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Chemical Composition and Enzymatic Inhibitory Activity of *Melicope pteleifolia* Leaf Essential Oil: A Comparative Study Between Two Vietnamese Regions

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Essential oil (EO) from *Melicope pteleifolia* leaf samples, collected from two geographically and climatically distinct regions in Vietnam, namely Quang Tri and Lam Dong provinces, exhibited different compositions and yields of 0.12 % and 0.14 % (EO1 and EO2, respectively). Gas Chromatography-Mass Spectrometry (GC-MS) analysis identified 54 compounds (94.99 %) in EO1 and 43 compounds (88.31 %) in EO2, with significant regional differences in major constituents. EO1 was rich in α-pinene (11.99 %), *β*-(*Z*)-ocimene (5.58 %), *β*-caryophyllene (11.15 %), and aromadendrene (6.86 %), while EO2 contained *trans*-*α*-bergamotene (22.2 %), *δ*-cadinene (10.88 %), and humulene epoxide II (7.47 %). Both oils demonstrated *α*-amylase inhibitory activity, with IC_{50} values of 72.60 ± 2.00 µg/mL (EO1) and 73.63 ± 1.84 µg/mL (EO2), surpassing acarbose (IC_{50} = 121.05 ± 3.96 µg/mL). However, tyrosinase inhibition activity was weak, failing to reach 50 % inhibition. Molecular docking confirmed humulene epoxide II as a key *α*-amylase inhibitor, with a binding energy of -8.038 kcal/mol, interacting with crucial residues. These findings highlight the potential of *M. pteleifolia* EO for antidiabetic applications, warranting further investigation.

Keywords: *Melicope pteleifolia*; essential oil; chemical composition; enzyme inhibition; molecular docking

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Melicope pteleifolia (Champion ex Bentham) T.G. Hartley, also known by its synonym *Euodia lepta* (Spreng.) Merr. and commonly referred to as "Ba chạc" in Vietnam, belongs to the Rutaceae family. This plant is widely distributed across tropical regions of Asia, particularly in South China and Southeast Asian countries (Cambodia, Laos, Myanmar, Thailand, and Vietnam) [1]. It is a perennial herbaceous shrub or small tree, traditionally used in folk medicine to treat various ailments, including rheumatism, sore throat, dermatitis, eczema, and insect or snake bites [1]. Over the years, extensive research has been conducted on the phytochemical constituents and biological activities of *M. pteleifolia*. Studies have demonstrated that its extracts, essential oils (EOs), and isolated compounds exhibit a broad spectrum of pharmacological properties, including antioxidant, anti-inflammatory, antiviral, analgesic, and antimicrobial effects [2-5]. Several bioactive compounds have been identified in this plant, notably phenylpropanoids, terpenoids, benzopyran derivatives, flavonoids, and alkaloids, which contribute to its medicinal potential [1]. Recent investigations have further explored the chemical composition and bioactivity of *M. pteleifolia* EOs. For instance, fresh leaf EO samples collected in China in 2021 and 2022, extracted at different times, revealed that α-thujene was the predominant compound in the 2021 sample [6]. Bioactivity assays indicated that this EO exhibited strong repellent properties against *Lasioderma serricorne* and *Liposcelis bostrychophila*, both of which are significant agricultural and storage pests [6]. Another study, focusing on the EOs extracted from the leaves and fruits of *M. pteleifolia* in Pu'er, Yunnan, China, reported that *β*-*cis*-ocimene was the primary compound, accounting for 26.96 % in leaf oil and 65.99 % in fruit oil. Meanwhile, the EO from the stems was found to be rich in origanene, which made up 77.00 % of its total composition [7]. In fumigation bioassays, the fruit-derived EO and *β*-*cis*-ocimene displayed notable toxicity against *L. serricorne*, while *β*-*cis*-ocimene was also highly toxic to *Tribolium castaneum*. Furthermore, both

the EOs and *β-cis*-ocimene demonstrated significant contact toxicity and repellent effects against three common pest species: *T. castaneum*, *L. serricorne*, and *L. bostrychophila* [7].

Despite the growing body of research on *M. pteleifolia*, there remains a gap in knowledge regarding the potential *α*-amylase and tyrosinase inhibitory effects of *M. pteleifolia* leaf essential oil. To the best of our knowledge, no studies have explored the *α*-amylase and tyrosinase inhibitory effects of *M. pteleifolia* leaf EO. Given the importance of these enzymes in metabolic disorders and skin-related conditions, this knowledge gap presents a crucial research opportunity. Specifically, *α*-amylase is a key enzyme involved in the breakdown of dietary starch into glucose, and its excessive activity is directly linked to postprandial hyperglycaemia and the progression of type 2 diabetes mellitus. Natural *α*-amylase inhibitors are consequently of great interest as safer alternatives to synthetic antidiabetic drugs. Similarly, tyrosinase plays a central role in melanin biosynthesis; its overactivity is associated with hyperpigmentation disorders and the deterioration in quality of food products. As a result, tyrosinase inhibitors are highly sought after for both cosmetic skin-whitening and pharmaceutical applications. Our study, therefore, aims to investigate the chemical composition of *M. pteleifolia* leaf essential oil samples from two distinct geographical regions in Vietnam (Quang Tri and Lam Dong provinces) and to evaluate their *in vitro α*-amylase and tyrosinase inhibitory activities. Furthermore, we propose that the observed enzyme-inhibitory effects are linked to the main chemical compounds, which will be explored through molecular docking studies. These investigations are expected to provide valuable insights into the plant's potential applications in the pharmaceutical and cosmetic industries.

MATERIAL AND METHODS

Plant Material

All parts of the fresh leaves of *M. pteleifolia* were collected in November 2023 from two locations in Vietnam: Hai Lang district, Quang Tri province (16°37'18.3"N, 107°09'29.5"E) and Lac Duong district, Lam Dong province (12°10'30.5"N, 108°41'55.9"E). These two areas were specifically chosen for comparative analysis due to their significant geographical and climatic differences, which are known to influence plant secondary metabolite profiles. The species was identified by Dang Minh Tu, a botanist from the National Institute of Medicinal Materials. Voucher specimens (MP.041.QT and MP.041.LD, respectively) were deposited at the Natural Products Laboratory, Vinh University.

Extraction of EO

Fresh leaves of *M. pteleifolia* (500 g per collection location) were washed, chopped into small pieces, and hydrodistilled for approximately 4 hours using a Clevenger-type apparatus, following the previously reported method [8]. The hydrodistillation was performed in triplicate. The EOs were collected, dried with anhydrous sodium sulfate, and stored at 4 °C in the dark until further use.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The EOs of *M. pteleifolia* were analyzed using an Agilent GC-MSD system. Briefly, an HP-5MS UI column (30 m × 0.25 mm i.d. × 0.25 µm film thickness) was employed with helium as the carrier gas at a flow rate of 1.0 mL/min (pressure: 8.23 psi). The GC oven temperature program was as follows: initial hold at 60 °C for 4 min, followed by a temperature ramp to 240 °C at 5 °C/min, then a hold at 240 °C for 10 min. The inlet temperature was 300 °C, while the mass spectrometer source and quadrupole temperatures were set at 230 °C and 150 °C, respectively. A 1.2 µL sample was injected with a split ratio of 30:1. Mass spectra were recorded at 70 eV over an m/z range of 50 to 550. Retention indices (RIs) were determined by co-injection with a C_7-C_{30} n-alkane series (Merck). Component identification was achieved by comparing their mass spectra and RIs with literature data (NIST 17 and Adams book [9]). The relative percentages of compounds were calculated by the peak area normalization method without taking into account the response factors of individual compounds.

α-Amylase Inhibitory Activity

The inhibitory activity of *M. pteleifolia* EOs against porcine pancreatic *α*-amylase (0.14 U/mL) was determined using a starch-iodine assay, as previously described [10]. The EO samples were dissolved in 5% DMSO (using a vortex mixer) at concentrations ranging from 31.25 to 1000 µg/mL. For each assay, 750 µL of EO solution was mixed with 150 µL of *α*-amylase solution and incubated at 37 °C for 15 minutes. The reaction was initiated by adding 225 µL of 0.25 % starch solution, followed by a further 15-minute incubation at 37 °C. The reaction was then quenched with 750 µL of HCl 1M and 1.5 mL of KI_3 solution. Absorbance was measured at 590 nm. Blanks were prepared without the enzyme, and acarbose was used as a positive control. All assays were performed in triplicate. Percent inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = [(A_0 - A_b)/(A_0 - A_s)] \times 100$$

where A_0, A_b, and A_s are absorbance values of the enzyme control, blank, and test sample, respectively. IC_{50} values were derived *via* non-linear regression (Microsoft Excel 365).

Tyrosinase Inhibitory Activity

To assess tyrosinase inhibitory activity, the EO samples were prepared by dissolving in 5 % DMSO at concentrations ranging from 125 to 1000 µg/mL using a vortex mixer. For the assay, 100 µL of each EO solution was mixed with 40 µL of tyrosinase (80 U/mL in buffer) in a 96-well plate and incubated at 25 °C for 10 minutes. The enzymatic reaction was initiated by adding 40 µL of 2.5 mM L-DOPA (in buffer), followed by a 20-minute incubation at 37 °C. Absorbance was subsequently measured at 490 nm with a microplate reader. IC_{50} values were used to evaluate inhibitory activity. Kojic acid was used as a positive control [11, 12].

Molecular Docking

The main compounds in the EO of *Melicope pteleifolia* leaves include *α*-farnesene, *α*-pinene, *β*-(*Z*)-ocimene, *β*-(*E*)-ocimene, *β*-caryophyllene, *trans*-*α*-bergamotene, aromadendrene, *δ*-cadinene, caryophyllene oxide, and humulene epoxide II, which were drawn using Marvin JS software and subsequently energy-optimized through the MMFF94s force field using Avogadro software [13, 14]. The structure of the human pancreatic *α*-amylase in complex with mini-montbretin A was downloaded from the RCSB Protein Data Bank (https://www.rcsb.org/structure/5E0F) with the access code 5E0F. This structure was preprocessed before molecular docking simulation, which involved removing co-crystallized molecules not relevant to this simulation, adding polar hydrogen atoms, and calculating Kollman charges. The AutoDockTools v1.5.6 software was used to define the grid box with the centre coordinates at x = -8.042 Å, y = 6.123 Å, z = -22.998 Å, a box size of 22 x 22 x 22 $Å^3$, and a grid spacing of 1. Molecular docking simulations were performed using AutoDock Vina v1.2.3 with parameters set as previously reported [11, 15, 16]. The results obtained from this simulation were analyzed and visualized using BIOVIA Discovery Studio Visualizer to represent 2D protein-ligand interactions.

RESULTS AND DISCUSSION

Yields and Chemical Compositions

The EO samples extracted from the leaves of *Melicope pteleifolia* collected from two different provinces, Quang Tri and Lam Dong, exhibited a pale-yellow colour and yielded 0.12 ± 0.01 and 0.14 ± 0.02 mL/g, respectively.

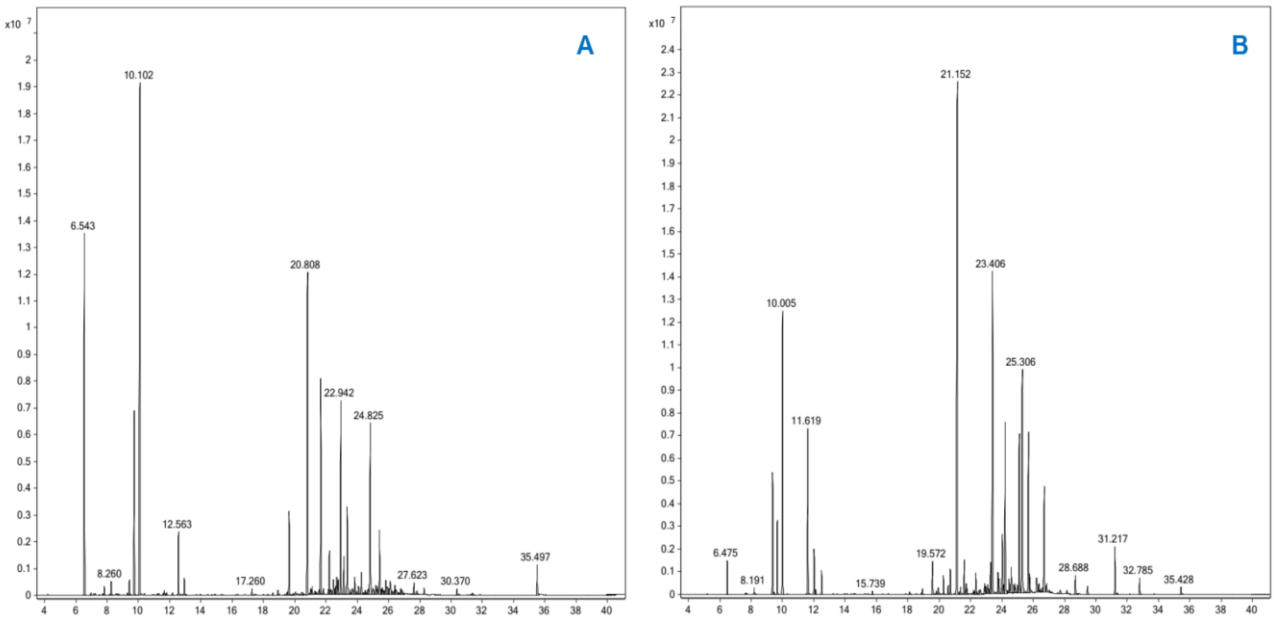


Figure 1. Gas chromatogram - mass spectrum of the essential oil samples of *Melicope pteleifolia* leaves from Quang Tri (**A**) and Lam Dong (**B**) provinces.

Gas chromatography-mass spectrometry (GC-MS) analysis revealed the two samples had distinct chemical compositions (**Figure 1**). The EO from Quang Tri province (EO1) contained 54 compounds, representing 94.99 % of its total composition, whereas the Lam Dong province sample (EO2) comprised 43 compounds, accounting for 88.31 % of its total content (Table 1). Comparing the key ingredients showed big differences. In EO1, the predominant constituents were *α*-pinene (11.99 %), *β*-(*Z*)-ocimene (5.58 %), *β*-caryophyllene (11.15 %), aromadendrene (6.86 %), *α*-farnesene (5.9 %), and caryophyllene oxide (6.24 %). Notably, these key compounds were either present in significantly lower concentrations or absent in EO2. For instance, *α*-pinene, abundant in EO1, constituted a negligible percentage in EO2. In contrast, EO2 was characterized by its richness in *trans*-*α*-bergamotene (22.2 %), *δ*-cadinene (10.88 %), *trans*-nerolidol (5.15 %), and humulene epoxide II (7.47 %). These compounds were found in much lower amounts or not at all in EO1, indicating a clear differentiation based on geographical origin. Despite these differences, both EOs were primarily composed of monoterpene hydrocarbons (44.76 % in EO1, 15.32 % in EO2), sesquiterpene hydrocarbons (35.10 % in EO1, 39.18 % in EO2), and oxygenated sesquiterpenes (13.56 % in EO1, 24.49 % in EO2). Within these general classifications, significant quantitative variations were also observed; for example, the monoterpene hydrocarbon content in EO1 was nearly three times higher than in EO2. Additionally, both samples contained a notable amount of *β*-(*E*)-ocimene, with 23.25 % in EO1 and 8.26 % in EO2. Several other compounds exceeded 1 % in concentration. In EO1, these included (4*E*,6*Z*)-allo-ocimene (1.83 %), *α*-copaene (2.54 %), *γ*-muurolene (1.41 %), cubebol (1.28 %), *δ*-cadinene (2.87 %), and humulene epoxide II (2.32 %). Meanwhile, EO2 featured *α*-limonene (3.28 %), *β*-(*Z*)-ocimene (1.91 %), *β*-linalool (4.78 %), *p*-ethylanisole (1.22 %), elemicine (1.99 %), ledene oxide II (4.74 %), and *β*-bisabolol (3.69 %). These overall profiles indicate distinct chemotypes for *M. pteleifolia* EOs from Quang Tri and Lam Dong provinces.

Table 1. Chemical constituents of *M. pteleifolia* leaf EOs from two provinces of Vietnam.

No.	RT (min)	RI (exp.)	RI (lit.)	Compounds	%	
					EO1	EO2
1	6.543	939	937	*α*-Pinene	**11.99**	0.91
2	6.978	954	952	Camphene	0.07	-
3	7.665	976	974	Sabinene	-	0.05
4	7.819	980	979	*β*-Pinene	0.27	-
5	8.260	993	991	*β*-Myrcene	0.48	0.18
6	8.666	1006	1005	*α*-Phellandrene	0.05	-
7	9.301	1028	1025	*p*-Cymene	0.07	-
8	9.433	1033	1030	*α*-Limonene	0.48	3.28
9	9.736	1043	1038	*β-(Z)*-Ocimene	**5.58**	1.91
10	10.102	1054	1049	*β-(E)*-Ocimene	**23.35**	**8.26**
11	11.309	1090	1088	Terpinolene	0.06	-
12	11.584	1098	1093	*α*-Naginatene	0.06	-
13	11.664	1100	1099	*β*-Linalool	0.15	4.78
14	11.802	1106	1104	Nonanal	0.07	-
15	12.019	1113	1110	*p*-Ethylanisole	-	1.22
16	12.563	1133	1131	*(4E,6Z)*-allo-Ocimene	1.83	0.63
17	12.935	1145	1144	*(4E,6E)*-Alloocimene	0.53	0.10
18	18.125	1324	1323	Methyl geraniate	-	0.12
19	18.599	1342	1338	*δ*-Elemene	0.07	-
20	18.926	1355	1351	*α*-Cubebene	0.15	-
21	19.521	1377	1372	*α*-Ylangene	0.11	-
22	19.641	1381	1376	*α*-Copaene	2.54	0.91
23	19.939	1392	1389	*β*-Cubebene	-	0.26
24	20.053	1396	1391	*β*-Elemene	0.18	-
25	20.270	1403	1402	Methyleugenol	-	0.55

26	20.579	1417	1415	*cis*-*α*-Bergamotene	-	0.26
27	20.711	1422	1420	*α*-Santalene	-	0.80
28	20.808	1426	1419	*β*-Caryophyllene	**11.15**	-
29	21.026	1435	1432	*β*-Copaene	0.17	-
30	21.117	1439	1435	*trans*-*α*-Bergamotene	0.28	**22.20**
31	21.283	1446	1443	*β*-Gurjunene	0.10	0.07
32	21.380	1449	1444	*cis*-*β*-Farnesene	0.06	0.21
33	21.552	1456	1453	*trans*-Geranylacetone	0.16	-
34	21.592	1458	1454	Guaia-6,9-diene	-	0.95
35	21.661	1461	1457	Aromadendrene	**6.86**	-
36	21.730	1463	1462	*β*-Santalene	-	0.39
37	21.838	1467	1466	9-epi-trans-Caryophyllene	0.19	0.15
38	22.216	1482	1477	*γ*-Muurolene	1.41	0.12
39	22.307	1486	1482	*α*-Amorphene	0.22	0.60
40	22.473	1492	1486	*β*-Eudesmene	0.55	0.06
41	22.599	1497	1493	Epicubebol	0.25	0.22
42	22.685	1500	1494	*α*-Selinene	0.78	-
43	22.788	1505	1499	*α*-Muurolene	0.54	-
44	22.897	1509	1509	*β*-Bisabolene	-	0.31
45	22.942	1511	1508	*α*-Farnesene	**5.90**	0.22
46	23.143	1520	1515	Cubebol	1.28	0.38
47	23.354	1529	1524	*δ*-Cadinene	2.87	**10.88**
48	23.572	1539	1532	Cubenene	0.17	-
49	23.698	1544	1543	*cis*-Sesquisabinene hydrate	0.32	0.70
50	23.835	1548	1542	*α*-Calacorene	0.55	0.57
51	24.030	1558	1554	Elemicine	-	1.99
52	24.258	1569	1564	trans-Nerolidol	0.76	**5.15**
53	24.510	1578	1576	Spathulenol	0.17	0.98
54	24.676	1585	1580	Globulol	0.19	-
55	24.825	1591	1581	Caryophyllene oxide	**6.24**	0.28
56	25.277	1611	1606	Humulene epoxide II	2.32	**7.47**
57	25.683	1630	1631	Ledene oxide II	0.15	4.74
58	25.821	1635	1627	Epicubenol	0.56	0.72
59	26.015	1644	1637	Caryophylladienol II	0.19	-
60	26.101	1647	1642	Cubenol	0.73	-
61	26.221	1653	1654	Isoelemicin	0.13	0.66
62	26.702	1675	1671	*β*-Bisabolol	0.08	3.69
63	26.868	1682	1677	Zizanol	0.19	0.33
64	27.623	1716	1715	Pentadecanal	0.38	0.05
65	35.497	2118	2114	*(E)*-Phytol	1.00	-
	Monoterpene hydrocarbons (No. 1-11, 16, 17)				44.76	15.32
	Oxygenated monoterpenes (No. 12,13,18, 33				0.37	4.90
	Sesquiterpene hydrocarbons (No. 19-24, 26-32, 34-45, 47, 48, 50)				35.10	39.18
	Oxygenated sesquiterpenes (No. 46, 49, 52-60, 62-64)				13.56	24.49
	Others (No. 14, 15, 25, 51, 61, 65)				1.20	4.42
	Total				94.99	88.31

Note: RT = Retention time (min); RI (exp.) = Experimental retention indices (HP-5MS UI column); RI (lit.) = Literature retention indices; Bold values indicate main components.

Table 2. *α*-Amylase inhibitory activity of *M. pteleifolia* leaf Eos.

Concentration (µg/mL)	Inhibition, %		
	EO1	**EO2**	**Acarbose**
15.63	4.45 ± 2.27	8.26 ± 2.58	
31.25	11.56 ± 3.23	16.36 ± 2.54	
62.5	43.44 ± 1.71	37.02 ± 2.35	
125	91.57 ± 0.87	90.54 ± 0.36	
IC_{50}	72.60 ± 2.00	73.63 ± 1.84	121.05 ± 3.96

Note: Data are presented as mean ± standard deviation; Acarbose (12.5 – 200 µg/mL) was used as a positive control.

A study of the literature showed that the EO from *M. pteleifolia* leaves collected in Pu'er, Yunnan, China, was found to contain *β-cis*-ocimene (26.9 6%) and (*E*)-*α*-bergamotene (8.85 %) as major components [7]. Another report identified methyl 8,11,14,17-eicosatetraenoate (17.40 %), elsholtzione (11.68 %), and *α*-copaene (9.78 %) as key constituents [17]. These variations highlight the influence of environmental factors and geographic location on the chemical composition of *M. pteleifolia* EOs.

In Vitro α-amylase and Tyrosinase Inhibition of the EOs

The research results indicate that both EO1 and EO2 exhibited *α*-amylase inhibitory activity in a concentration-dependent manner, as described in Table 2. At the lowest concentration (15.63 µg/mL), the inhibition levels of EO1 and EO2 were 4.45 % and 8.26 %, respectively, indicating a weak effect. As the concentration increased to 31.25 µg/mL and 62.5 µg/mL, the inhibition levels also increased, with EO1 reaching 43.44 % and EO2 reaching 37.02 %, demonstrating that EO1 was more effective at this concentration. Notably, at 125 µg/mL, both samples achieved nearly maximal inhibition (~91 %), suggesting enzyme saturation. Compared to the reference compound acarbose, the IC_{50} of acarbose (121.05 ± 3.96 µg/mL) was significantly higher than that of EO1 (72.60 ± 2.00 µg/mL) and EO2 (73.63 ± 1.84 µg/mL). This indicates that EO1 and EO2 had stronger *α*-amylase inhibitory activity than acarbose in the current work. Since *α*-amylase is a key enzyme in the breakdown of starch into simple sugars, its inhibition helps slow down carbohydrate digestion, reducing postprandial blood glucose spikes. With their stronger effect compared to acarbose, EO1 and EO2 have the potential to be promising candidates for blood glucose control. However, further in-depth studies are needed to determine their active components and specific mechanisms of action for potential application in type 2 diabetes treatment.

In addition, both EOs exhibited weak tyrosinase inhibition activity, showing some level of enzyme inhibition (<50%) within the tested concentration range, as shown in Table 3.

To date, no studies have been conducted on the anti-*α*-amylase and antityrosinase inhibitory activities of *M. pteleifolia* leaf EO. However, several plant species belonging to the Rutaceae family have been reported to exhibit these activities. For instance, *β*-caryophyllene has been suggested to play a crucial role in the *α*-amylase inhibitory activity of *Paramignya scandens* EO, a property also observed in the EO of *Acronychia pedunculata* [11, 18]. Additionally, *α*-pinene, a major constituent in the EOs of *Citrus* plants, has been shown to be an effective *α*-amylase inhibitor [19]. Alongside these compounds, (*E*)-*β*-ocimene has also been reported to contribute to *α*-amylase inhibition [10]. Moreover, the observed anti-*α*-amylase activity in these plant species may not solely result from individual compounds. Still, it could also stem from synergistic or additive effects arising from the diverse constituents within the complex mixture of EOs.

Table 3. Tyrosinase inhibitory activity of *M. pteleifolia* leaf Eos.

Concentration (µg/mL)	Inhibition, %		
	EO1	**EO2**	**Kojic acid** [12]
62.5	5.42 ± 0.43	11.14 ± 1.41	
125	20.55 ± 0.91	20.86 ± 1.37	
250	22.74 ± 1.27	N.i.	
500	N.i.	N.i.	
IC_{50}	N.d.	N.d.	72.65 ± 5.98

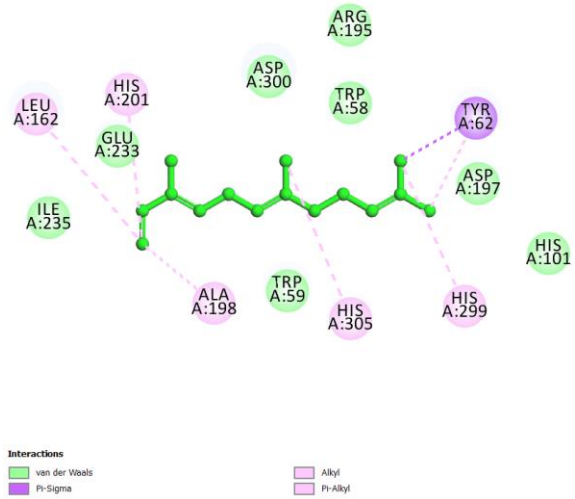
Note: Data are presented as mean ± standard deviation; N.i.= No inhibition; N.d.: Not determined; Kojic acid (31.25 - 250 µg/mL) was used as a positive control [12].

Docking Results

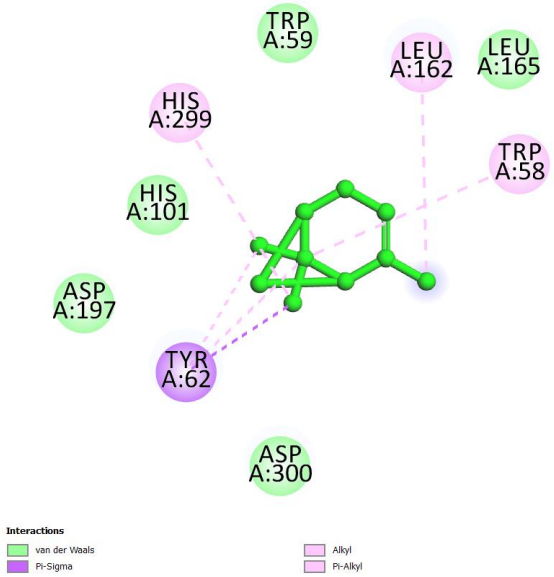
First, the docking protocol was validated through re-docking, with the calculated RMSD value of 1.87325 < 2 Å confirming the high reliability of the prediction results. Therefore, this protocol was subsequently used to evaluate the major compounds hypothesized to contribute significantly to the α-amylase inhibitory activity of *M. pteleifolia* leaf EO. The results of the molecular docking study showed that the major compounds in the EO of *M. pteleifolia* leaves had significant interaction potential with the α-amylase enzyme, with binding affinity values ranging from -8.038 kcal/mol to -5.112 kcal/mol as shown Table 4. Notably, humulene epoxide II exhibited the highest affinity, indicating it as the most promising compound for inhibiting α-amylase activity. In-depth protein-ligand interaction analysis revealed that the major compounds formed alkyl and pi-alkyl interactions with critical residues on α-amylase, such as His299, Leu162, Tyr62 (**Figure 2**). Additionally, these compounds established stable pi-sigma interactions with Tyr62, contributing to enhanced binding affinity (**Figure 2**). Specifically, humulene epoxide II interacted with residues Leu162, Leu165, Trp58, and Tyr62, demonstrating a strong and stable interaction potential. Other compounds, such as α-farnesene, β-caryophyllene, and aromadendrene, also exhibited good binding capacity when interacting with residues like His299, Leu162, Tyr62, and Trp58. Compared to the reference compound mini-montbretin A, the major compounds in the EO primarily focused on interactions with Tyr62, Leu162, Leu165 and His299, showing similar interaction modes that lead to α-amylase inhibition potential. Previous computational docking studies have reported that caryophyllene oxide, a major compound in the EO of *Knema globularia* leaves, exhibited the most potent α-amylase inhibitory activity [10]. Similarly, the EO of *Paramignya scandens*, with *(E)*-β-caryophyllene as its primary compound, showed superior binding affinity against the α-amylase enzyme [11]. Furthermore, *in silico* molecular docking studies on *Schinopsis lorentzii* EO identified β-caryophyllene, terpinen-4-ol, and sabinene as compounds with favourable binding affinities for both α-amylase and α-glucosidase [20]. Overall, this study enhanced the understanding of the interaction mechanisms between EO compounds and the α-amylase enzyme, suggesting the potential application of these compounds in the development of antidiabetic agents. However, molecular docking, while valuable for predicting binding interactions, does not account for factors such as metabolism, bioavailability, or pharmacokinetics *in vivo*. Therefore, further experimental studies are essential to validate these findings and assess their translational potential.

Table 4. The docking results of major compounds in the EO of *M. pteleifolia* leaves with enzyme α-amylase.

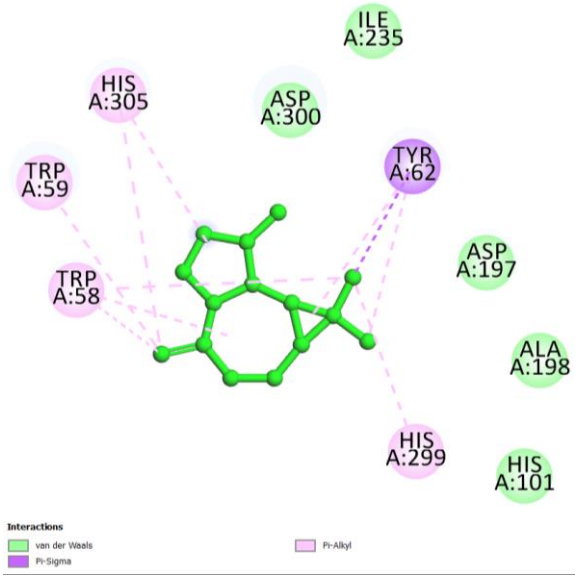
Compounds	Binding affinity (kcal/mol)	Alkyl and pi-alkyl interactions	Pi-sigma interactions
α-Farnesene	-5.987	Leu162, His201, Ala198, His305, His299, Tyr62	Tyr62
α-Pinene	-5.508	His299, Leu162, Trp58, Tyr62	Tyr62
Aromadendrene	-7.095	His305, Trp59, Trp58, His299, Tyr62	Tyr62
β-Caryophyllene	-7.629	Trp59, Leu162, Leu165, Tyr62	Tyr62
β-(*E*)-Ocimene	-5.112	His101, Ala198, Trp59, His299, Leu162	Tyr62
β-(*Z*)-Ocimene	-5.477	Leu165, His101, His299	Tyr62
Caryophyllene oxide	-7.711	Trp58, Trp59, His299, Tyr62	-
δ-Cadinene	-7.475	His299, Leu165, Hiss305, Trp58, Trp59, Tyr62	Tyr62
Humulene epoxide II	-8.038	Leu162, Leu165, Trp58	Tyr62
trans-α-Bergamotene	-6.864	Trp58, Trp59, Leu165, His299	Tyr62



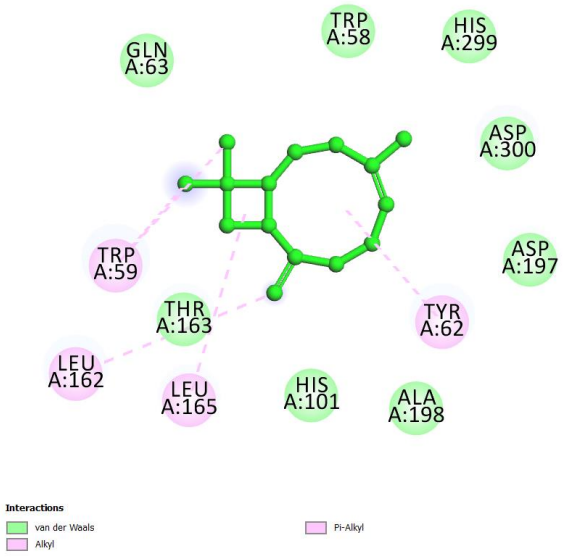
5E0F-α-Farnesene



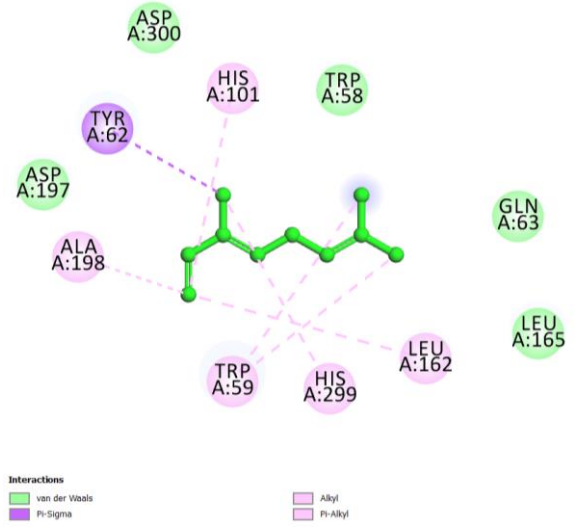
5E0F-α-Pinene



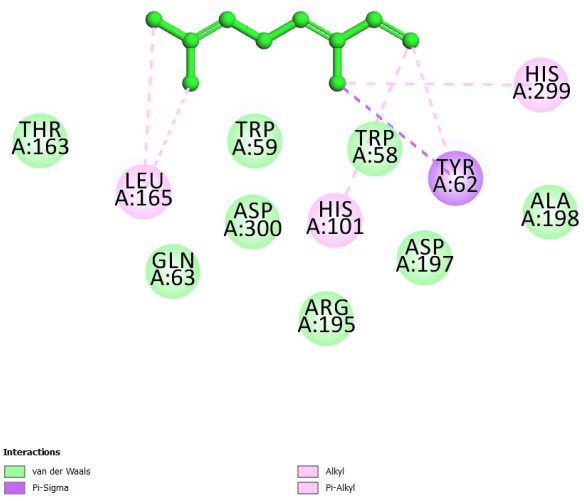
5E0F-Aromadendrene



5E0F-β-Caryophyllene



5E0F-β-(E)-Ocimene



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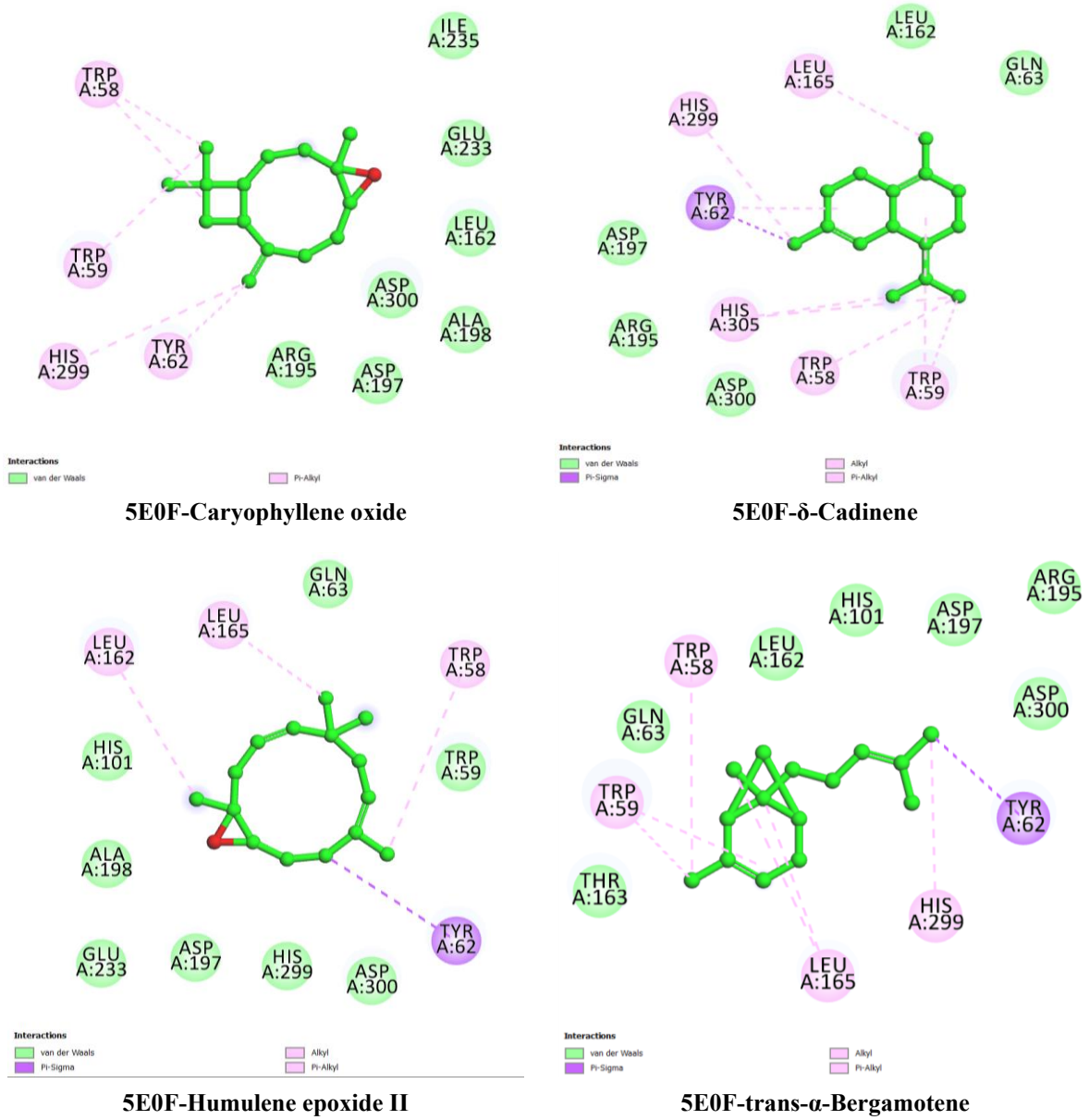

Figure 2. 2D interaction views of major compounds in the essential oil of *M. pteleifolia* leaves within the active site of the enzyme *α*-amylase.

CONCLUSION

Essential oils extracted from *M. pteleifolia* leaves collected from Quang Tri and Lam Dong provinces exhibited significant chemical and biological differences. GC-MS analysis confirmed various differences in the compositional profiles of these two samples, demonstrating the influence of geographical and environmental factors on their phytochemical content. Both essential oils had encouraging *α*-amylase inhibitory action, significantly outperforming acarbose. Molecular docking analysis further corroborated the strong interaction potential of key constituents, particularly humulene epoxide II, with the *α*-amylase enzyme. These findings suggest that *M. pteleifolia* essential oil is a potential natural source for *α*-amylase inhibitors, warranting further investigation into its use for blood sugar control. Additionally, neither of the essential oil samples showed strong tyrosinase inhibitory effects.

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