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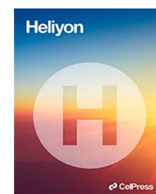

Xanthine oxidase, α-glucosidase and α-amylase inhibitory activities of the essential oil from *Piper lolot*: *In vitro* and in silico studies

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A R T I C L E I N F O

Keywords:
Piper lolot
Essential oil
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A B S T R A C T

Introduction: Piper lolot is a species of herb used as a popular food in Vietnam. Furthermore, the species has been used as a Vietnamese traditional medicine to treat many diseases.
Methods: Chemical constituents in the essential oil from leaves of *Piper lolot* were determined using GC/MS analysis. The anti-gout and anti-diabetic activities of the essential oil were determined through the inhibitory assays against xanthine oxidase, α-glucosidase and α-amylase enzymes. In addition, molecular docking simulations were used to elucidate the inhibitory mechanism between the main compounds and the enzymes.
Results: The dominant constituents of the *Piper lolot* essential oils were determined as *β*-caryophyllene (20.6%), *β*-bisabolene (11.6%), *β*-selinene (8.4%), *β*-elemene (7.7%), *trans*-muurola-4(14),5-diene (7.4%), and (*E*)-*β*-ocimene (6.7%). The essential oil displayed xanthine oxidase, *α*-amylase, and *α*-glucosidase inhibitory activities with IC_{50} values of 28.4, 130.6, and 59.1 μg/mL, respectively. The anti-gout and anti-diabetic activities of the essential oil from the *P. lolot* species are reported for the first time. Furthermore, molecular docking simulation was consistent to *in vitro* experiments.
Conclusion: The present study provides initial evidence that the essential oil of *P. lolot* may be a potential natural source to develop new diabetes preparations.

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1. Introduction

Gout and diabetes are two common diseases in middle-aged people. Studies have shown an association between these two diseases [1]. Treatment is prolonged and strongly dependent on dietary and lifestyle modifications. Discovering common ingredients that can effectively support the treatment of diabetes and gout is thus considered to be a good significant strategy. Xanthine oxidase (XO) is necessary for oxidizing hypoxanthine to xanthine and converting xanthine from purine nucleotides to uric acid [2,3]. As a consequence, XO activity causes uric acid to accumulate in the various tissues, resulting in gout.

Meanwhile, α-glucosidase and α-amylase are enzymes involved in the breakdown of carbohydrates. When these enzymes are active, the complex carbohydrates are broken down, which increases blood sugar levels. In individuals with type 2 diabetes, their ability to regulate blood sugar levels is impaired due to insulin resistance or insufficient insulin production. Therefore, inhibiting the activities of alpha-glucosidase and alpha-amylase can be beneficial for individuals with type 2 diabetes in controlling blood sugar levels by postponing the digestion of complex carbohydrates in the intestine, leading to a slower release of glucose into the bloodstream [4–6]. In this study, we focused on finding natural ingredients that can inhibit the activity of these protein targets to support the treatment of gout and diabetes.

Genus *Piper* (Piperaceae) is extensively spread in tropical and subtropical areas with over 700 species, out of which 16 species were found in Vietnam [7,8]. *Piper lolot*, a herb is known as "la lot" or "tat bat" in Vietnam. The whole plant of *P. lolot* has been used as a traditional medicine to treat toothache, headache, diarrhea, rheumatism, lumbago, purulent rhinitis, and digestive disorders [7]. Previous phytochemical investigations of this species have led to the isolation of amide alkaloids, phenanthrene-type alkaloids, steroids, chalcones, amides, benzenoids, and ionones [8–12]. Furthermore, pharmacological studies on extracts from *P. lolot* showed anti-platelet [8], anti-inflammatory [9], anti-diabetic [10], and anti-oxidant [11] activities.

Since the beginning of civilization, plants have provided constant support for food, medicine, and other essentials. Recently, the production of functional foods from medicinal herbs is a current trend. *P. lolot* is frequently used in Vietnamese cuisine as a food or healthcare supplements. The *P. lolot* essential oils were previously reported including monoterpenes, sesquiterpenes, and their oxygenated derivatives [13]. Generally, the chemical constituents and biological effects of *P. lolot* essential oil have not been thoroughly investigated. Therefore, we reported herein the chemical composition as well as xanthine oxidase, α-amylase, and α-glucosidase inhibition of the leaf essential oil of *P. lolot*. To our best knowledge, this is the first scientific report regarding the anti-gout and anti-diabetic activities of the essential oil extracted from *P. lolot* species.

2. Materials and methods

2.1. Plant material

Leaves of *Piper lolot* species (2.5 kg) were collected from Nam Dong, Thua Thien Hue, Vietnam in June 2022, and were identified by Dr. Do Van Truong (Vietnam National Museum of Nature, VAST). The voucher specimen (H-51) was deposited at the herbarium of Mientrung Institute for Scientific Research, VNMN, VAST, Vietnam.

2.2. Extraction of the essential oil

Fresh leaves of *Piper lolot* were cut into small pieces and their oils were yielded by steam distillation using a glass apparatus for 4 h at normal pressure. The essential oil was then collected, dried with sodium sulfate, and then stored in sealed vials at 4 °C until further analysis.

2.3. Analysis of essential oil

The GC-MS analysis was performed with a Shimadzu GCMS-QP2010 Plus system (Shimadzu, Kyoto, Japan). Equity-5 capillary column (30 m × 0.25 mm, film thickness 0.25 μm, Supelco, USA) was used with helium as carrier gas (flow rate: 1.5 mL/min). The GC oven temperature was operated at 60 °C for 2 min and programmed to 240 °C at a rate of 4 °C/min, and kept constant at 240 °C for 10 min, and programmed to 280 °C at a rate of 5 °C/min. The sample was injected using a splitless mode. The injector temperature was set to 280 °C. Mass spectra were recorded at 70 eV. The mass range was from 40 to 500 amu at a sampling rate of 0.5 scan/s. The essential oil components were identified by comparison of their relative retention index (RI) to a series of *n*-alkanes (RI determined with reference to homologous series of *n*-alkanes C_7–C_{40}). Computer matching against commercial (WILEY7 Library and NIST11 Library) and components of known oils, as well as MS and RI data from the literature, were used for the identification [14,15].

2.4. Biological material

Xanthine oxidase from bovine milk (X4376), α-glucosidase from *Saccharomyces cerevisiae* (G5003), porcine pancreas α-amylase (A3176), xanthine, 4-nitrophenyl β-D-glucopyranoside (pNPG), starch azure, acarbose, and allopurinol were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Other reagents were acquired from Kanto Chemical Co., Inc., Tokyo, Japan.

2.4.1. α-Glucosidase inhibition assay

The α-glucosidase enzyme inhibition assay was performed by the protocol described by Ranilla et al. (2010) with minor modifications [16]. Briefly, the reaction mixture consisting of 50 μL of the sample was incubated with 100 μL of 0.1 M potassium phosphate buffer (pH 6.8) containing α-glucosidase solution (0.5 U/mL) in 96-well plates at 37 °C for 10 min. After pre-incubation, 50 μL of 5 mM pNPG was added to each well to start the reaction. After incubating at 37 °C for 5 min, the absorbance was recorded at 405 nm in a microplate reader (Biotek, USA). The inhibitory activity was calculated by the following equation: α-glucosidase inhibition (%) = $(1 - A/A_c) \times 100$, where A is the absorbance of the sample and A_c is the absorbance of the control, respectively. The absorbance of blank samples (reaction solutions without enzyme) is subtracted before calculating the equation. The IC_{50} value was calculated by GraphPad Prism.

Table 1
Chemical components of the essential oil from the leaves of *Piper lolot*.

No	Compounds	RT (min)	SI (%)	RI[a]	RI[b]	Content (%)
1	α-Pinene	6.700	96	932	932	0.1
2	β-Pinene	8.108	96	975	974	0.7
3	Myrcene	8.583	93	990	988	0.1
4	Limonene	10.017	94	1028	1024	0.2
5	(Z)-β-Ocimene	10.375	97	1036	1032	0.4
6	**(E)-β-Ocimene**	10.867	97	1048	1044	**6.7**
7	Linalool	12.958	98	1100	1095	0.4
8	Allo-ocimene	14.242	95	1129	1128	0.5
9	(E)-Anethole	21.142	94	1285	1282	0.2
10	2-Undecanone	21.542	96	1294	1293	0.2
11	Bicycloelemene	23.433	93	1338	1338	0.2
12	α-Cubebene	23.975	95	1351	1348	0.2
13	Hydrocinnamyl acetate	24.850	92	1371	1366	0.1
14	α-Copaene	25.117	96	1377	1374	1.8
15	**β-Elemene**	25.900	93	1396	1389	**7.7**
16	**β-Caryophyllene**	27.200	97	1427	1422	**20.6**
17	β-Copaene	27.375	91	1431	1430	0.3
18	γ-Elemene	27.542	95	1435	1434	0.2
19	α-Guaiene	27.750	92	1440	1437	0.1
20	α-Humulene	28.425	97	1457	1452	4.5
21	*trans*-β-Farnesene	28.525	93	1459	1454	1.2
22	Allo-aromadendrene	28.667	82	1463	1458	0.1
23	γ-Muurolene	29.358	86	1479	1478	0.2
24	Germacrene D	29.508	95	1483	1480	1.2
25	**β-Selinene**	29.833	96	1491	1489	**8.4**
26	***trans*-Muurola-4(14),5-diene**	29.958	85	1494	1493	**7.4**
27	α-Zingiberene	30.125	90	1498	1493	4.5
28	α-Selinene	30.192	93	1500	1498	3.5
29	α-Muurolene	30.292	91	1502	1500	0.3
30	δ-Guaiene	30.508	91	1508	1509	0.3
31	**β-Bisabolene**	30.775	96	1515	1505	**11.6**
32	α-Panasinsene	31.017	92	1521	1519	0.5
33	δ-Cadinene	31.250	93	1527	1522	1.6
34	*trans*-γ-Bisabolene	31.542	88	1534	1529	0.3
35	Elemol	32.217	95	1551	1548	0.5
36	*trans*-Nerolidol	32.808	98	1567	1561	2.9
37	Caryophyllene oxide	33.542	94	1585	1582	0.6
38	Viridiflorol	33.867	89	1594	1592	0.1
39	*trans*-β-Elemenone	34.133	88	1600	1602	1.2
40	α-Acorenol	35.250	81	1630	1632	0.2
41	γ-Eudesmol	35.383	91	1634	1630	0.1
42	Cubenol	35.775	83	1644	1645	0.1
43	Torreyol	35.925	91	1648	1644	0.3
44	Intermedeol	36.392	86	1661	1665	0.4
45	(E)-Asarone	37.225	93	1683	1675	0.7
46	α-Bisabolol	37.383	87	1688	1685	0.1
47	(E,E)-Geranyl linalool	48.933	94	2030	2026	0.5
	Sesquiterpene hydrocarbons					76.7
	Monoterpene hydrocarbons					8.7
	Oxygenated sesquiterpenes					6.3
	Oxygenated diterpenes					0.5
	Oxygenated monoterpenes					0.4
	Other compounds					1.3
	Total					93.9

RI[a]: Retention indices relative to C_7–C_{40} *n*-alkanes calculated on Equity-5 capillary column; RI[b]: Literature retention indices [14,15,25].

2.4.2. α-Amylase inhibition assay

The α-amylase enzyme inhibitory assay was performed by the protocol described by Hansawasdi et al. (2000) with slight modifications [17]. Starch azure was suspended in 0.05 M Tris–HCl buffer (pH = 6.8) containing 0.01 M $CaCl_2$. The tubes containing the substrate solution were boiled for 5 min and then pre-incubated at 37 °C for 5 min. A total of 100 μL of each sample, 100 μL of the substrate solution, and 50 μL of porcine pancreatic amylase in Tris–HCl buffer (2 U/mL) were incubated at 37 °C for 15 min. Then, 250 μL of acetic acid 50% was added to each tube to stop the reaction. After, the reaction tubes were centrifuged at 3000 rpm for 5 min at 4 °C, and the absorbance of the supernatant was measured at 595 nm using a microplate reader (Biotek, USA). The inhibitory activity was calculated by the following equation: α-amylase inhibition (%) = $(1–A/A_c) \times 100$, where A is the absorbance of the sample and A_c is the absorbance of the control, respectively. The absorbance of blank samples (reaction solutions without enzyme) is subtracted before calculating the equation. The IC_{50} value was calculated by GraphPad Prism.

2.4.3. Xanthine oxidase inhibition assay

The xanthine oxidase inhibitory assay was performed by the protocol described by Noro et al. (1983) with minor modifications [18]. The reaction mixture consisting of 50 μL of the sample, 35 μL of 70 mM phosphate buffer (pH = 7.5), and 30 μL of enzyme solution (0.01 U/ml in 70 mM phosphate buffer, pH = 7.5) was prepared immediately before use. After pre-incubation at 25 °C for 15 min, the reaction was initiated by adding 60 μL of 150 mM xanthine in the same buffer. The reaction mixture was incubated at 25 °C for 30 min. The reaction was stopped by adding 25 μL of 1 N HCl, and the absorbance was measured at 290 nm using a microplate reader (Biotek, USA). The inhibitory activity was calculated by the following equation: xanthine oxidase inhibition (%) = $(1–A/A_c) \times 100$, where A is the absorbance of the sample and A_c is the absorbance of the control, respectively. The absorbance of blank samples (reaction solutions without enzyme) is subtracted before calculating the equation. The IC_{50} value was calculated by GraphPad Prism.

2.5. Molecular docking simulations

2.5.1. Protein and ligand preparation

The crystal structures of xanthine oxidase (PDB ID: 3NRZ), α-Amylase (PDB ID: 4W93), and α-glucosidase (PDB ID: 5NN8) were downloaded from the Research Collaboratory for Structural Bioinformatics Protein Data Bank. The attached HETATM was removed and energy minimization was performed by the standard optimization parameter of Swiss PDB Viewer [19]. Then, we added polar hydrogen atoms and Kollman charges to the protein using Auto Dock tools 1.5.6. Finally, the macromolecule was exported into a dockable pdbqt format for molecular docking. 3D structures of candidates were downloaded from the PubChem library and converted to dockable pdbqt format utilizing Open Babel 3.1.1 [20].

2.5.2. Molecular docking

A molecular docking process was carried out using AutoDock Vina (version 1.1.2) [21]. The grid box covered the active sites of the 3D ligand structure, with Docking scores reported in kcal/mol. Finally, BIOVIA Discovery Studio Visualizer 2020 was used to visualize the molecular interactions between proteins and ligands.

3. Results and discussion

3.1. Chemical composition of essential oil

The obtain of essential oil from the fresh leaves of *Piper lolot* was 0.061%. The essential oil was yielded as a pale yellow liquid with a typical aromatic odor and lighter than water. The GC/MS experiment indicated that the leaf oil contained forty-seven volatile constituents representing 93.9% of the oil content. Most components belonged to sesquiterpene hydrocarbons (76.7%) and monoterpene hydrocarbons (8.7%) (Table 1). Among these, β-caryophyllene (20.6%), β-bisabolene (11.6%), β-selinene (8.4%), β-elemene (7.7%), *trans*-muurola-4(14),5-diene (7.4%), and (*E*)-β-ocimene (6.7%) were found as major components (Fig. 1). Literature surveys indicated

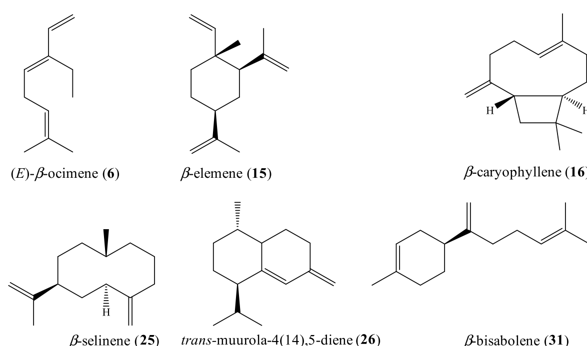

Fig. 1. Major compounds of *Piper lolot* essential oil. The number of compounds in the figure corresponds to their number in Table 1.

that the most abundant compound, *β*-caryophyllene, has a broad pharmacological spectrum including antibacterial, antioxidant, anti-proliferative [22], cytotoxicity [23], anticancer, and anti-carcinogenic activities [24]. According to Dung and co-workers, the leaf oil of *P. lolot* collected in Thua Thien Hue province comprised twenty-seven constituents (accounting for about 70–80%). The major components included *β*-caryophyllene (26.1%), *α*-copaen (8.9%), *α*-cadinol (8.6%), *δ*-cadinol (6.9%), and *δ*-cadinene (6.8%) [13]. The observed difference in qualities and contents might be due to the distribution of species, analysis and identification method.

3.2. Anti-gout and anti-diabetic activity of the essential oil

The inhibitory activity of the leaf essential oil of *P. lolot* against xanthine oxidase, *α*-amylase, and *α*-glucosidase was evaluated in the current study. The results (Table 2) indicated the inhibition of the essential oil against three tested enzymes with IC_{50} values of 28.4, 130.6, and 59.1 μg/mL, respectively. The above IC_{50} values implied that the leaf oil of *P. lolot* shows different preferences toward the *α*-amylase and *α*-glucosidase enzymes. Remarkably, the essential oil exhibited potent anti-*α*-glucosidase activity which was approximately four times stronger than the positive control (acarbose, IC_{50} = 201.4 μg/mL). Recently, some authors reported that essential oils from the *Piper* species inhibit enzymes associated with diabetes and gout such as xanthine oxidase (*P. cubeba*, *P. nigrum*, and *P. betle*), *α*-amylase (*P. nigrum* and *P. guineense*), and *α*-glucosidase (*P. guineense* and *P. longum*) [26–30]. The essential oil from *P. guineense*, composed mainly of *β*-pinene (41.24%), 1,8-cineole (17.22%), *α*-pinene (13.63%), *γ*-terpinene (5.68%), myrcene (4.37%), *cis*-ocimene (3.63%), allo-ocimene (3.43%), *α*-thujene (2.98%), and pinene-2-ol (2.79%), displayed inhibitory activities against *α*-amylase and *α*-glucosidase enzymes with EC_{50} values of 86.06 and 68.29 mL/L, respectively [30]. Furthermore, the *P. nigrum* essential oil, containing *β*-caryophyllene (11.5%), limonene (10.8%), *β*-phellandrene (8.6%), *β*-pinene (7.4%), *α*-pinene (3.6%), 3-carene (2.5%), and sabinene (2.3%), showed good inhibition against *α*-amylase enzyme with an IC_{50} value of 125.41 μg/mL [28]. Recently, Andriana et al. (2019) reported that the essential oil from *P. nigrum*, comprising mainly *β*-caryophyllene (51.12%), *β*-thujene (20.58%), *β*-selinene (5.59%), *δ*-elemene (5.03%), and *α*-copaene (4.79%), and the essential oil from *P. cubeba*, with the main chemical constituents being terpinen-4-ol (42.41%), *α*-copaene (20.04%), *γ*-elemene (17.68%), *α*-cubebene (6.54%), and D-germacrene (2.50%), exhibited potent inhibition against xanthine oxidase enzyme with IC_{50} values of 77.11 and 54.87 μg/mL, respectively [26]. The above results indicate that the main chemical composition of essential oils extracted from species belonging to the genus *Piper* is quite diverse. Among them, *β*-caryophyllene is often present as a major chemical component in these essential oils, and it may be an important contributor to the inhibitory effects on xanthine oxidase, α-amylase, and α-glucosidase enzymes exhibited by the essential oils extracted from *Piper* species. This is consistent with the findings of our study. The *P. lolot* essential oil, which demonstrated good activity against xanthine oxidase, α-amylase, and α-glucosidase enzymes, also contains *β*-caryophyllene (20.6%). However, the available results are limited to establishing a definitive relationship. This is the first report on the anti-gout and anti-diabetic activities of the leaf essential oil from *P. lolot*. Several *in vivo* studies have reported the anti-diabetic effects of the *P. lolot* extracts on diabetic rats [31–34]. The previous *in vivo* studies along with *in vitro* results in the present study confirm that *P. lolot* is a potential source of anti-diabetic natural products.

3.3. Molecular docking analysis

Molecular docking is a crucial method for identifying the interaction between proteins and drug candidates [35]. To further investigate the molecular mechanism, the molecular docking method was used to validate *in vitro* results. Following our previous finding, the 3D structure of the XO enzyme was selected and prepared [36]. Meanwhile, the 3D structure of α-amylase and *α*-glucosidase were downloaded from the protein data bank (RCSB). The grid box was set to cover the interactive site between crystal compounds and target protein (Fig. 2A).

The chemical composition of *P. lolot* essential oil is very diverse. However, the bioactivity of essential oils is often determined by the dominant constituents. Therefore, six compounds with ratios greater than 5% including (*E*)-*β*-ocimene, *β*-elemene, *β*-caryophyllene, *β*-selinene, *trans*-muurola-4(14),5-diene and *β*-bisabolene were selected for molecular docking process. Two commercial compounds, acarbose and allopurinol, were also selected as references. The results were shown in Table 3.

Regarding XO and *α*-amylase, it can be seen that of the six compounds, most have similar or weaker binding affinity than the reference compound. This could be explained for the ability to inhibit the *in vitro* activity of the essential oil of *P. lolot* on these two proteins. Otherwise, all of the principal constituents have an affinity for *α*-glucosidase that is equivalent to or greater compared to acarbose. This result could be also explained for the strong inhibitory activity of the essential oil of *P. lolot* on these two proteins on *α*-glucosidase.

The interaction between six compounds and the targets was shown in **Supplementary S1**. It is highlighted that *β*-bisabolene had a

Table 2
Xanthine oxidase, *α*-amylase, and *α*-glucosidase inhibitory activities of the essential oil from the leaves of *Piper lolot*.

Samples	IC_{50} (μg/mL) ± SD		
	Xanthine oxidase	*α*-Amylase	*α*-Glucosidase
P. lolot essential oil	28.4 ± 1.7	130.6 ± 5.7	59.1 ± 2.0
Allopurinol[a]	13.3 ± 0.9	–	–
Acarbose[a]	-	55.5 ± 4.0	201.4 ± 5.8

[a] Positive control.

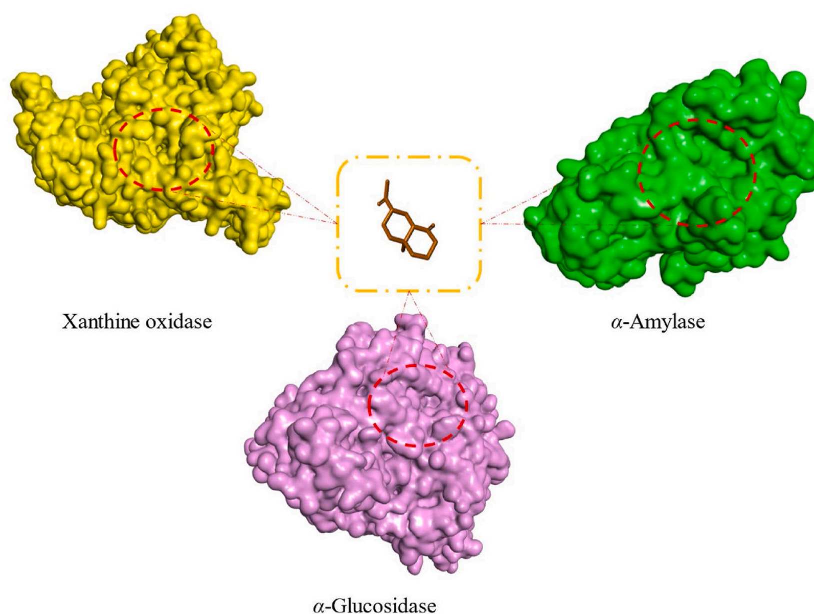

Fig. 2. Docking site of ligands to the target proteins.

Table 3
Docking scores between the compounds and the proteins.

No.	Compounds	Docking score (kcal/mol)		
		Xanthine oxidase	α-Amylase	α-Glucosidase
1	(*E*)-β-Ocimene	−5.4	−5.1	−5.3
2	β-Elemene	−6.3	−6.8	−5.6
3	β-Caryophyllene	−5.8	−7.4	−6.2
4	β-Selinene	−7.2	−7.3	−6.1
5	*trans*-Muurola-4(14),5-diene	−6.5	−6.9	−6.8
6	β-Bisabolene	−7.8	−7	−6.7
7	Acarbose		**−8.1**	**−5.6**
8	Allopurinol	**−7.1**		

strong affinity to three targets.

3.3.1. Interaction between β-bisabolene and xanthine oxidase

β-Bisabolene interacted with XO via six hydrophobic interactions (Fig. 3A). Three key residues at the active site of XO, Ala-910, Phe-1009, and Phe-914, were found to interact with β-bisabolene via Pi-Sigma and Pi-Alkyl. Meanwhile, the amino acids Ala-1078 and Ala-1079 interacted with β-bisabolene at distances of 4.27 Å and 3.81 Å, respectively. Aromatic structure of β-bisabolene also formed Pi-Alkyl interaction with Arg-912 with a minimum distance of 4.1 Å. In order to explore the binding mode of β-bisabolene, the active site of XO is shaped like a small and narrow gate located quite deep inside the protein surface. This is suitable for small molecules in space to bind to the protein.

3.3.2. Interaction between β-bisabolene and α-Amylase

β-Bisabolene interacted with α-amylase via three hydrophobic interactions (Fig. 3B). The amino acids Trp-59 and His-299 interacted with β-bisabolene at minimum distances of 4.56 Å and 6.12 Å, respectively. Meanwhile, the aromatic structure of β-bisabolene also formed Pi-Alkyl interaction with Tyr-62 with a minimum distance of 5.14 Å. The active site of α-Amylase is shaped like a small and narrow gate located quite deep inside the protein surface. This is suitable for small molecules in space to bind to the protein. The active site is a wide hole on the outside of the protein's surface. This was convenient for ligands to bind to proteins. This explained that a bulky molecule, acarbose, has a stronger binding affinity for proteins than essential oil molecules.

3.3.3. Interaction between β-Bisabolene and α-Glucosidase

β-Bisabolene interacted with α-glucosidase via seven hydrophobic interactions (Fig. 3C). The amino acids Trp-516 and His-674 interacted with β-bisabolene at minimum distances of 6.72 Å and 6.15 Å, respectively. Meanwhile, Phe-649 and Trp-613 had Alkyl interaction with β-bisabolene at minimum distances of 5.63 Å and 6.59 Å, respectively. In addition, Leu-677 and Leu-678 were found to

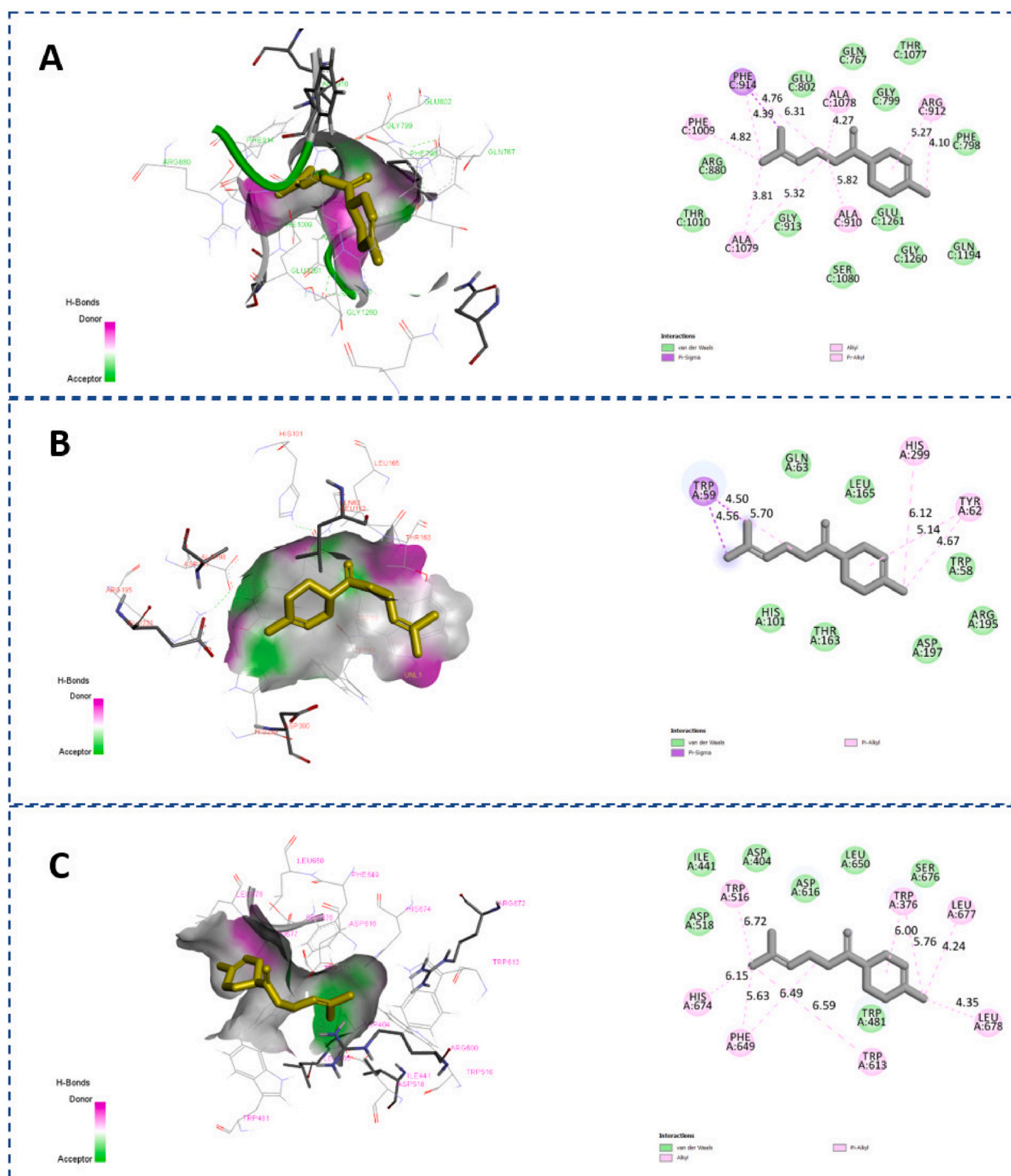

Fig. 3. Interaction between *β*-bisabolene and protein (**A**) Xanthine oxidase (**B**) *α*-Amylase (**C**) *α*-Glucosidase.

interact with the -CH3 group of *β*-bisabolene. The aromatic structure of *β*-bisabolene also formed to interact with Trp-376 with a distance of 6.00 Å.

Functional and structural studies of *α*-glucosidase revealed different active sites and allosteric sites [37]. Ding et al. (2018) indicated five interactive sites and interactions between oleanolic acid and ursolic and α-glucosidase at allosteric sites [38]. In this case, the position that compounds bind to *α*-glucosidase was simulated at the active site, where acarbose binds to the 3D crystal structure. The binding site is a narrow hole on the protein surface. Previous studies have demonstrated that acarbose is selective for *α*-glucosidase at this active site [39]. Therefore, most of the major components of the essential oil *P. lolot* had stronger affinities than acarbose is expected to explain for its strong inhibitory capacity *in vitro*.

β-Bisabolene is a sesquiterpene that is present in the composition of many plants. Previous studies have shown that *β*-bisabolene has cytotoxicity in breast cancer cell lines. In addition, *β*-bisabolene is effective in reducing the growth of transplanted 4T1 mammary tumors *in vivo* [40]. Besides, many synthetic compounds have been developed based on the structural framework of *β*-bisabolene against *Staphylococcus aureus* [41]. However, studies on the biological activity of *β*-bisabolene are still limited. From this result, further studies are needed to evaluate the ability of this compound to inhibit XO, *α*-amylase and *α*-glucosidase activities in experimental

experiments. This suggests that *β*-bisabolene has the potential to become hit compounds to develop drugs capable of treating gout and diabetes. In addition, essential oil components containing *β*-bisabolene also showed remarkable biological activities, for example, the antimicrobial and antioxidant potential of the essential oils of *Psammogeton canescens* and *Bupleurum longiradiatum* [42,43]. In previous studies, *β*-caryophyllene also showed inhibitory effects on *α*-glucosidase and *α*-amylase activities [44]. Additionally, essential oils containing *β*-caryophyllene exhibited anti-inflammatory activity, inhibiting XO activity [45,46]. From the GC-MS results, it can be seen that *β*-caryophyllene and *β*-bisabolene are the two components that account for the highest percentage of the essential oil content. Therefore, we also predict that the ability of essential oil *P. lolot* to inhibit the activity of XO, *α*-amylase and *α*-glucosidase is based on the synergistic effect of *β*-bisabolene and *β*-caryophyllene. *P. lolot* is used commonly in Vietnam in dishes. The species has a short life cycle, and it is easy to grow and collect this plant in different places. Although developing a health-promoting product needs long-time processes and effort, our results support further potential research on this plant.

4. Conclusions

The leaf essential oil of *P. lolot* collected from Thua Thien Hue province, Vietnam was composed of forty-seven constituents, in which *β*-caryophyllene (20.6%), *β*-bisabolene (11.6%), *β*-selinene (8.4%), *β*-elemene (7.7%), *trans*-muurola-4(14),5-diene (7.4%), and (*E*)-*β*-ocimene (6.7%) were six main components. The leaf oil showed significant inhibitory activities against xanthine oxidase, *α*-amylase, and *α*-glucosidase enzymes. Molecular docking simulation was used to further investigate the inhibitory mechanism between compounds and proteins. Our study suggests that *β*-bisabolene has the potential to become hit compounds to develop drugs capable of treating gout and diabetes. In addition, we also provide initial evidence that the essential oil of *P. lolot* may be a potential natural source to develop new diabetes preparations.

Author contribution statement

Tan Khanh Nguyen, Dat Ton That Huu, Linh Tran Thi Thuy: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper. </p>

Cuong Le Canh Viet: Conceived and designed the experiments; Wrote the paper. </p>

Thai Pham Hong, Ha Tran Phuong, Ty Pham Viet: Conceived and designed the experiments; Analyzed and interpreted the data. </p>

Duc Ho Viet, Duc Le Phu: Analyzed and interpreted the data. </p>

5. Data availability statement

Data included in article/supplementary material/referenced in article.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e19148.

References

[1] A. Pan, et al., Bidirectional association between diabetes and gout: the Singapore Chinese Health Study, Sci. Rep. 6 (1) (2016) 25766.
[2] F. Haidari, et al., Effects of onion on serum uric acid levels and hepatic xanthine dehydrogenase/xanthine oxidase activities in hyperuricemic rats, Pak J Biol Sci 11 (14) (2008) 1779–1784.
[3] A. Mittal, et al., The potential role for xanthine oxidase inhibition in major intra-abdominal surgery, World J. Surg. 32 (2) (2008) 288–295.
[4] T.N.T. Nguyen, et al., α-Glucosidase inhibitory activity and quantitative contribution of phenolic compounds from Vietnamese Aquilaria crassna leaves, Nat. Prod. Commun. 17 (3) (2022), 1934578X221080326.
[5] A. Sen, et al., Chemical composition, antiradical, and enzyme inhibitory potential of essential oil obtained from aerial part of Centaurea pterocaula Trautv, J. Essent. Oil Res. 33 (1) (2021) 44–52.
[6] R. Tundis, M.R. Loizzo, F. Menichini, Natural products as alpha-amylase and alpha-glucosidase inhibitors and their hypoglycaemic potential in the treatment of diabetes: an update, Mini Rev. Med. Chem. 10 (4) (2010) 315–331.
[7] V.V. Chi, The dictionary of medicinal plants in Vietnam. Medical publishing house, Hanoi 1 (2012) 1264–1265.
[8] C.Y. Li, et al., Isolation and identification of antiplatelet aggregatory principles from the leaves of Piper lolot, J. Agric. Food Chem. 55 (23) (2007) 9436–9442.

[9] N.H. Nam, Y.Y. Jae, *NF-κB inhibitory activities of the methanol extracts and some constituents therein of some Vietnamese medicinal plants.* Sci, Pharm. Times 77 (2) (2009) 389–399.
[10] M.T.T. Nguyen, et al., Screening of α-glucosidase inhibitory activity of Vietnamese medicinal plants: isolation of active principles from Oroxylum indicum, Nat. Prod. Sci. 18 (2012) 47–51.
[11] N.N. Thu, et al., The polyphenol content and antioxidant activities of the main edible vegetables in northern Vietnam, J. Nutr. Sci. Vitaminol. 50 (3) (2004) 203–210.
[12] P. Luger, et al., The crystal structure of 3-(4′-Methoxyphenyl)propanoyl pyrrole of Piper lolot C.DC from Vietnam, Cryst. Res. Technol. 37 (6) (2002) 627–633.
[13] N.X. Dung, et al., Compositional Analysis of the leaf, stem and rhizome oils of Piper lolot C. DC. from Vietnam, J. Essent. Oil Res. 8 (6) (1996) 649–652.
[14] D. Joulain, W.A. Koenig, The Atlas of Spectral Data of Sesquiterpene Hydrocarbons, E.B. Verlag, Hamburg, 1989.
[15] R.P. Adams, in: Identification of essential oil components by gas chromatography/mass spectrometry, 4.1th Edition, Allured Publishing, Carol Stream, Illinois, 2017, pp. 1–184.
[16] L.G. Ranilla, et al., Phenolic compounds, antioxidant activity and in vitro inhibitory potential against key enzymes relevant for hyperglycemia and hypertension of commonly used medicinal plants, herbs and spices in Latin America, Bioresour. Technol. 101 (12) (2010) 4676–4689.
[17] C. Hansawasdi, J. Kawabata, T. Kasai, Alpha-amylase inhibitors from roselle (Hibiscus sabdariffa linn.) tea, Biosci., Biotechnol., Biochem. 64 (5) (2000) 1041–1043.
[18] T. Noro, et al., Inhibitors of xanthine oxidase from the flowers and buds of Daphne genkwa, Chem. Pharmaceut. Bull. 31 (11) (1983) 3984–3987.
[19] W. Kaplan, T.G. Littlejohn, Swiss-PDB viewer (deep view), Brief Bioinform 2 (2) (2001) 195–197.
[20] N.M. O'Boyle, et al., Open Babel: an open chemical toolbox, J. Cheminf. 3 (1) (2011) 33.
[21] O. Trott, A.J. Olson, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading, J. Comput. Chem. 31 (2) (2010) 455–461.
[22] S.S. Dahham, et al., The anticancer, antioxidant and antimicrobial properties of the sesquiterpene β-caryophyllene from the essential oil of Aquilaria crassna, Molecules 20 (7) (2015) 11808–11829.
[23] K. Fidyt, et al., β-caryophyllene and β-caryophyllene oxide-natural compounds of anticancer and analgesic properties, Cancer Med. 5 (10) (2016) 3007–3017.
[24] J. Legault, A. Pichette, Potentiating effect of β-caryophyllene on anticancer activity of α-humulene, isocaryophyllene and paclitaxel, J. Pharm. Pharmacol. 59 (12) (2007) 1643–1647.
[25] V. Babushok, P. Linstrom, I. Zenkevich, Retention indices for frequently reported compounds of plant essential oils, J. Phys. Chem. Ref. Data 40 (2011) 1–47.
[26] Y. Andriana, et al., Biological activities and chemical constituents of essential oils from Piper cubeba Bojer and Piper nigrum L, Molecules (10) (2019) 24.
[27] S. Kumar, S. Sharma, N. Vasudeva, Screening of antidiabetic and antihyperlipidemic potential of oil from Piper longum and piperine with their possible mechanism, Expert Opin Pharmacother 14 (13) (2013) 1723–1736.
[28] M.R. Loizzo, et al., Anti-rancidity effect of essential oils, application in the lipid stability of cooked Turkey meat patties and potential implications for health, Int. J. Food Sci. Nutr. 66 (1) (2015) 50–57.
[29] F. Mahomoodally, et al., Antiglycation, comparative antioxidant potential, phenolic content and yield variation of essential oils from 19 exotic and endemic medicinal plants, Saudi J. Biol. Sci. 26 (7) (2019) 1779–1788.
[30] G. Oboh, et al., Antioxidative properties and inhibition of key enzymes relevant to type-2 diabetes and hypertension by essential oils from black pepper, Adv Pharmacol Sci 2013 (2013) 926047.
[31] C. Krisanapun, et al., In vitro evaluation of anti-diabetic potential of Piper sarmentosum Roxb. extract, Faseb. J. 26 (S1) (2012), 6867-686.7.
[32] P. Peungvicha, et al., Hypoglycemic effect of the water extract of Piper sarmentosum in rats, J. Ethnopharmacol. 60 (1) (1998) 27–32.
[33] Z.C. Thent, et al., Histological changes in the heart and the proximal aorta in experimental diabetic rats fed with Piper sarmentsoum, Afr J Tradit Complement Altern Med 9 (3) (2012) 396–404.
[34] Z.C. Thent, et al., Effect of Piper sarmentosum extract on the cardiovascular system of diabetic sprague-dawley rats: electron microscopic study, Evid Based Complement Alternat Med 2012 (2012) 628750.
[35] L.T.T. Tran, et al., In Silico and in vitro evaluation of alkaloids from Goniothalamus elegans Ast. for breast cancer treatment, Nat. Prod. Commun. 17 (3) (2022), 1934578X221088110.
[36] L.T.T. Tran, et al., Virtual screening and in vitro evaluation to identify a potential xanthine oxidase inhibitor Isolated from Vietnamese Uvaria cordata, Nat. Prod. Commun. 17 (2) (2022), 1934578X221080339.
[37] V. Roig-Zamboni, et al., Structure of human lysosomal acid α-glucosidase–a guide for the treatment of Pompe disease, Nat. Commun. 8 (1) (2017) 1111.
[38] H. Ding, et al., Inhibitory mechanism of two allosteric inhibitors, oleanolic acid and ursolic acid on α-glucosidase, Int. J. Biol. Macromol. 107 (2018) 1844–1855.
[39] N. Ur Rehman, et al., α-Glucosidase inhibition and molecular docking studies of natural brominated metabolites from marine macro Brown alga dictyopteris hoytii, Mar. Drugs 17 (12) (2019) 666.
[40] S.K. Yeo, et al., β-Bisabolene, a sesquiterpene from the essential oil extract of opoponax (commiphora guidottii), exhibits cytotoxicity in breast cancer cell lines, Phytother Res. 30 (3) (2016) 418–425.
[41] A.M.A. Nascimento, et al., Synergistic bactericidal activity of Eremanthus erythropappus oil or β-bisabolene with ampicillin against Staphylococcus aureus, Antonie Leeuwenhoek 92 (1) (2007) 95–100.
[42] B. Shi, et al., Chemical composition, antibacterial and antioxidant activity of the essential oil of Bupleurum longiradiatum, Nat. Prod. Commun. 5 (7) (2010) 1139–1142.
[43] M. Kazemi, H. Rostami, Chemical composition, antimicrobial and antioxidant activities of the essential oil of Psammogeton canescens, Nat. Prod. Res. 29 (3) (2015) 277–280.
[44] D.W. Yan, et al., Meroterpene-like α-glucosidase inhibitors based on biomimetic reactions starting from β-caryophyllene, Molecules (2) (2020) 25.
[45] M.H. Mahnashi, et al., Phytochemical analysis, α-glucosidase and amylase inhibitory, and molecular docking studies on persicaria hydropiper L. Leaves essential oils, Evid Based Complement Alternat Med 2022 (2022), 7924171.
[46] M.M. Hudaib, et al., Xanthine oxidase inhibitory activity of the methanolic extracts of selected Jordanian medicinal plants, Pharmacogn Mag 7 (28) (2011) 320–324.