1099	1099	β -Linalool	0.10±0.01
18	16.061	1145	1142	Camphor	0.14±0.01
19	17.026	1166	1166	Borneol	3.66±0.05
20	17.587	1177	1177	Terpinen-4-ol	0.68±0.01
21	18.2	1190	1189	α -Terpineol	0.71±0.01
22	18.452	1195	1193	Myrtenal	0.08±0.00
23	20.255	1237	1235	Methyl thymyl ether	0.06±0.01
24	22.504	1286	1285	Bornyl acetate	1.03±0.01
25	28.165	1417	1419	β -(E)-Caryophyllene	0.28±0.01
26	33.989	1566	1564	Nerolidol	0.05±0.00
27	34.424	1578	1576	Spathulenol	0.05±0.01
28	34.597	1582	1581	β -Caryophyllene epoxide	0.31±0.02
29	35.829	1619	1618	epi-Cedrol	0.60±0.03
30	36.18	1631	1631	γ -Eudesmole	0.15±0.02
31	36.337	1636	1635	Hinesol	0.22±0.03
32	36.594	1645	1645	Agarospinol	0.46±0.02
33	36.851	1654	1653	α -Eudesmol	1.56±0.03
34	37.375	1671	1672	Isolongifolol, methyl ether	0.19±0.02
Monoterpene hydrocarbons (No.1–12, 14, 15)					49.95±0.28
Oxygenated monoterpenes (No.13, 16–24)					45.84±0.43
Sesquiterpene hydrocarbons (No.25)					0.28±0.01
Oxygenated sesquiterpenes (No 26–34)					3.60±0.17
Total					99.67±0.90

RT = Retention time (min); RI (exp.) = Experimental retention indices (HP-5MS UI column); RI (lit.) = Literature retention indices; Bold values indicate main components; Data are presented as mean $\pm$ standard deviation.

EO. The leaf EO contained β -pinene in more than half and thus monoterpene hydrocarbons were the major compounds (80.28 ± 0.50). In contrast, both monoterpene hydrocarbons and oxygenated monoterpene hydrocarbons were found abundantly in the rhizome EO. This study provides the first chemical characterization of the *H. malayanum* rhizome EO.

These results are consistent to previous studies of other *Hedychium* essential oils extracted in Vietnam. The leaf EO of *H. Malayanum* from Malaysia (0.038% yield, 16 compounds) was dominated by β -pinene (39.1%), α -pinene (22.3%) and β -caryophyllene (13.7%), while the stem EO (0.009% yield, 7 compounds), was primarily composed of β -pinene (46.7%), 1,8-cineole (17.7%) and α -pinene

Table 2. Chemical constituents of *H. malayanum* leaf EO from Vietnam

No.	RT (min)	RI (exp.)	RI (lit.)	Compounds	%
1	6.877	924	925	Tricyclene	0.05 $\pm$ 0.00
2	7.055	929	929	α -Thujene	0.10 $\pm$ 0.00
3	7.286	936	937	α -Pinene	14.93$\pm$0.13
4	7.799	951	952	Camphene	0.69 $\pm$ 0.00
5	8.759	976	974	Sabinene	2.75 $\pm$ 0.05
6	8.9	979	979	β -Pinene	57.8$\pm$0.25
7	9.456	992	991	β -Myrcene	0.43 $\pm$ 0.01
8	10.19	1010	1011	3-Carene	0.05 $\pm$ 0.00
9	10.462	1017	1017	α -Terpinene	0.14 $\pm$ 0.00
10	10.793	1026	1025	p-Cymene	0.32 $\pm$ 0.01
11	10.966	1030	1030	α -Limonene	2.19 $\pm$ 0.03
12	11.065	1033	1032	1,8-Cineole	3.78 $\pm$ 0.06
13	11.422	1041	1038	β -(Z)-Ocimene	0.05 $\pm$ 0.01
14	11.867	1052	1049	β -(E)-Ocimene	0.11 $\pm$ 0.01
15	12.281	1061	1060	γ -Terpinene	0.55 $\pm$ 0.01
16	13.581	1088	1088	α -Terpinolene	0.13 $\pm$ 0.01
17	16.91	1163	1164	α -Pinocarvone	0.08 $\pm$ 0.01
18	17.036	1166	1166	Borneol	0.11 $\pm$ 0.01
19	17.592	1178	1177	Terpinen-4-ol	0.06 $\pm$ 0.01
20	18.436	1194	1193	Myrtenal	0.26 $\pm$ 0.01
21	22.509	1286	1285	Bornyl acetate	0.39 $\pm$ 0.01
22	28.175	1418	1419	β -(E)-Caryophyllene	9.43$\pm$0.17
23	29.564	1452	1454	α -Caryophyllene	0.43 $\pm$ 0.01
24	29.853	1459	1461	Alloaromadendrene	0.3 $\pm$ 0.01
25	30.702	1480	1481	Germacrene D	0.14 $\pm$ 0.00
26	30.885	1484	1486	β -Eudesmene	0.09 $\pm$ 0.03
27	31.326	1494	1495	β -Cyclogermacrene	0.02 $\pm$ 0.03
28	31.871	1508	1509	β -Bisabolene	0.12 $\pm$ 0.01
29	32.143	1516	1517	7-epi- α -Selinene	0.05 $\pm$ 0.00
30	32.427	1524	1524	δ -Cadinene	0.06 $\pm$ 0.01
31	33.994	1566	1564	Nerolidol	0.12 $\pm$ 0.01
32	34.35	1576	1576	Spathulenol	0.08 $\pm$ 0.01
33	34.597	1582	1581	β -Caryophyllene epoxide	1.03 $\pm$ 0.04
34	35.818	1619	1618	epi-Cedrol	0.21 $\pm$ 0.01
35	36.185	1631	1631	γ -Eudesmole	0.11 $\pm$ 0.01
36	36.342	1637	1635	Hinesol	0.20 $\pm$ 0.01
37	36.594	1645	1645	Agarospinol	0.35 $\pm$ 0.05
38	36.851	1654	1653	α -Eudesmol	1.01 $\pm$ 0.04
39	37.385	1672	1672	Isolongifolol, methyl ether	0.11 $\pm$ 0.01
40	39.267	1741	1738	Isolongifolol	0.06 $\pm$ 0.01
Monoterpene hydrocarbons (No. 1–11, 13–16)					80.28 $\pm$ 0.50
Oxygenated monoterpenes (No. 12, 17–21)					4.68 $\pm$ 0.09
Sesquiterpene hydrocarbons (No. 22–30)					10.83 $\pm$ 0.26
Oxygenated sesquiterpenes (No. 31–40)					3.3 $\pm$ 0.19
Total					99.09$\pm$1.05

RT = Retention time (min); RI (exp.) = Experimental retention indices (HP-5MS UI column); RI (lit.) = Literature retention indices; Bold values indicate main components; Data are presented as mean $\pm$ standard deviation.

(16.9%).²¹ Because Vietnam and Malaysia are Southwest Asia countries and methods for oil extraction are similar, the similar chemotype of EOs is reasonable. The rhizome EO of *H. yunnanense* (0.21% yield, 51 compounds) mainly contains β -pinene (21.07%), 1,8-cineole (20.63%), α -pinene (8.80%), and camphene (6.35%), while the leaf EO of this species (0.09% yield, 33 compounds) was rich in β -pinene (45.92%), α -pinene (19.48%), and β -caryophyllene (18.26%).²² In another report on chemical compositions of leaf and rhizome essential oils of *H. stenopetalum* Lodd (0.15% yield, 29 compounds for the leaf oil; 0.35% yield, 21 compounds for the rhizome oil) and *H. villosum* (0.14% yield, 27 compounds for the leaf oil; 0.22% yield, 31 compounds for the rhizome oil), the major components of both essential oils were α -pinene (8.5%–21.6%) and β -pinene (32.2%–52.2%). In addition, linalool (28.5%) and 1,8-cineole (10.7%) were identified in the rhizome EOs of *H. stenopetalum* and *H. villosum*, respectively.²³ In a study described by Thanh *et al.*, composition of essential oils of four *Hedychium* species from Vietnam was demonstrated. α -Pinene (52.5%) and β -pinene (31.8%) were characterized as main ingredients in the leaf oil of *H. stenopetalum* Lodd., while linalool (45.2%), (E)-nerolidol (8.7%) and α -pinene (5.0%) were found in high content in the root oil. The leaf of *H. coronarium* J. König was rich in β -pinene (20.0%), linalool (15.8%), 1,8-cineole (10.7%), α -pinene (10.1%) and α -terpineol (8.6%), while β -pinene (23.6%), α -humulene (17.1%) and β -caryophyllene (13.0%) were dominant in the root oil of this plant. *H. flavum* Roxb. leaf oil was characterized by β -pinene (22.5%), α -humulene (15.7%) and β -caryophyllene (10.4%), whilst α -humulene (18.9%), β -caryo-

phyllene (11.8%) and β -pinene (11.2%) were abundant in the stem oil, as well as β -pinene (21.8%), linalool (17.5%) and 1,8-cineole (13.5%) rich in the root oil. Additionally, *H. ellipticum* Buch.-Ham. ex Smith leaf oil was dominated by (E)-nerolidol (15.9%), β -pinene (11.8%) and bornyl acetate (9.2%), while the root oil was rich in 1,8-cineole (40.8%), α -pinene (18.3%) and β -pinene (11.0%).²⁴

3.2. *In vitro* α -amylase and α -glucosidase inhibition of the EOs

The bioassay results indicate that both essential oils exhibit α -amylase inhibitory activity in a concentration-dependent manner, as described in Table 3. At the lowest concentration (62.5 $\mu\text{g/mL}$), the inhibition levels of rhizome EO and leaf EO were 9.73 ± 1.33 and 1.45 ± 0.70 , respectively, representing a weak activity. When the concentration increased to 125 $\mu\text{g/mL}$ and 250 $\mu\text{g/mL}$, the inhibition capacity also climbed, with rhizome EO reaching 30.98 ± 2.17 and leaf EO reaching 33.78 ± 2.33 . In general, the rhizome EO showed good inhibition at high concentration. However, the α -amylase inhibitory activity of the rhizome and leaf EOs were weaker than the reference compound acarbose (IC_{50} of acarbose, rhizome, and leaf EOs were 271.51 ± 13.51 , 609.04 ± 14.72 , and 499.10 ± 6.20 $\mu\text{g/mL}$, respectively).

In addition, regarding α -glucosidase inhibition activity, rhizome EO exhibited no activity at tested concentration, as shown in Table 4. The observed inactivity in the α -glucosidase assay is likely due to the enzyme's highly specific active site, which may limit the binding of the various compounds present in the essential oils.

Table 3. α -Amylase inhibitory activity of the *H. malayanum* essential oils

Samples	Inhibition percentage*, %					IC_{50} ($\mu\text{g/mL}$)
	62.5	125	250	500	1000	
RHM-EO	9.73 ± 1.33	22.48 ± 1.60	30.98 ± 2.17	39.64 ± 2.48	76.64 ± 3.60	609.04 ± 14.72
LHM-EO	1.45 ± 0.70	17.02 ± 3.99	33.78 ± 2.33	53.97 ± 3.00	93.76 ± 3.27	499.10 ± 6.20
Acarbose	31.25	62.5	125	250	500	
	9.10 ± 2.07	17.81 ± 2.94	29.15 ± 4.17	56.42 ± 3.68	81.21 ± 2.29	271.51 ± 13.51

RHM-EO: Rhizome *H. malayanum* essential oil; LHM-EO: Leaf *H. malayanum* essential oil; *Inhibition percentage was measured at various concentrations of the essential oils (62.5–1000 $\mu\text{g/mL}$) and acarbose (31.25–500 $\mu\text{g/mL}$); Data are presented as mean $\pm$ standard deviation.

Table 4. α -Glucosidase inhibitory activity of the *H. malayanum* essential oils

Samples	Inhibition percentage*, %					IC_{50} ($\mu\text{g/mL}$)
	62.5	125	250	500	1000	
RHM-EO	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
LHM-EO	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Acarbose	31.25	62.5	125	250	500	
	6.77 ± 0.68	16.35 ± 3.12	33.23 ± 1.62	51.92 ± 1.43	69.03 ± 1.43	234.85 ± 13.70

RHM-EO: Rhizome *H. malayanum* essential oil; LHM-EO: Leaf *H. malayanum* essential oil; *Inhibition percentage was measured at various concentrations of the essential oils (62.5–1000 $\mu\text{g/mL}$) and acarbose (31.25–500 $\mu\text{g/mL}$); Data are presented as mean $\pm$ standard deviation.

To date, no studies have been conducted on the chemical composition and bioactivities of *H. malayanum* EOs. However, several species belonging to the *Hedychium* genus have been reported to exhibit anti-enzymatic activities. *H. puerense* rhizome essential oil showed a moderate inhibitory effect on α -glucosidase ($IC_{50} = 5.42 \pm 0.32$ mg/mL) and tyrosinase ($IC_{50} = 3.23 \pm 0.21$ mg/mL).² The author proposed that linalool, the most abundant component in this EO, might contribute a significant antidiabetic effect and enhanced insulin resistance. In enzyme inhibitory assay, *Hedychium flavum* leaf essential oil exhibited a potent inhibition on α -glucosidase ($IC_{50} = 1.03 \pm 0.02$ mg/mL).¹⁰ Terpinen-4-ol and α -terpineol, two major compounds in this EO, might be responsible for excellent inhibition of α -glucosidase in these EO. In *H. malayanum* EOs, linalool, terpinen-4-ol, and α -terpineol are minor components. This can explain why these EOs did not show inhibition of α -glucosidase. In addition, water and ethanol extracts of *H. coccineum* rhizome displayed significant α -glucosidase inhibitory activity with IC_{50} values of 22.87 ± 2.17 μ g/mL 5.41 ± 0.44 μ g/mL, respectively, superior to that of acarbose IC_{50} of 226.69 ± 16.14 μ g/mL.²⁵

3.3. Molecular docking results

The molecular docking analysis revealed that the major compounds from the essential oils of *H. malayanum* exhibited negative binding energies toward the α -amylase enzyme (PDB ID: 5E0F) indicating favorable binding interactions, with affinities ranging from -5.51 to -7.08 kcal/mol (Table 5). The robustness of the docking protocol was confirmed through prior validation, where re-docking of the co-crystallized ligand yielded an RMSD of 1.873 Å, indicating high reliability of the predicted binding poses.²⁶ Among the tested compounds, β -(E)-caryophyllene showed the strongest binding affinity (-7.078 kcal/mol), suggesting a relatively higher inhibitory potential compared to the other terpenoid constituents. This compound primarily interacted with key hydrophobic residues within

the active site, including Trp59, Trp58, Tyr62, and Leu162, through π -alkyl interactions, which are known to contribute significantly to ligand stabilization in hydrophobic binding pockets of α -amylase enzyme (Figure S2, Supporting information). Monoterpenes such as α -pinene, β -pinene, α -limonene, and 1,8-cineole demonstrated comparable binding affinities, ranging from -5.51 to -5.83 kcal/mol. These compounds predominantly formed π -alkyl and π -sigma interactions with amino acid residues such as Tyr62, Leu165, Leu162, His101, and His299, indicating that hydrophobic interactions play a major role in their binding to α -amylase (Figure S2, Supporting information). Notably, Tyr62 appeared repeatedly in the interaction profiles of all docked ligands, highlighting its importance in ligand recognition within the enzyme's binding pocket.

In comparison with the reference compound, several key amino acid residues, namely Tyr62, Leu162, Leu165, and His299, were also observed to participate in ligand binding. This overlap in interacting residues suggests that the essential oil constituents and the reference inhibitor occupy a similar binding region within the α -amylase active site. These findings are consistent with previous reports on essential oils from *K. globularia*, *P. scandens*, *S. chinensis*, and *P. tabulaeformis*, where sesquiterpenes such as (E)- β -caryophyllene, caryophyllene oxide, longifolene, and spathulenol exhibit notable binding affinity toward α -amylase and contribute to the observed *in vitro* inhibitory activity.^{26,27} The present docking results suggest that the α -amylase inhibitory potential of *H. malayanum* essential oil is likely associated with the combined action of multiple terpenoid constituents, particularly hydrophobic sesquiterpenes.

4. Conclusion

In this study, the chemical composition and bioactivities of essential oil from the rhizome and leaf of *H. ma-*

Table 5. Binding affinities and interacting amino acid residues of *H. malayanum* rhizome and leaf essential oil constituents with α -amylase (PDB ID: 5E0F).

No.	Compounds	Binding affinity (kcal/mol)	Interacting amino acid residues
1	α -Pinene	-5.614	π -sigma: Tyr62 π -alkyl: Trp58, His305, Leu165, His101, Leu162
2	β -Pinene	-5.51	π -sigma: Tyr62 π -alkyl: His299, Leu162, Leu165
3	α -Limonene	-5.76	π -alkyl: Leu165, Trp59, Tyr62, His299
4	1,8-Cineole	-5.825	π -alkyl: Tyr62, Leu165, Leu162, His101
5	β -(E)-Caryophyllene	-7.078	π -alkyl: Trp59, Trp58, Tyr62, Leu162
6	Reference compound	-10.57	Conventional hydrogen bond: Glu233, Asp300, Tyr151, His201, Asp197 π -ion: Asp300 π -donor hydrogen bond: His299 π - π T-shaped: Tyr62 π -alkyl: Ile235, Leu162, Leu165

layanum are demonstrated. GC-MS analysis identified 1,8-cineole (39.33 ± 0.34), β -pinene (23.36 ± 0.13), α -pinene (9.65 ± 0.03), and α -limonene (5.57 ± 0.06) as the major compounds in the rhizome oil. The leaf oil was rich in β -pinene (57.8 ± 0.25), α -pinene (14.93 ± 0.13), and β -(*E*)-caryophyllene (9.43 ± 0.17). Both the rhizome and leaf EOs demonstrated α -amylase inhibitory activity, with IC_{50} values of 609.04 ± 14.72 μ g/mL and 499.10 ± 6.20 μ g/mL, respectively, which were lower than the reference compound, acarbose ($IC_{50} = 271.50$ μ g/mL). However, these EOs were inactive with α -glucosidase. This is the first time, a study on composition of rhizome oil and bioactivities of EOs from *H. malayanum* is presented. Chemical composition of nonvolatile compounds is underway in our lab and will be reported in due course.

Supplementary material:

Supporting material is available.

Conflict of interests:

We confirm that there is no conflict of interests for this article.

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Povzetek

V tej študiji so predstavljene sestava eteričnih olj (EO) iz koreninskih poganjkov in listov rastline *H. malayanum*, zbranih v narodnem parku Bidoup-Núi Bà v južnem Vietnamu, ter njihova aktivnost α -amilaze in α -glukozidaze. Analiza s plinsko kromatografijo sklopljena z masno spektrometrijo (GC-MS) je identificirala 34 spojin (99,54 %) v EO korenine in 40 spojin (99,13 %) v EO listov. Glavne sestavine EO korenine so vključevale 1,8-cineol ($39,33 \pm 0,34$), β -pinen ($23,36 \pm 0,13$), α -pinen ($9,65 \pm 0,03$), in α -limonen ($5,57 \pm 0,06$), medtem ko je eterično olje listov bogato z β -pinenom ($57,8 \pm 0,25$), α -pinenom ($14,93 \pm 0,13$) in β -(E)-kariofilenom ($9,43 \pm 0,17$). Obe olji sta pokazali zaviralno aktivnost proti α -amilazi, z vrednostmi IC_{50} $499,10 \pm 6,20$ $\mu\text{g/mL}$ (LHM-EO) oziroma $609,04 \pm 14,72$ $\mu\text{g/mL}$ (RHM-EO), ki so bile nižje od referenčne spojine, akarbose ($IC_{50} = 271,51 \pm 13,51$ $\mu\text{g/mL}$). Na žalost se je izkazalo, da sta bila oba eterična olja *H. malayanum* v testiranih eksperimentalnih pogojih neaktivna proti α -glukozidazi. Po našem najboljšem vedenju je to prvo poročilo o sestavi eteričnega olja iz korenine in biološki aktivnosti *H. malayanum* iz Vietnama.



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