

# Essential Oils of Two Species of Zingiberaceae Family from Vietnam: Chemical Compositions and $\alpha$ -Glucosidase, $\alpha$ -Amylase Inhibitory Effects

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## Abstract

The ginger family (Zingiberaceae) is one of the most diverse and abundant families in the plant kingdom in terms of number of species. In terms of chemistry, this is a family of plants containing much essential oil with mainly monoterpene and sesquiterpene derivatives, which has been widely used in medicine, food, and flavor industries. In our series of studies on ginger family plants, the two new species including *Wurfbainia schmidtii* (K. Schum.) Škorničk. & A.D. Poulsen and *Zingiber atroporphyreus* Škorničk. & Q.B. Nguyen has been investigated for the first time. This study aimed to identify the chemical compositions of essential oils extracted from the rhizomes and leaves of these two species using gas chromatography-mass spectrometry (GC/MS) analysis and evaluate their hypoglycemic effects through the  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory assays. In terms of chemical composition, in general, the main compounds appearing in the essential oils of both leaves and rhizomes of each species are similar but they are differences in the percentage of the main components, for example, 1,8-cineole is a main component in leaves whereas fenchyl acetate is the main one in rhizomes of *W. schmidtii*. Several distinctive compounds, in comparison with other members in the genus *Zingiber*, namely  $\beta$ -pinene,  $\alpha$ -pinene,  $\beta$ -clemene, and sabinene, were found in the essential oil of *Z. atroporphyreus*. Regarding hypoglycemic effects, the essential oils from the two species tested possessed weak  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory effects. For the first time, this study was designed to investigate the chemical composition as well as the hypoglycemic effect of essential oils distilled from the rhizomes and leaves of two species *W. schmidtii* and *Z. atroporphyreus* have been reported, serving as a premise for further systematic research on their phytochemistry and pharmacological effects being conducted.

## Keywords

*Wurfbainia schmidtii*, *Zingiber atroporphyreus*, essential oil, GC/MS,  $\alpha$ -glucosidase,  $\alpha$ -amylase

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## Introduction

The Zingiberaceae family, the largest family in the order Zingiberales, is a monocotyledon-bearing aromatic and medicinal family with roughly 52 genera and 1300 species that may be found in the tropics from Malaysia to India.<sup>1,2</sup> The native Zingiberaceae species were important traditional medicinal plants as well as providing sources of spices and food for ethnic groups in many countries around the world, such as China, India, Thailand, Laos, and Vietnam.<sup>2,3</sup> Several species of the Zingiberaceae family (eg, *Alpinia galanga*, *Curcuma longa*, *Zingiber officinale*, *Hedychium coronarium*, *Kaempferia galanga*, etc) were well-known for their effects in treating various diseases, such as digestive disorders, hypertension, diabetes mellitus (DM), infection, inflammation, antioxidant, anti-microbial, anti-cancer, antiparasitic, analgesic, hepatoprotective activities, etc, as

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reported in previous studies.<sup>3,4</sup> In terms of chemical composition profiles, some species (eg, *A. galanga*, *C. longa*, *Curcuma thorbailii*, *Curcuma rhabdota*, *Curcuma petiolata*, *Distichochlamys citrea*, *Z. officinale*, *H. coronarium*, *K. galanga*, etc) were previously reported to be rich in essential oils. Moreover, various chemical components, such as flavonoids, tannins, resins, phenols, volatile fatty acids, etc, were isolated from their different parts.<sup>4-7</sup> Essential oils (EOs) are volatile substances with low molecular weight and various biological activities that have been known.<sup>8</sup> As a source of natural chemicals for treating illnesses including diabetes, anti-cancer, hepatoprotective, antioxidant, and antimicrobial activities,<sup>4</sup> EOs are currently gaining popularity as a natural substitute in the health industry. EOs are therefore extremely important in the pharmaceutical, food, agricultural, cosmetic, and health industries.<sup>9,10</sup>

In the process of finding new medicinal sources from the Zingiberaceae family, two aromatic species named *Wurfbainia schmidtii* (K.Schum.) Škorničk. & A.D.Poulsen and *Zingiber atroporphyreus* Škorničk. & Q.B. Nguyễn were studied. The genus *Wurfbainia* Giseke is widely distributed from the Himalayas to South China and Western and Central Malesia,<sup>11</sup> especially among them, *W. schmidtii* is the native range of Indo-China, that is, Cambodia, Laos, Thailand, and Vietnam. It is a rhizomatous geophyte and grows primarily in the wet tropical biome.<sup>12</sup> Meanwhile, the genus *Zingiber* Mill is native to tropical and subtropical Asia and includes 208 accepted species.<sup>12</sup> In 2015, *Z. atroporphyreus*, a new *Zingiber* species from Vietnam, was reported by Leong-Skornickova and colleagues.<sup>13</sup> In the literature, all species in these two genera are utilized extensively for medicine, food, and ornamental purposes. Specifically, *W. schmidtii* was used for tonic, carminative, and stomachic properties and for treating the symptoms of dizziness and digestive disorders. Moreover, it is also used for herbal soap and spas due to its very attractive aromatic smell. Additionally, the young leaves were used for food, as previously documented.<sup>3,11</sup> However, until now, there have been no studies that have noted the chemical composition or pharmacological effects of *Z. atroporphyreus*.

Currently, DM, a chronic disease condition characterized by unusually high blood glucose levels, is brought on by an endocrine metabolic imbalance in the body.<sup>14</sup> It is expected that by 2045, the International Diabetes Federation (IDF) will have estimated globally about 12.2% (783.2 million) of the 20-79 age group with diabetes, and especially, global diabetes-related health spending is estimated at \$1.054 billion. Additionally, IDF estimates that diabetes prevalence is expected to increase in middle-income, high-income, and low-income countries by 21.1%, 12.2%, and 11.9%, respectively.<sup>15</sup> In traditional medical systems, numerous varieties of herbs have been used to treat diabetes for a very long period. These herbs are thought to have a high economic benefit and low adverse effects when used to treat diabetes. Therefore, natural medicinal sources that support and treat diabetes are increasingly receiving attention from scientists. Among herbal sources, the members of Zingiberaceae family are also used to support and treat

diabetes, such as *Aframomum melegueta*, *C. longa*, *D. citrea* (Black Ginger), and *Z. officinale*.<sup>4,14</sup> Therefore, natural medicinal sources that support and treat diabetes are increasingly receiving attention from scientists. The objective of the present study, EOs from two plant species (*W. schmidtii* and *Z. atroporphyreus*) were evaluated for their chemical profile and inhibitory activities against carbohydrate-hydrolyzing enzymes, namely  $\alpha$ -glucosidase and  $\alpha$ -amylase. The findings of this study will provide information about the antidiabetic potential of two plant species collected in Vietnam.

## Results and Discussion

### *Chemical Components of Essential Oils*

The GC/MS analysis (Supplemental Figures 1-4) provided information on the chemical components of the EOs from the leaves and rhizomes of *W. schmidtii* and *Z. atroporphyreus*, the results of which are presented in Table 1.

As for *W. schmidtii* EOs, the most dominant components of the leaf EO were oxygenated monoterpenes (44.1%), followed by monoterpene hydrocarbon (22.2%), while the rhizome EO contained mostly monoterpene hydrocarbons (41.1%) and oxygenated monoterpenes (29.3%). Specifically, the major components of *W. schmidtii* leaf EO were 1,8-cineol (18.5%), *endo*-borneol (14.3%), camphene (10.8%), and 2-bornanone (7.6%), while the rhizome EO contained mostly fenchyl acetate (15.8%) camphene (15.0%),  $\beta$ -pinene (8.7%), isobornyl acetate (6.1%),  $\alpha$ -pinene (5.2%), and spathulenol (5.1%). Noticeably, camphene,  $\alpha$ -pinene, and spathulenol can be found in both leaf and rhizome EOs of *W. schmidtii* with significant contents (5%-10%). The GC/MS results also showed the resemblance between *Z. atroporphyreus* leaf and rhizome EOs in terms of chemical composition. To be more specific, the major chemical components of the leaf and rhizome EOs were  $\beta$ -pinene (40.6% and 48.0%),  $\alpha$ -pinene (12.0% and 14.7%),  $\beta$ -elemene (11.7% and 4.4%), and sabinene (6.1% and 3.8%), respectively.

The main chemical compositions of EOs of *Zingiber* species were  $\alpha$ -zingiberene, ar-curcumen, geranial, sabinene, and camphene. Interestingly, the EOs of *Z. atroporphyreus* expressed the distinction, in comparison to those of other *Zingiber* species, with  $\beta$ -pinene,  $\alpha$ -pinene,  $\beta$ -elemene, and sabinene as the most dominant components.<sup>17</sup> As for the *Wurfbainia* genus, there has been some information on the chemical composition of the EOs of these species. According to a chemical review of the essential oils prepared from several species of the *Amomum* genus, which are synonyms with several species of the *Wurfbainia* genus, there were several differences in the chemical compositions.<sup>18</sup> Specifically,  $\alpha$ -pinene (10.4%-31.3%) and  $\beta$ -pinene (29.9%-58.5%) were the major compounds of *Wurfbainia uliginosa* (syn: *Amomum uliginosum*) rhizomes and *Wurfbainia villosa* (syn: *Amomum villosum*) leaves while  $\beta$ -caryophyllene (26.6%-37.4%) and  $\alpha$ -humulene (12.5%-16.5%) were the most dominant in the EOs of

**Table 1.** Chemical Components of Essential Oils from Leaves and Rhizomes of *W. schmidtii* and *Z. atroporphyreus*.

No.	RT (min)	Compounds	RI <sup>a</sup>	RI <sup>b</sup>	<i>W. schmidtii</i>		<i>Z. atroporphyreus</i>	
					Leaf EO	Rhizome EO	Leaf EO	Rhizome EO
1	5.296	Tricyclene	928	925	0.2	0.5	0.1	0.2
2	5.382	$\alpha$ -Thujene	932	929	0.1	0.2	0.2	0.3
3	5.559	$\alpha$ -Pinene	940	937	4.2	<b>5.2</b>	<b>12.0</b>	<b>14.7</b>
4	5.925	Camphene	955	952	<b>10.8</b>	<b>15.0</b>	1.7	4.4
5	6.520	Sabinene	978	974	0.3	0.1	<b>6.1</b>	3.8
6	6.618	$\beta$ -Pinene	982	979	2.1	<b>8.7</b>	<b>40.6</b>	<b>48.0</b>
7	6.944	$\beta$ -Myrcene	993	991	0.2	0.7	1.5	1.8
8	7.339	$\alpha$ -Phellandrene	1007	1005	0.1	0.1	0.2	0.1
9	7.505	3-Carene	1014	1011	-	-	0.8	0.5
10	7.671	$\alpha$ -Terpinene	1020	1017	-	0.1	0.1	0.4
11	7.917	<i>p</i> -Cymene	1029	1025	0.3	3.5	0.1	0.3
12	8.043	Limonene	1034	1030	3.5	4.7	3.1	3.5
13	8.151	1,8-Cineol	1038	1032	<b>18.5</b>	3.3	0.3	0.4
14	8.266	( <i>Z</i> )- $\beta$ -Ocimene	1042	1038	-	0.2	0.1	0.1
15	8.586	( <i>E</i> )- $\beta$ -Ocimene	1052	1049	0.1	0.2	0.7	0.4
16	8.924	$\gamma$ -Terpinene	1063	1060	0.2	1.6	0.2	0.7
17	9.845	Terpinolene	1091	1088	0.1	0.3	0.1	0.6
18	10.183	Linalool	1100	1099	0.8	0.1	0.1	-
19	10.629	Fenchol	1116	1113	-	0.4	-	0.2
20	10.909	<i>cis</i> -2- <i>p</i> -Menthen-1-ol	1126	1122	0.1	-	-	-
21	11.470	<i>trans</i> -Pinocarveol	1144	1139	-	-	-	0.1
22	11.682	2-Bornanone	1150	1143	<b>7.6</b>	0.5	-	-
23	12.059	Isoborneol	1162	1157	1.7	0.5	-	-
24	12.254	Pinocarvone	1167	1164	-	-	-	0.1
25	12.408	<i>endo</i> -Borneol	1172	1167	<b>14.3</b>	0.8	0.3	0.3
26	12.735	Terpinen-4-ol	1181	1177	0.4	0.2	-	0.3
27	13.164	$\alpha$ -Terpineol	1193	1189	0.7	0.1	-	0.3
28	13.364	Myrtenal	1198	1193	-	-	0.1	0.2
29	14.177	Fenchyl acetate	1225	1223	-	<b>15.8</b>	-	-
30	15.298	Linalyl acetate	1259	1257	-	0.2	-	-
31	15.784	( <i>E</i> )-Citral	1274	1270	-	-	0.1	-
32	16.362	Isobornyl acetate	1290	1286	-	<b>6.1</b>	0.1	0.3
33	16.683	<i>trans</i> -Pinocarvyl acetate	1299	1297	-	-	0.1	-
34	16.769	Methyl myrtenate	1301	1301	-	-	0.1	-
35	17.593	Myrtenyl acetate	1329	1327	-	-	0.2	-
36	17.982	$\delta$ -Elemene	1341	1338	1.5	0.7	0.4	0.1
37	18.354	$\alpha$ -Terpinyl acetate	1353	1350	-	1.3	-	0.1
38	19.218	$\alpha$ -Copaene	1379	1376	-	0.2	0.9	0.4
39	19.441	Geranyl acetate	1386	1382	-	-	1.1	-
40	19.750	$\beta$ -Elemene	1395	1391	4.2	3.0	<b>11.7</b>	4.4
41	20.448	<i>cis</i> - $\alpha$ -Bergamotene	1418	1415	-	-	0.2	0.1
42	20.608	Caryophyllene	1423	1419	2.3	1.4	3.5	1.7
43	21.020	$\gamma$ -Elemene	1437	1433	-	-	0.4	0.2
44	21.077	$\alpha$ -Bergamotene	1439	1435	-	-	-	0.2
45	21.335	Selina-5,11-diene	1448	1447	-	0.1	0.1	0.1
46	21.444	<i>Epi</i> - $\beta$ -Santalene	1451	1448	-	-	0.1	0.2
47	21.655	Humulene	1458	1454	0.3	0.8	0.2	-
48	21.718	( <i>E</i> )- $\beta$ -Famesene	1460	1457	-	-	0.3	0.4
49	21.878	Alloaromadendrene	1465	1461	0.1	0.3	0.2	0.1
50	22.308	$\gamma$ -Selinene	1479	1481	-	0.2	0.3	0.2
51	22.508	$\alpha$ -Curcumene	1485	1483	-	-	1.1	-
52	22.611	Aristolochene	1488	1487	1.6	1.6	1.0	2.7
53	22.662	$\beta$ -Eudesmene	1490	1486	2.2	1.0	-	-
54	22.903	$\alpha$ -Zingiberene	1497	1495	-	-	4.0	2.1
55	22.971	$\beta$ -Bicyclgermacrene	1499	1495	3.6	2.1	-	-

(Continued)

Table 1. Continued

No.	RT (min)	Compounds	RI <sup>a</sup>	RI <sup>b</sup>	<i>W. schmidtii</i>		<i>Z. atroporphyreus</i>	
					Leaf EO	Rhizome EO	Leaf EO	Rhizome EO
56	23.292	<i>β</i> -Bisabolene	1511	1509	-	-	0.8	0.3
57	23.589	7- <i>epi</i> - <i>α</i> -Selinene	1521	1517	-	0.2	0.2	0.2
58	23.749	<i>δ</i> -Cadinene	1527	1524	-	-	1.5	0.4
59	23.784	<i>α</i> -Panasinsen	1528	1527	0.7	-	-	-
60	23.990	( <i>E</i> )- <i>γ</i> -Bisabolene	1536	1533	-	-	0.1	-
61	24.511	Elemol	1554	1549	0.1	0.1	-	-
62	24.905	Nerolidol	1567	1564	-	0.1	-	-
63	25.352	Spathulenol	1582	1576	4.1	<b>5.1</b>	-	-
64	25.518	Caryophyllene oxide	1587	1581	-	0.6	-	0.4
65	25.912	Guaiol	1600	1596	-	-	0.1	0.1
66	26.262	Humulene epoxide II	1613	1606	-	0.5	-	-
67	26.942	Longifolenaldehyde	1638	1631	0.4	-	-	-
68	27.057	Isospathulenol	1642	1638	-	0.4	-	-
69	27.400	<i>β</i> -Eudesmol	1655	1549	0.4	-	-	0.1
70	27.520	Pogostole	1659	1660	1.3	1.5	0.4	-
71	27.635	Intermedeol	1663	1667	-	1.1	-	0.6
72	28.333	<i>β</i> -Costal	1688	1691	1.8	1.0	-	-
73	28.436	<i>α</i> -Costal	1691	1695	1.4	0.8	-	-
74	31.005	Methyl isocostate	1788	1792	0.1	0.1	-	-
75	31.406	Ambrial	1803	1809	-	0.6	-	-
76	32.218	Dehydrosaussurea lactone	1836	1838	0.2	-	-	-
Total					92.6	93.9	97.6	97.1
Monoterpene hydrocarbons (No. 1-12, 14-17)					22.2	41.1	67.6	79.8
Oxygenated monoterpenes (No. 13, 18-35, 37, 39)					44.1	29.3	2.5	2.3
Sesquiterpene hydrocarbons (No. 36, 38, 40-60)					16.5	11.6	27	13.8
Oxygenated sesquiterpenes (No. 61-76)					9.8	11.9	0.5	1.2

Abbreviations: RT (min), retention time (min); RI<sup>a</sup>, retention indices relative to n-alkanes (C<sub>8</sub>-C<sub>30</sub>) on HP-5MS UI column; RI<sup>b</sup>, retention indices from NIST17 and Adams' book.<sup>16</sup>

Major components are given in boldface type.

Table 2. Inhibitory Effects of the Essential Oils and Acarbose on *α*-Amylase.

Samples	Percentage inhibition <sup>a</sup> , %					
	0.25 mg/mL	0.5 mg/mL	1.0 mg/mL	2.0 mg/mL	4.0 mg/mL	IC <sub>50</sub> (mg/mL)
Leaf EO of <i>W. schmidtii</i>	6.50 ± 0.62	7.50 ± 0.43	19.89 ± 0.65	31.12 ± 0.44	50.14 ± 0.22	3.83 ± 0.04
Rhizome EO of <i>W. schmidtii</i>	n.i.	5.78 ± 1.42	16.07 ± 1.27	57.01 ± 0.69	70.74 ± 1.30	1.85 ± 0.07
Leaf EO of <i>Z. atroporphyreus</i>	40.88 ± 1.77	58.22 ± 1.62	84.11 ± 1.43	89.32 ± 0.96	>99	0.39 ± 0.03
Rhizome EO of <i>Z. atroporphyreus</i>	43.52 ± 2.75	68.79 ± 1.51	83.46 ± 1.78	88.11 ± 0.84	96.41 ± 1.63	0.30 ± 0.03
Acarbose	n.a.	n.a.	n.a.	n.a.	n.a.	0.09 ± 0.00

Abbreviations: n.i., no inhibition; n.a., not available.

<sup>a</sup>The percentage inhibition was calculated at the different EO concentrations (0.25-4.0 mg/mL). Data are shown as mean ± standard deviation (n = 3).

*Wurfbainia longiligularis* (syn: *Amomum longiligulare*) leaves, stems, and roots. The fruit EO of *Wurfbainia villosa* was found to contain camphor (22.3%-37.9%), borneol acetate (15.5%-30.5%), and camphene (6.7%-7.6%). Some studies on *Wurfbainia vera* (syn: *Amomum kravanh*) fruit revealed that 1,8-cineole was present in the EO with the highest content (34.0%-78.0%). The present study revealed that the chemical compositions of *W. schmidtii* EOs were similar to that of *W. vera*, *W. aromatica*, *Amomum subulatum*, and *Amomum tsao-ko* with 1,8-cineole as the most major compound.<sup>18</sup>

### *In Vitro α-Glucosidase and α-Amylase Inhibitory Effects*

The EOs from *W. schmidtii* and *Z. atroporphyreus* leaves and rhizomes were tested for their ability to inhibit *α*-amylase, and the results are shown in Table 2 and Supplemental Table 1. At concentrations ranging between 0.25 and 1.0 mg/mL, the EO from *W. schmidtii* leaves appeared to be slightly better at inhibiting the enzyme compared to the EO from its rhizomes. However, the effect is not concentration-dependent manner with the leaf EO no longer exerting stronger inhibitory activity than the rhizome

**Table 3.** Inhibitory Effects of the Essential Oils and Acarbose on  $\alpha$ -Glucosidase.

Samples	Percentage inhibition <sup>a</sup> , %					IC <sub>50</sub> (mg/mL)
	0.25 mg/mL	0.5 mg/mL	1.0 mg/mL	2.0 mg/mL	4.0 mg/mL	
Leaf EO of <i>W. schmidtii</i>	5.07 ± 0.93	9.10 ± 2.85	8.82 ± 3.05	3.81 ± 0.22	n.i.	n.a.
Rhizome EO of <i>W. schmidtii</i>	2.11 ± 1.48	8.18 ± 1.94	4.43 ± 1.90	n.i.	n.i.	n.a.
Leaf EO of <i>Z. atroporphyreus</i>	n.i.	n.i.	n.i.	n.i.	n.i.	n.a.
Rhizome EO of <i>Z. atroporphyreus</i>	n.i.	n.i.	n.i.	n.i.	n.i.	n.a.
Acarbose	n.a.	n.a.	n.a.	n.a.	n.a.	0.07 ± 0.00

Abbreviations: n.i., no inhibition; n.a., not available.

<sup>a</sup>The percentage inhibition was calculated at the different EO concentrations (0.25-4.0 mg/mL). Data are shown as mean ± standard deviation (n = 3).

EO. In detail, the former inhibited  $\alpha$ -amylase by 31.12% on average while the latter inhibited the enzyme by 57.01% at the concentration of 2.0 mg/mL. Similarly, the inhibitory effect of the rhizome EO was estimated to be about 40% higher than that of its leaf counterpart at a concentration of 4 mg/mL. As a result, the IC<sub>50</sub> value of rhizome EO (1.85 ± 0.07 mg/mL) was significantly lower than that of the leaf EO (3.83 ± 0.04 mg/mL), signifying its stronger inhibitory activity against  $\alpha$ -amylase. The results also demonstrated comparable inhibition percentages between the two EOs from *Z. atroporphyreus* across the monitored concentration range. As presented in Table 2, no significant difference in the IC<sub>50</sub> values of the two EOs was noted. In comparison with the *W. schmidtii* EOs, the *Z. atroporphyreus* EOs had much lower IC<sub>50</sub> values and accordingly exhibited higher inhibitory effects against the enzyme. In general, all the examined EO samples may possess a weaker anti- $\alpha$ -amylase activity compared to acarbose (IC<sub>50</sub> = 0.09 ± 0.00 mg/mL).

Previous studies have documented the inhibitory potential of *Zingiber* species against  $\alpha$ -amylase, but there is currently no existing literature that provides data on this specific activity for EOs from the *Wurfbainia* genus. For example, the EO from *Z. officinale* was shown to inhibit  $\alpha$ -amylase.<sup>19</sup> Several plant species within the Zingiberaceae family have been investigated for their potential in this bioactivity. For example, the EO obtained from both fresh and dried rhizomes of *Curcuma longa* demonstrated a significantly lower IC<sub>50</sub> value compared to acarbose, a well-known  $\alpha$ -amylase inhibitor.<sup>20</sup> Additionally, a recent study demonstrates EO of *Curcuma caesia* rhizomes exhibits profound  $\alpha$ -amylase inhibitory activity.<sup>21</sup> Reports also indicate that the EOs extracted from the seeds of *A. melegueta* and *A. danielli* exerted  $\alpha$ -amylase inhibition, although their efficacy was not as potent as acarbose.<sup>20</sup> The inhibitory effect of the EOs from *W. schmidtii* may be due to the presence of monoterpenes in their chemical composition. In our study, the major constituents identified in the *W. schmidtii* EOs were pinenes and camphene. Previous research has suggested that these compounds could potentially contribute to inhibitory effects against  $\alpha$ -amylase.<sup>20,22</sup> The anti- $\alpha$ -amylase activity observed in this species could also be due to synergistic or additive effects of the various constituents present in the EOs.

As for  $\alpha$ -glucosidase inhibitory activity of the EOs, the *Z. atroporphyreus* EOs showed no inhibition in the tested

concentrations while the *W. schmidtii* EOs exhibited very weak inhibition against  $\alpha$ -glucosidase (Table 3). According to the findings of our study, if the EOs had an inhibitory effect on  $\alpha$ -amylase but not  $\alpha$ -glucosidase, they would have impacts on the early stages of carbohydrate digestion, specifically the breakdown of starch. However, it would have limited effects on the later stages, where glucose is released from disaccharides and oligosaccharides. This selective inhibition could potentially lead to a slower and more controlled release of glucose from foods rich in starch. Such an effect could be valuable in the development of natural-based medications for managing type 2 diabetes. In a previous study, the flower EO of *Hornstedtia scyphifera* showed potential  $\alpha$ -glucosidase inhibitory activity with the IC<sub>50</sub> value of 21.44  $\mu$ g/mL.<sup>23</sup> The leaf EO of *Alpinia vietnamica* was reported to the  $\alpha$ -glucosidase inhibitory assay with the IC<sub>50</sub> value of 115.18 ± 4.87  $\mu$ g/mL.<sup>24</sup>

## Conclusion

This study presents the first report on the volatile compositions and carbohydrate-hydrolyzing enzyme inhibitory effects of the EOs from *W. schmidtii* and *Z. atroporphyreus*. The findings indicated that oxygenated monoterpenes were the most abundant chemical class in the leaf EO while monoterpene hydrocarbons predominated in the rhizome EO of *W. schmidtii*. As for *Z. atroporphyreus*, monoterpene hydrocarbons accounted for the highest percentages in its EO contents. The in vitro bioassay results showed the *Z. atroporphyreus* EOs exhibited higher inhibitory activity against  $\alpha$ -amylase compared to the *W. schmidtii* EOs. However, no  $\alpha$ -glucosidase inhibition was observed for any of the EOs. These findings imply potential industrial applications for these EOs in the food and pharmaceutical sectors. Future investigations should focus on the isolation and characterization of non-volatile compounds from these plant species and assess their bioactivities.

## Materials and Methods

### Plant Materials

The fresh leaves and rhizomes of *W. schmidtii* were collected from Dinh Mountain, Tan Thanh District, Ba Ria-Vung Tau Province, Vietnam in July-2022, while the fresh leaves and



rhizomes of *Z. atropurpureus* were collected from Xuan Son National Park, Tan Son District, Phu Tho Province, Vietnam in September-2022. The plants were identified by Assoc. Prof. Dr Nguyen Hoang Tuan. Voucher specimens HCWS-028 and HCZA-038, respectively, were deposited at the Laboratory of the Department of Chemistry, Vinh University, Nghe An Province, Vietnam.

### Extraction of the Essential Oils

The EOs were obtained by hydrodistillation which was carried out in a Clevenger-type distillation as previously described with slight modifications.<sup>7,25</sup> The experiment was repeated three times and carried out until no further EO could be distilled (approximately 3.5 h each). The leaf and rhizome EOs were treated with anhydrous sodium sulfate to remove the traces of water and stored in sealed vials in the dark at 4°C for further analysis.

### GC/MS Analysis

GC/MS analysis was performed using an Agilent Technologies 7890B GC system equipped with an HP-5MS UI column (30 m × 0.25 mm, 0.25 μm film thickness) and coupled to an Agilent 5977B MSD. Helium was used as the carrier gas (1.0 mL/min). The EO was dissolved in dichloromethane (1:100 v/v) then the sample (1.0 μL) was injected, a split ratio of 25:1. The GC oven temperature was kept at 60°C for 1 min and programmed to 240°C at a rate of 4°C/min, and maintained at 240°C for 4 min. Mass spectra were taken at 70 eV. The injector, MS Quad, MS source, and transfer line temperatures were set at 300°C, 150°C, 230°C, and 300°C, respectively. The mass range was from  $m/z$  50 to 550 (2.0 scan/s).

Constituent identification of the EO was based on a comparison of their mass spectra and retention indices (RI) with those reported by the NIST17 database and Adams' book. The relative percentages of compounds were calculated by the relative area of the total ion chromatogram (TIC) peaks of volatile components.

### In Vitro $\alpha$ -Amylase Inhibitory Effect

A diluted EO (0.25-4 mg/mL) in 5% DMSO was combined with 10 μL of  $\alpha$ -amylase solution in a phosphate buffer with pH 6.9 and then subjected to incubation at 37°C for 15 min. To initiate the reaction, 15 μL of a 0.25% starch solution was introduced, followed by another 15-min incubation at 37°C. A blank sample was prepared similarly, except that the  $\alpha$ -amylase solution was omitted. To terminate the reaction, 50 μL of 1 M HCl was introduced. Subsequently, 100 μL of KI<sub>3</sub> solution was added. The spectrophotometer was used to analyze the absorbance at 595 nm.<sup>26</sup> The percentage inhibition of enzymatic activity was computed as follows:

$$\text{Percentage inhibition} = [1 - A/B] \times 100$$

where A and B represent the absorbance of the test sample and the blank, respectively. IC<sub>50</sub> values (expressed in mg/mL) were employed to assess the activity of the EOs. Acarbose served as a reference standard.

### In Vitro $\alpha$ -Glucosidase Inhibitory Effect

The assay was carried out by mixing 50 μL of an EO diluted in 5% DMSO with 40 μL of  $\alpha$ -glucosidase (0.05 U) in phosphate buffer (pH 6.8), followed by a 20-min incubation at 37°C. After this step, 40 μL of 5 mM 4-nitrophenyl- $\beta$ -D-glucopyranoside (p-NPG) was added and another incubation (20 min, 37°C) was performed. To stop the reaction, 130 μL of 0.2 M sodium carbonate solution was added, and the changes in absorbance were measured at 405 nm.<sup>27</sup> The concentration range was 0.25-4 mg/mL. Acarbose served as a reference standard.

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### Author Contributions

HTT: Methodology and writing—review & editing; DGL: Investigation and methodology; VTH: Experiment and formal analysis; DCV: Experiment and formal analysis; TDT: Investigation and methodology; HNN: Writing—original draft; CTV: Writing—original draft; TTN: Writing—original draft; NHT: Investigation and methodology; THDN: Writing—review & editing and supervision; All authors have read and agreed to the published version of the manuscript.

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### Statement of Human and Animal Rights

This article does not contain any studies with human or animal subjects.

## Informed Consent

There are no human subjects in this article and informed consent is not applicable.

## Supplemental Material

Supplemental material for this article is available online.

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