Journal of Essential Oil Bearing Plants

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/teop20

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To cite this article: Hieu Tran-Trung, Phan Thi Thuy, Vi Thi Thuan, Nguyen Xuan Ha, Nguyen Van Hue, Hieu Nguyen-Ngoc, Trang H.D. Nguyen, Nguyen Hoang Tuan, Tran Van Chen, Nguyen Thi Thu Hien & Le Duc Giang (2023) Chemical composition and antimicrobial activity of essential oil obtained from the rhizomes of *Kaempferia champasakensis*: *in vitro* and molecular docking studies, Journal of Essential Oil Bearing Plants, 26:4, 958-969, DOI: 10.1080/0972060X.2023.2251512

To link to this article: https://doi.org/10.1080/0972060X.2023.2251512

Journal of Essential Oil Bearing Plants
https://www.tandfonline.com/journals/teop
DOI: 10.1080/0972060X.2023.2251512

Taylor & Francis
Taylor & Francis Group
ISSN Print: 0972-060X; ISSN Online: 0976-5026

Research Article

Chemical composition and antimicrobial activity of essential oil obtained from the rhizomes of *Kaempferia champasakensis*: *in vitro* and molecular docking studies

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Received 3 July 2023
Revised 20 July 2023
Accepted 15 August 2023

Abstract
Kaempferia champasakensis Picheans. & Koonterm (Zingiberaceae) is locally known as "Dia Lien hoa trang" in Vietnam. Despite a previous study on the essential oils (EO) of *K. champasakensis* leaves, no other studies have reported the chemical components and biological activities of *K. champasakensis*. Therefore, this study aims to identify the chemical constituents of the EO from the rhizomes of *K. champasakensis* and evaluate their antimicrobial activity, which has been re-confirmed through molecular docking studies. The gas chromatography-mass spectrometry (GC/MS) analysis revealed sixty-one constituents (95.4%) present in the EO of *K. champasakensis* rhizomes. Among them, camphene (40.7%), α-pinene (10.2%), and β-pinene (7.6%) were identified as the main compounds in the EO. Bioassays indicated its antimicrobial activity in the order of inhibitory effectiveness: *Staphylococcus aureus* > *Enterococcus faecalis* ≈ *Candida albicans* > *Bacillus cereus* ≈ *Escherichia coli* > *Salmonella enterica* > *Pseudomonas aeruginosa*. The molecular docking study of ligand-protein interactions involving the major constituents of EO was conducted against target proteins related to *Staphylococcus aureus*, *Enterococcus faecalis, Candida albicans*, *Bacillus cereus*, and *Escherichia coli* main activities. The results revealed that α-pinene exhibited strong binding affinity against *E. coli*, β-pinene demonstrated more effective inhibition against *E. faecalis* and *B. cereus*, and camphene primarily exhibited activity against *C. albicans* and *S. aureus*. Furthermore, the analysis of interactions between the main compounds and the selected proteins was elucidated in the current study. This is the first time that chemical components of the EO of *K. champasakensis* rhizomes and their antimicrobial activity have been reported. These findings contributed to the basis for further studies on the chemical compositions and biological effects of *K. champasakensis*.

Keywords
Antimicrobial activity, Essential oil, *Kaempferia champasakensis*, Molecular docking

Introduction

Kaempferia L. is a genus of plants that can be found across Southeast Asia, China and India, with approximately 60 different species[1]. Some of these species have been commonly used in traditional medicine. For example, *K. galanga* has been traditionally used to treat a range of conditions in China, including cholera, contusions, constipation, and stomachaches[2].

In Bangladesh's traditional medicine, *K. rotunda* is used to manage elevated blood sugar levels that are frequently seen in individuals with diabetes[3]. Besides, the essential oil (EO) extracted from the rhizome of *K. rotunda* is utilized as a preservative for food due to its capacity to prevent the growth of microbes, protein degradation, and lipid oxidation in fish fillets[4]. Research has indicated that EO obtained from *Kaempferia* species possesses multiple bioactivities that are important to human health. For instance, *K. galanga* rhizome EO contains ethyl *p*-methoxycinnamate which reportedly played an important role in its flavor and fragrances, making it useful in aromatherapy[2,5,6]. In addition, this compound was shown to exhibit inhibitory effects on cyclooxygenase enzymes 1 (COX-1) and 2 (COX-2) by 42.9% and 57.8%, respectively, comparable with indomethacin used as a positive control (82.8% and 54.6%)[7]. The essential oils from *K. parviflora* showed potential antioxidant, anti-inflammatory, anti-cholinesterase, tyrosinase, and antidiabetic activities[8].

K. champasakensis Picheans. & Koonterm, a new species from Champasak - Southern Laos, was recognized in 2008[9]. It was later found in Vietnam during a ginger study conducted in Binh Thuan and Ba Ria-Vung Tau provinces from 2015 to 2016[10]. This plant, locally known as "Dia Lien hoa trang" in Vietnam, is a perennial herb with a long, slender, creeping rhizome. The EO from *K. champasakensis* leaves has recently been investigated, showing multiple major constituents, such as β-caryophyllene (16.1%), β-elemenone (14.5%), β-pinene (13.8%), germacrone (9.9%), myrcene (9.5%) and diisooctyl phthalate (8.6%)[11]. This research also showed a distinctive volatile profile of *K. champasakensis* leaf EO in comparison with those from the other *Kaempferia* species. In the search for natural compounds with antibacterial activity from essential oil-bearing plants, *K. champasakensis* was one of the plants that we investigated based on its available information. Although species of the genus *Kaempferia* (e.g., *K. galanga, K. rotunda*, etc.) have been widely used in traditional medicine, so far, information on *K. champasakensis* has been still limited in the literature. Especially, to the best of our knowledge, no additional information about the chemical composition and antimicrobial activity of the EO of the species rhizomes has been reported. In this study, the EO from the rhizomes of *K. champasakensis* was analyzed and evaluated for its antimicrobial activity, and molecular docking studies were conducted to validate the results of *in vitro* activity. The results of the study will enhance our comprehension of the bioactive constituents present in the species, as well as the potential therapeutic benefits of its EO.

Materials and methods

Plant material

With the help of local villagers, *K. champasakensis* rhizomes were collected from Big Mountain, Ward 1, Vung Tau City, Ba Ria - Vung Tau Province, Vietnam in June 2022 and were identified by Assoc. Prof. Dr. Nguyen Hoang Tuan from the Faculty of Pharmacognosy and Traditional Medicine, Hanoi University of Pharmacy. The voucher specimen (NHTuan 006) has been deposited at the Herbarium of Hanoi National University, Vietnam.

Extraction of the EO

The EO of *K. champasakensis* rhizomes (500 g fresh sample) was extracted using a Clevenger-type apparatus connected to a cooling bath system for 4 hours (beginning from the water boiling point until the amount of obtained EO was constant). The isolation of EO was repeated three times. To remove excessive water, the EO was dried with anhydrous sodium sulfate (Na_2SO_4) and stored in a refrigerator at 4°C before analysis of chemical composition and evaluation of antimicrobial activity[12-14].

Gas chromatography/mass spectrometry (GC/MS) analysis and identification of individual components

The *K. champasakensis* rhizomes EO was analyzed using an Agilent Technologies 7890B-GC gas chromatography equipped with an Agilent 5977B MSD model mass spectrometer

and an HP5-MS UI column (30 m × 0.25 mm i.d., film thickness: 0.25 µm), as reported by Hieu Tran-Trung *et al.*[11]. The carrier gas used was Helium (He) with a flow rate of 1.0 mL/min. The diluted sample of 1.0 µL was injected in the split/splitless (50:1 split) mode. The injector temperature was set at 300°C, MS Quad temperature was fixed at 150°C, while the transfer line temperature and MS source were set at 300°C and 230°C, respectively. The oven temperature program was fixed from 50 (held for 2 min) to 150°C with a rate of 5°C/min, kept at 150°C for 10 min and then increased to 280°C with a rate of 10°C/min and finally kept at this temperature for 10 min. The ionization energy was 70 eV (EI), with a scan mass range of 50-550 m/z at 2.0 scan/s. Compounds identification in the EO of *K. champasakensis* rhizomes was performed by comparing their retention indices (RI) and mass spectra with those in the literature (NIST17 and Adams book[15]), while the RI values were calculated as previously reported[13,14].

Finally, the relative peak area percentage was used to quantify the EO compounds, as mentioned in previous studies[11,12,16].

In vitro antimicrobial assay

The antimicrobial screening test of the EO was conducted according to Hadacek and Greger's method as previously described[17,18]. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enterica* ATCC 13076, *Enterococcus faecalis* ATCC 299212, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 14579, and *Candida albicans* ATCC 10231 were provided by the National Institute for Food Control. The stock solution of the EO was diluted with 1% DMSO. Briefly, the bacterial and yeast strains were incubated to obtain approximately 2×10^5 CFU/mL, after which a volume of 50.0 µL of broth was inoculated and maintained in Luria-Bertani (LB) medium with each different dilutions (equivalent to a range of concentrations: 256.0, 128.0, 64.0, 32.0, 16.0, 8.0, 4.0, and 2.0 µg/mL) of the EO and EO-free solutions, all of which were incubated overnight at 37°C. The minimum inhibitory concentration (MIC value) was determined to be the lowest concentration of the EO with no visible growth of microorganisms after 24 hours of incubation. The mixtures of bacterial cells-tetracycline and yeast cells-cycloheximide were used in the positive control. All assays were performed in triplicate.

Molecular docking study

To provide an overview of the binding mechanisms between the suggested compounds and active site amino acid residues, molecular docking is performed for the examined compounds against the active site of *C. albicans* N-myristoyltransferase, *E. coli* enoyl reductase-nad+-triclosan, *E. faecalis* Carbamate kinase, *S. aureus* TyrRS, and *B. cereus* PatB1. The AutoDock Vina v1.2.3 software is utilized for molecular docking in the present study[19]. The structures of the three main compounds in the EO from *K. champasakensis* rhizome, including α-pinene, β-pinene and camphene, are drawn using Marvin Sketch software, and the MMFF94s force field in the Avogadro program is employed for their optimization[20]. The crystal structures of the *C. albicans* N-myristoyltransferase, *E. coli* enoyl reductase-nad+-triclosan, carbamate kinase (*Enterococcus faecalis*), *S. aureus* TyrRS, and *B. cereus* PatB1 are obtained from the RCSB protein data bank with corresponding PDB IDs 1IYL, 1C14, 2WE5, 1JIJ, and 5V8E[21-25]. The protein is prepared for docking by removing co-crystallized ligands, adding hydrogen atoms, assigning Kollman partial charges, and converting it to the PDBQT format using AutodockTools v1.5.6 software. The grid box for the 1IYL, 1C14, 2WE5, 1JIJ, and 5V8E proteins is set to encompass the active sites with a corresponding size of 22x22x22 Å, and the box center is located at the co-crystallized ligand central coordinates. The exhaustiveness parameter used in this study is set to 400, as reported in previous studies[26,27]. The co-crystallized ligands are redocked into the protein's active site to confirm the suitability and reliability of the docking protocol. The best poses of each compound from the molecular docking simulations are selected for analyzing the protein-ligand interactions using Discovery Studio Visualize software.

Results and discussion

Chemical composition of the EO

The EO hydrodistilled from *K. champasakensis* rhizome was pale-yellow and lighter than water. By using hydrodistillation, the yield of the EO from *K. champasakensis* rhizome was approximately 0.12% (w/w) of fresh weight. The chemical composition of the rhizome EO was analyzed by the GC/MS method (Fig. S1). The result showed that sixty-one constituents were identified in the EO of *K. champasakensis* rhizome, representing 95.4% of the total EO (Table 1). Besides, the present results indicated that monoterpene hydrocarbons were the major volatile compound group with a percentage of 69.5% in total content, followed by sesquiterpene hydrocarbons (15.2%) and oxygenated hydrocarbons (10.6%). The major compound was identified as camphene (40.7%), and the other two main compounds were α-pinene (10.2%) and β-pinene (7.6%). These major chemical constituents are also found in the essential oils of *K. larsenii* rhizome (e.g., camphene (48.0%), α-pinene (15.09%), β-pinene (6.63%))[28], *K. daklakensis* rhizome (e.g., camphene (23.63%), α-pinene (3.22%), camphor (4.42%))[29]. The chemical composition of *K. champasakensis* rhizome EO was considerably different from that of the leaf EO with the major chemical compositions of β-caryophyllene (16.1%), β-elemenone (14.5%), β-pinene (13.8%), germacrone (9.9%), myrcene (9.5%) and diisooctyl phthalate (8.6%)[11]. Furthermore, the main composition of *K. champasakensis* rhizome oil was also different from that of *K. rotunda* rhizome oil (e.g., benzoic acid (58.25 ± 0.14%), bornyl acetate (14.4±0.35%), zingiberene (5.75 ± 0.08%), and β-myrcene (3.87 ± 0.08%))[30], *K. galanga* rhizome oil from Thailand (e.g., ethyl-p-methoxy-cinnamate (31.77%), methylcinnamate (23.23%), carvone (11.13%), eucalyptol (9.59%), and pentadecane (6.41%))[31], *K. galanga* rhizome oil from India (e.g., *trans*-ethyl-p-methoxycinnamate (28.4-70.0%), *trans*-ethyl cinnamate (11.5-26.6%), δ-3-carene (0.1-6.5%), 1,8-cineole (0.2-5.2%), borneol (1.0-2.4%), and pentadecane (6.0-16.5%))[32].

Evaluation of in vitro antimicrobial activity

The antimicrobial activity of EO from *K. champasakensis* rhizomes against the pathogenic microorganisms tested via the MIC values is presented in Table 2. This EO was inhibitory toward all the examined strains. The results demonstrated the antibacterial and anti-yeast activities of *K. champasakensis* rhizome EO with a high activity level (MIC of 8 - 128 µg/mL). Specifically, considering the antimicrobial assay, the EO proved to have the strongest inhibition against *S. aureus* (MIC = 8 µg/mL), followed by *E. faecalis* and *C. albicans* (MIC = 16 µg/mL). Moreover, the EO of *K. champasakensis* rhizomes showed better inhibition against *B. cereus* and *E. coli* (MIC = 32 µg/mL) than *S. enterica* (MIC = 64 µg/mL) and *P. aeruginosa* (MIC = 128 µg/mL). For the positive control, the minimum bacterial and yeast inhibitory concentrations of tetracycline and cycloheximide ranged from 4 µg/mL to 256 µg/mL. Previous studies have reported that the EO has various biological effects such as antibacterial, antifungal, and antioxidant activities, etc[33]. Thus, numerous EO-bearing herbs have been reported as potential sources of antimicrobial agents[33,34]. In particular, the EO of *K. champasakensis* rhizomes, especially monoterpene hydrocarbons (such as camphene, α-pinene, β-pinene, limonene, etc), is the significant pharmacological basis for the antimicrobial activity of *K. champasakensis* rhizomes. This argument has been confirmed in previous studies[35,36]. Interestingly, the important chemical compounds (e.g., camphene, 1,8-cineol, camphor, limonene, etc.) are similar to those found in other EO-bearing plants (e.g., *K. rotunda*, *K. angustifolia*, *K. galanga*, *K. larsenii*, *K. daklakensis*, etc.)[28,29,37,38]. Camphene is found abundantly in the EOs. In previous biological investigations, camphene has been shown to possess various pharmacological effects such as antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, antidiabetic, and anti-leishmanial properties, etc[39]. Moreover, in another work, Badawy *et al.* reported camphene, α-pinene, β-myrcene, terpinen-4-ol, camphor, and linalool, as potential antibacterial agents against pathogenic strains such as *Escherichia coli* and *Staphylococcus aureus*[40]. Similarly, limonene is one of the common monoterpene hydrocarbons in volatile oils of various plants

Table 1. Chemical composition of the EO of *K. champasakensis* rhizomes

No.	RT (min)	RI (obsd.)	RI (lit.)	Compounds	Content (%)
1	6.824	926	925	Tricyclene	3.1
2	6.967	932	929	3-Thujene	0.1
3	7.156	939	937	α-Pinene	10.2
4	7.596	955	952	Camphene	40.7
5	8.260	978	974	4(10)-thujene	0.5
6	8.346	980	979	β-Pinene	7.6
7	8.752	993	991	β-Myrcene	1.9
8	9.135	1006	1005	α-Phellandrene	0.1
9	9.307	1012	1011	δ-3-Carene	0.1
10	9.725	1028	1025	p-Cymene	0.2
11	9.851	1033	1030	Limonene	4.2
12	9.931	1036	1032	1,8-Cineol (= Eucalyptol)	0.7
13	10.263	1047	1045	2-Heptanol, acetate	0.1
14	10.423	1053	1049	(E)-β-ocimene	0.1
15	10.738	1063	1060	γ-Terpinene	0.2
16	11.613	1091	1088	Terpinolene	0.5
17	11.945	1100	1099	Linalool	0.1
18	13.267	1149	1145	Camphor	2.4
19	13.885	1170	1167	Endo-borneol	0.4
20	14.222	1181	1177	Terpinen-4-ol	0.1
21	14.783	1199	1193	Myrtenal	0.1
22	15.676	1233	1226	Bornyl formate	0.4
23	17.284	1289	1285	Bornyl acetate	1.4
24	18.674	1343	1338	δ-Elemene	0.3
25	19.710	1381	1376	α-Copaene	0.2
26	20.122	1396	1391	β-Elemene	0.5
27	20.351	1405	1399	Cyperene	2.9
28	20.597	1415	1409	α-Gurjunene	0.2
29	20.854	1426	1419	β-Caryophyllene	2.8
30	21.083	1435	1432	β-Copaene	0.1
31	21.163	1438	1433	γ-Elemene	0.2
32	21.341	1445	1440	Aromadendrene	0.1
33	21.438	1449	1447	Selina-5,11-diene	0.2
34	21.569	1454	1453	Aristolene	0.1
35	21.701	1460	1454	α-Humulene	0.5
36	21.890	1467	1461	Allo-aromadendrene	0.2
37	21.953	1469	1466	epi-β-Caryophyllene	1.3
38	22.227	1480	1477	γ-Muurolene	1.0
39	22.399	1486	1481	Germacrene D	0.7

table 1. (continued).

No.	RT (min)	RI (obsd.)	RI (lit.)	Compounds	Content (%)
40	22.479	1489	1487	Aristolochene	0.3
41	22.536	1491	1486	β-Selinene	0.4
42	22.771	1500	1494	α-Selinene	1.2
43	22.880	1504	1499	α-Muurolene	0.1
44	23.286	1517	1513	γ-Cadinene	0.5
45	23.372	1520	1514	β-Curcumene	0.2
46	23.544	1525	1524	Cadina-1(10),4-diene	1.0
47	23.978	1539	1533	4,10-dimethyl-7-isopropyl[4,4,0]-bicyclo-1,4-decadiene	0.1
48	24.327	1550	1538	α-Cadinene	0.1
49	24.751	1563	1559	Hedycaryol	0.2
50	25.329	1580	1577	Spathulenol	0.3
51	25.540	1587	1581	Caryophyllene oxide	0.5
52	25.912	1597	1591	Viridiflorol	0.1
53	26.130	1603	1593	*Cis*-β-elemenone	0.2
54	26.307	1607	1597	*Trans*-β-elemenone	2.1
55	26.731	1616	1614	Di-epi-1,10-cubenol	0.1
56	27.881	1641	1640	τ-Cadinol	0.1
57	28.470	1653	1653	α-Cadinol	0.2
58	30.570	1695	1693	Germacrone	0.8
59	33.603	1765	1762	Neocurdione	0.1
60	35.051	1797	1809	Ambrial	0.2
61	36.155	1845	1844	Curcumenone	0.1
	Identified				95.4
	Monoterpene hydrocarbons (No. 1-11, 14-16)				69.5
	Oxygenated monoterpenes (No. 12, 17-23)				5.6
	Sesquiterpene hydrocarbons (No. 24-48)				15.2
	Oxygenated sesquiterpenes (No. 49-61)				5.0
	Other (No. 13)				0.1

RT (min): Retention time; RI (obsd.): Retention indices of compounds on the HP-5MS UI column; RI(lit.): Retention indices of compounds obtained from the library

(e.g., *K. angustifolia*, *K. galanga*, *K. daklakensis*, etc)[29,37,38]. Limonene exhibits significant inhibitory activity against gram-negative and gram-positive bacteria as well as fungal activity such as *Aspergillus niger*, *P. aeruginosa*, *S. aureus* and *E. coli*[41,42] and it is safe and low in toxicity[43]; therefore, limonene has wide application prospects in antibacterial and food preservation[42]. In previous studies reported that 1,8-cineol is one of the oxygenated monoterpenes with various biological effects, such as analgesic, anti-inflammatory, anti-nociceptive, antibacterial, cytotoxic, antioxidant, anti-tumor, vasorelaxant and fumigant activities[31,38]. This component is also widely distributed in volatile oils from other plants such as *K. galanga*, *K. daklakensis,* etc[29,38]. In general, the essential oil of *K. champasakensis* exhibited significant activity against *S. aureus* like *K. larsenii*[28], *K. daklakensis*[29], *K. galanga*[6]. In particular,

Table 2. Antimicrobial activity of the EO of *K. champasakensis* rhizomes

Microorganisms	MIC (µg/mL)		
	EO	Tetracycline	Cycloheximide
E. faecalis ATCC 299212	16 ± 0.48	4	NT
S. aureus ATCC 25923	8 ± 0.13	16	NT
B. cereus ATCC 14579	32 ± 0.39	64	NT
E. coli ATCC 25922	32 ± 0.43	8	NT
P. aeruginosa ATCC 27853	128 ± 0.87	256	NT
S. enterica ATCC 13076	64 ± 0.14	64	NT
C. albicans ATCC 10231	16 ± 0.87	NT	32

NT: Not tested; Mean ± SD, n = 3

compared with *K. champanensis* rhizome EO in our study, *K. daklakensis* rhizome EO had no inhibitory effect on the growth of *E. coli*, *P. aeruginosa*, and *C. albicans*[29]. Additionally, the EOs from *Kaempferia* species such as *K. ganlanga*, *K. daklakensis*, *K. champasakensis*, etc., all showed stronger bacterial inhibition against gram-positive bacteria than gram-negative bacteria[6,29]. One of the important mechanisms of the antibacterial activity of EOs is the induction of protein denaturation on bacterial cell membranes[28]. This could explain why the EOs exhibit weak activity against Gram-negative bacteria possibly due to the complex and thick outer lipopolysaccharide membrane (e.g., peptidoglycan structure, porin channel) of Gram-negative bacteria[28,44]. The present study revealed that *K. champasakensis* rhizome essential oil is rich in camphene which has possible to isolate this important part as a source of camphene and use them in the pharmaceutical industry for different purposes. Again, this study demonstrated the strong antibacterial and antifungal properties of *K. champasakensis* rhizome EO. In light of this information, in-depth studies on *K. champasakensis* are needed to elucidate and confirm its uses in the pharmaceutical field.

Molecular docking study

In this study, the molecular docking process was performed using the AutoDock Vina v1.2.3 program to evaluate the inhibitory potential and interactions between the main components in the EO of *K. champasakensis* rhizomes and target proteins related to antibacterial and antifungal mechanisms. Prior to commencing the procedure, a re-docking process was conducted, followed by a comparison of the co-crystallized ligand with the predicted re-docked position to validate the docking process. The effectiveness of the procedure was confirmed by the root mean square deviation (RMSD) result of 1.17689 Å (RMSD < 2Å), corresponding to the co-crystallized ligand in the represented 1JIJ protein complex (Fig. 1).

Subsequently, the main components from the EO of *K. champasakensis* rhizomes, including α-pinene, β-pinene, and camphene, were docked into the active site of proteins 1IYL, 1C14, 2WE5, 1JIJ, and 5V8D. The obtained results are presented in Table 3. The binding affinities of the compounds ranged from -5.485 to -5.769 kcal/mol for protein 1IYL, -3.87 to -4.119 kcal/mol for protein 2WE5, -4.574 to -5.348 kcal/mol for protein 1JIJ, -5.939 to -6.161 kcal/mol for protein 1C14 and -4.364 to -4.649 for protein 5V8E, respectively. The monoterpene hydrocarbon compound, α-pinene exhibited the highest binding affinity compared to other compounds when docked into the active site of protein enoyl ACP reductase (*E. coli*). On the other hand, β-pinene demonstrated the most effective inhibitory potential against the proteins carbamate kinase (*E. faecalis*) and thiophene-2-carboxyaldehyde methane-sulfonyl-hydrazone (*B. cereus*). Finally, camphene exhibited higher evaluation scores than α-pinene and β-pinene due to its strong binding affinity with the proteins

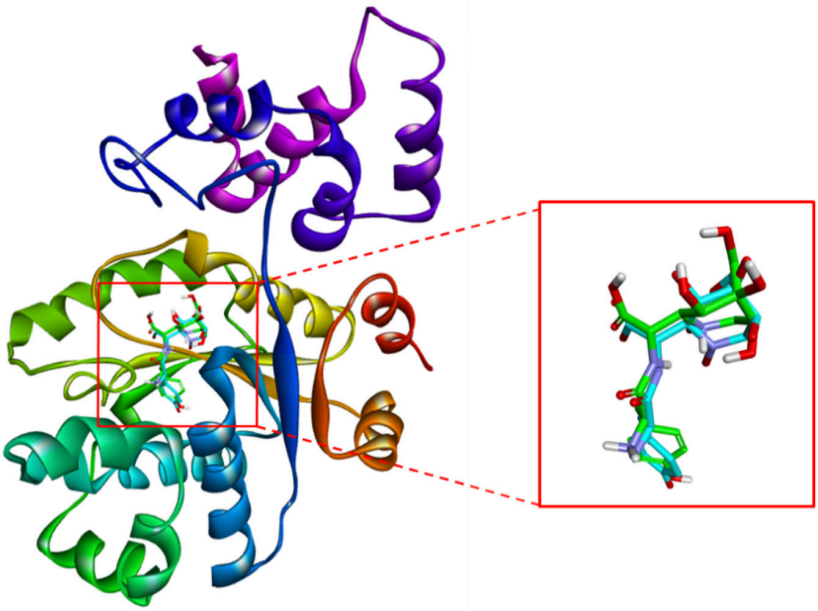

Figure 1. The re-docking result of the SB-239629 and superimposing their binding poses at the active pocket of 1JIJ protein

Table 3. Binding affinity of the major constituents with *C. albicans* N-myristoyltransferase (PDB ID 1IYL), *E. coli* enoyl reductase-nad+-triclosan (PDB ID 1C14), *E. faecalis* Carbamate kinase (PDB ID 2WE5), *S. aureus* TyrRS (PDB ID 1JIJ), and *B. cereus* PatB1 (PDB ID 5V8E) protein targets

Compounds	Binding affinity ΔG (kcal/mol)				
	1IYL	2W5E	1JIJ	1C14	5V8E
α-Pinene	-5.485	-3.909	-4.694	-6.161	-4.499
β-Pinene	-5.565	-4.119	-4.574	-6.070	-4.649
Camphene	-5.769	-3.870	-5.348	-5.939	-4.364

N-myristoyltransferase (*C. albicans*) and tyrosyl-tRNA synthetase (*S. aureus*).

Furthermore, the interactions between the selected proteins and compounds were visualized in Fig. S2. The result showed that the ligands formed several hydrophobic interactions with different amino acid residues at the active site of *S. aureus* tyrosyl-tRNA synthetase. With α-pinene, three interactions were formed, including one π-σ interaction and one π-alkyl interaction. Residues His50 and Pro53 established π-alkyl interactions with the ligand, while residue Phe54 formed a π-σ interaction. Another monoterpene, β-pinene, formed a similar π-alkyl interaction with α-pinene. However, the binding positions in the molecule were arranged differently, resulting in the absence of a π-σ interaction for β-pinene. Instead, two new π-alkyl interactions were observed with residues Ala39 and Cys37. Among the compounds, camphene formed the highest number of π-alkyl interactions at the active site of *S. aureus* tyrosyl-tRNA synthetase. This compound also exhibited π-alkyl interactions similar to α-pinene and β-pinene at residues His50, Ala39, Cys37, Phe54, and Pro53. Notably, residues His50 and Ala39 were found to be important active positions of *S. aureus* tyrosyl-tRNA synthetase[24].

For enoyl reductase (*E. coli*) residues α-pinene (PDB ID: 1C14) formed five hydrophobic interactions. Four of these interactions were π-alkyl interactions with the following residues: Ile200, Ala197, Ala196, and Ala189. Additionally,

an additional π-σ interaction was formed with Tyr146 (Fig. S3). On the other hand, β-pinene and camphene only formed π-alkyl interactions. β-Pinene established six interactions with residues Ile20, Ala197, Tyr146, Ile192, Ala189, and Tyr156. Meanwhile, camphene formed three interactions at Ala189, Tyr146, and Ala197.

The interaction of the ligands with the binding site of carbamate kinase (*E. faecalis*) (PDB ID 2WE5) can be observed in Fig. S4. Hydrophobic interactions of alkyl and π-alkyl types were observed. α-Pinene and β-pinene formed two π-alkyl interactions, while camphene formed only one π-alkyl interaction.

Fig. S5 exhibits the docking results for the major components of the EO with the 1IYL protein. As can be observed, α-pinene formed four pi-alkyl interactions at residues His227, Tyr225, Phe240 and one pi-sigma bond at residue Phe115 while β-pinene differs only from α-pinene when interacts with Phe339 to form π-alkyl bonds. Furthermore, camphene possessed the most π-alkyl interactions at residues Tyr225, His227, Phe115, Phe240, and Phe339.

All interactions formed with residues of Thiophene-2-carboxyaldehyde methane-sulfonyl-hydrazone (*B. cereus*) (PDB ID 5V8E) were of the π-alkyl and alkyl types. These interactions were established with the following residues: His201 (for all three compounds), Phe338 (for β-Pinene), and Met391 (for camphene) (Fig. S6).

Molecular docking studies have played a crucial role in identifying potential compounds from *Kaempferia* species and their mechanisms of action. Previous studies have successfully employed this method to model the potential binding modes of phytochemicals found in *K. parviflora* with proteins involved in cell toxicity and metastasis, namely Bcl-2, Bcl-XL, ERK2, and FAK[45]. Another research demonstrated the strong binding affinity of methoxyflavones derived from *K. parviflora* with various small receptors, such as COX-1, P2Y12, and GPVI, as evidenced by docking simulations[46]. On the other hand, the applications of molecular docking for essential oils of *Kaempferia* species are limited. These docking simulations provide valuable scientific insights into the potential of *K. champasakensis* and its receptor-ligand interactions, especially with bacterial targets, offering promising prospects for the development of natural antibacterial products.

Conclusions

In conclusion, the chemical constituents and antimicrobial activity of the EO from *K. champasakensis* rhizomes were reported for the first time. The GC/MS analysis detected sixty-one volatile constituents in the EO, of which camphene, α-pinene, and β-pinene predominated over the other compounds. The results also demonstrated that the EO showed a potential antimicrobial activity, comparable to that of tetracycline. Molecular docking studies were also conducted to investigate the effective inhibitory properties of three main compounds (α-pinene, β-pinene, and camphene) against target proteins associated with these activities. These findings suggested that *K. champasakensis* has the potential for a large- scale cultivation to study its important compounds. Future research should prioritize the isolation and characterization of non-volatile compounds from these species, along with the assessment of their biological activities.

Competing interests

No potential conflict of interest was reported by the authors.

Supplementary data

Figures S1-S6 are provided as supplementary information.

References

1. **Techaprasan, J., Klinbunga, S., Ngamriab-sakul, C., Jenjittikul, T. (2010).** Genetic variation of *Kaempferia* (Zingiberaceae) in Thailand based on chloroplast DNA (psbA-trnH and petA-psbJ) sequences. Genet. Mol. Res. 9: 1957-1973.
2. **Srivastava, N., Singh, S., Gupta, A.C., Shanker, K., Bawankule, D.U., Luqman, S.J.T.r. (2019).** Aromatic ginger (*Kaempferia galanga* L.) extracts with ameliorative and protective potential as a functional food, beyond its flavor and nutritional benefits. Toxicol. Rep. 6: 521-528.

3. **Atun, S., Arianingrum, R. (2015).** Anti-cancer activity of bioactive compounds from *Kaempferia rotunda* rhizome against human breast cancer. Int. J. Pharmacogn. Phytochem. 7: 262-269.
4. **Diastuti, H., Chasani, M., Suwandri, S. (2020).** Antibacterial activity of benzyl benzoate and crotepoxide from *Kaempferia rotunda* L. Rhizome. Indones. J. Chem. 20: 9-15.
5. **Lal, M., Munda, S., Begum, T., Pandey, S.K. (2023).** Identification and Registration of the Novel High-Rhizome-Yielding Variety Bharamputra-1 of *Kaempferia galanga* L. Agriculture. 13: 482.
6. **Begum, T., Gogoi, R., Sarma, N., Pandey, S.K., Lal, M. (2023).** Novel ethyl p-methoxy cinnamate rich *Kaempferia galanga* (L.) essential oil and its pharmacological applications: special emphasis on anti-cholinesterase, anti-tyrosinase, α-amylase inhibitory, and genotoxic efficiencies. PeerJ. 11: e14606.
7. **Umar, M.I., Asmawi, M.Z., Sadikun, A., Atangwho, I.J., Yam, M.F., Altaf, R., Ahmed, A. (2012).** Bioactivity-guided isolation of ethyl-p-methoxycinnamate, an anti-inflammatory constituent, from *Kaempferia galanga* L. extracts. Molecules. 17: 8720-8734.
8. **Begum, T., Gogoi, R., Sarma, N., Pandey, S.K., Lal, M. (2022).** Direct sunlight and partial shading alter the quality, quantity, biochemical activities of *Kaempferia parviflora* Wall., ex Baker rhizome essential oil: A high industrially important species. Ind. Crops Prod. 180: 114765.
9. **Picheansoonthon, C., Koonterm, S. (2008).** A new species of *Kaempferia* (Zingiberaceae) from Southern Laos. Taiwania. 53: 406-409.
10. **Van, T.T.K., Nga, N.P., Viet, H. (2018).** *Kaempferia champasakensis* pichean. & Koonterm- A new record species for Vietnam. Vnuhcm J. Nat. Sci. 2: 13-18.
11. **Hieu, T.T., Duc, D.X., Hieu, N.N., Danh, N.D., Tuan, N.H., Van Trung, H., Thang, T.D., Giang, L.D. (2023).** Chemical Composition of the Volatile Oil from the Leaves of *Kaempferia champasakensis* Picheans. & Koonterm. (Zingiberaceae). J. Essent. Oil-Bear. Plants. 26: 108-114.
12. **Trung, H.T., Giang, L.D., Thuan, V.T., Van Trung, H., Hieu, N.N., Duc, N.D., Cuong, T.Q., Duc, D.X. (2023).** Chemical Composition of Essential Oils Extracted from the Leaves and Rhizomes of *Alpinia hongiaoensis* Tagane. (Zingiberaceae) growing Wild in Vietnam. J. Essent. Oil-Bear. Plants. 26: 396-402.
13. **Paw, M., Begum, T., Gogoi, R., Pandey, S. K., Lal, M. (2020).** Chemical composition of *Citrus limon* L. Burmf peel essential oil from North East India. J. Essent. Oil-Bear. Plants. 23: 337-344.
14. **Sarma, N., Begum, T., Pandey, S.K., Gogoi, R., Munda, S., Lal, M. (2020).** Chemical composition of *Syzygium cumini* (L.) Skeels leaf essential oil with respect to its uses from North East region of India. J. Essent. Oil-Bear. Plants. 23: 601-607.
15. **Tuan, D.Q., Dinh, D., Tran, T.N., Pham, T.V., Ho, D.V., Le, N.T., Le, A.T., Nguyen, H.T. (2022).** Chemical Composition of Essential Oil from the *Zingiber monophyllum* (Zingiberaceae) from Vietnam. J. Essent. Oil-Bear. Plants. 25: 987-993.
16. **Trung, H.T., Van Chen, T., Hieu, N.N., Dang, V.S., An, N.T.G., Thang, T.D., Minh, L.T.H., Van Trung, H., Duc, D.X., Giang, L.D. (2023).** Chemical components and antimicrobial properties of essential oil distilled from *Siliquamomum oreodoxa* N.S. Lý & Škornick (Zingiberaceae) rhizomes. J. Essent. Oil-Bear. Plants. 26: 547-555.
17. **Tran-Trung, H., Dau, X.D., Nguyen, T.C., Nguyen-Thi-Thu, H., Nguyen-Ngoc, H., Nguyen, T.G.A., Hoang, V.T., Nguyen, D.-K., Nguyen, D.D., Tran Van, C. (2023).** Phytochemical Analysis of the Essential Oils From the Rhizomes of Three Vietnamese Curcuma Species and Their Antimicrobial Activity. Nat. Prod. Commun. 18: 1934578X231167229.
18. **Tran-Trung, H., Giang, L.D., Trang, D.T.H., An, N.T.G., Hieu, N.N., Vu, D.C., Nguyen, T.H., Nguyen-Thi-Thu, H., Van**

Trung, H. (2023). Chemical Examination and Antimicrobial Activity of Essential Oils from the Leaves and Rhizomes of *Meistera caudata* Šída f. & Škorničk.(Zingiberaceae). J. Biol. Act. Prod. Nat. 13: 68-75.

19. **Eberhardt, J., Santos-Martins, D., Tillack, A.F., Forli, S. (2021).** AutoDock Vina 1.2. 0: New docking methods, expanded force field, and python bindings. J. Chem. Inf. Model. 61: 3891-3898.
20. **Halgren, T.A. (1999).** MMFF VI. MMFF94s option for energy minimization studies. J. Comput. Chem. 20: 720-729.
21. **Sogabe, S., Masubuchi, M., Sakata, K., Fukami, T.A., Morikami, K., Shiratori, Y., Ebiike, H., Kawasaki, K., Aoki, Y., Shimma, N. (2002).** Crystal structures of *Candida albican*s N-myristoyltransferase with two distinct inhibitors. Chem. Biol. 9: 1119-1128.
22. **Qiu, X., Abdel-Meguid, S.S., Janson, C.A., Court, R.I., Smyth, M.G., Payne, D. (1999).** Molecular basis for triclosan activity involves a flipping loop in the active site. Protein Sci. 8: 2529-2532.
23. **Ramón-Maiques, S., Marina, A., Guinot, A., Gil-Ortiz, F., Uriarte, M., Fita, I., Rubio, V. (2010).** Substrate binding and catalysis in carbamate kinase ascertained by crystallographic and site-directed mutagenesis studies: movements and significance of a unique globular subdomain of this key enzyme for fermentative ATP production in bacteria. J. Mol. Biol. 397: 1261-1275.
24. **Qiu, X., Janson, C.A., Smith, W.W., Green, S.M., McDevitt, P., Johanson, K., Carter, P., Hibbs, M., Lewis, C., Chalker, A. (2001).** Crystal structure of *Staphylococcus aureus* tyrosyl-tRNA synthetase in complex with a class of potent and specific inhibitors. Protein Sci. 10: 2008-2016.
25. **Sychantha, D., Little, D.J., Chapman, R.N., Boons, G.-J., Robinson, H., Howell, P.L., Clarke, A.J. (2018).** PatB1 is an O-acetyltransferase that decorates secondary cell wall polysaccharides. Nat. Chem. Biol. 14: 79-85.
26. **Mostefa, N., Djebli, N., Khanh, P.N., Ha, N.X., Anh, H.N., Ha, V.T., Huong, T.T., Anh, D.V., Cuong, N.M. (2023).** Anti-Alzheimer's Activity of Polyphenolic Stilbenes-Rich Acetone Fraction of the Oil-Removed Seeds of *Passiflora edulis*: *in vivo* and *in silico* Studies. Chem. Biodivers. e202201051.
27. **Tuan, N.A., Khanh, P.N., Ha, N.X., Binh, T.C., Khanh, N.D., Oanh, T.T. (2022).** Compounds Isolated from *Lawsonia inermis* L. Collected in Vietnam and Evaluation of Their Potential Activity Against the Main Protease of SARS-CoV-2 Using *In silico* Molecular Docking and Molecular Dynamic Simulation. Nat. Prod. Commun. 17: 1934578X221125161.
28. **Theanphong, O., Mingvanish, W., Jenjittikul, T. (2023).** Antimicrobial and Radical Scavenging Activitıes of Essential Oils from *Kaempferia larsenii* Sirirugsa. Trends Sci. 20: 5212.
29. **Tuan, N.H., Tung, N.T., Khanh, P.N. (2019).** Research on chemical compositions and anti-microbial activity of the essential oil of the rhizome of *Kaempferia daklakensis* N.H.Tuan & N.D.Trong- A new record from Vietnam flora. J. King Saud Univ. Sci. 31: 1505-1510.
30. **Sahoo, S., Dash, M., Sahoo, D., Kar, S.K., Kar, B., Nayak, S. (2023).** Chemical Composition and Biological Activities of Essential Oil from *Kaempferia rotunda.* Proceedings of the National Academy of Sciences, India Section B: Biological Sciences.
31. **Tewtrakul, S., Supreeya, Y., Kummee, S., Atsawajaruwan, L. (2005).** Chemical components and biological activities of volatile oil of *Kaempferia galanga* Linn. Songklanakarin J. Sci. Technol. 27: 503-507.
32. **Raina, A.P., Abraham, Z. (2016).** Chemical profiling of essential oil of *Kaempferia galanga* L. germplasm from India. J. Essent. Oil Res. 28: 29-34.
33. **Kumar, R., Pandey, A., Varshney, V.K. (2019).** Antifungal activity of the essential oil of *Tagetes minuta* against some fungi of forestry importance. J. Biol. Act. Prod. Nat. 9: 67-72.

34. **Bhalla, P., Tripathi, Y.C., Varshney, V.K. (2021).** Phytochemistry and biological activity of *Skimmia laureola* leaf essential oil-a review. J. Essent. Oil Res. 33: 315-329.
35. **Tsasi, G., Danalatos, G., Tomou, E.-M., Sakadani, E., Bethanis, M., Kolokouris, A., Samaras, Y., Ćirić, A., Soković, M., Skaltsa, H. (2022).** Chemical composition and antimicrobial activity of the essential oil of *Abies cephalonica* Loudon from Mount Ainos (Kefalonia, Greece). J. Essent. Oil Res. 34: 143-147.
36. **Aloui, F., Baraket, M., Jedidi, S., Hosni, K., Bouchnak, R., Salhi, O., Jdaidi, N., Selmi, H., Ghazghazi, H., Khadhri, A. (2021).** Chemical Composition, Anti-radical and Antibacterial Activities of Essential Oils from Needles of *Pinus halepensis* Mill., *P. pinaster* Aiton., and *P. pinea* L. J. Essent. Oil-Bear. Plants. 24: 453-460.
37. **Woerdenbag, H.J., Windono, T., Bos, R., Riswan, S., Quax, W.J. (2004).** Composition of the essential oils of *Kaempferia rotunda* L. and *Kaempferia angustifolia* Roscoe rhizomes from Indonesia. Flavour Fragr. J. 19: 145-148.
38. **Munda, S., Saikia, P., Lal, M. (2018).** Chemical composition and biological activity of essential oil of *Kaempferia galanga*: a review. J. Essent. Oil Res. 30: 303-308.
39. **Hachlafi, N.E., Aanniz, T., Menyiy, N.E., Baaboua, A.E., Omari, N.E., Balahbib, A., Shariati, M.A., Zengin, G., Fikri-Benbrahim, K., Bouyahya, A. (2021).** *In vitro* and *in vivo* biological investigations of camphene and its mechanism insights: A review. Food Rev. Int. 1-28.
40. **Badawy, M.E., Marei, G.I.K., Rabea, E.I., Taktak, N.E. (2019).** Antimicrobial and antioxidant activities of hydrocarbon and oxygenated monoterpenes against some foodborne pathogens through *in vitro* and *in silico* studies. Pestic. Biochem. Physiol. 158: 185-200.
41. **Vuuren, S.F.V., Viljoen, A.M. (2007).** Antimicrobial activity of limonene enantiomers and 1,8-cineole alone and in combination. Flavour Fragr. J. 22: 540-544.
42. **Han, Y., Sun, Z., Chen, W. (2019).** Antimicrobial Susceptibility and Antibacterial Mechanism of Limonene against *Listeria monocytogenes*. Molecules. 25: 33.
43. **Kim, Y.W., Kim, M.J., Chung, B.Y., Bang, D.Y., Lim, S.K., Choi, S.M., Lim, D.S., Cho, M.C., Yoon, K., Kim, H.S., Kim, K.B., Kim, Y.S., Kwack, S.J., Lee, B.-M. (2013).** Safety Evaluation And Risk Assessment of d-Limonene. J. Toxicol. Environ. Health - B: Crit. Rev. 16: 17-38.
44. **Li, Z.H., Cai, M., Liu, Y.S., Sun, P.L., Luo, S.L. (2019).** Antibacterial Activity and Mechanisms of Essential Oil from *Citrus medica* L. var. *sarcodactylis*. Molecules. 24: 1577.
45. **Hairunisa, I., Bakar, M.F.A., Da'i, M., Bakar, F.I.A., Syamsul, E.S. (2023).** Cytotoxic Activity, Anti-Migration and In Silico Study of Black Ginger (*Kaempferia parviflora*) Extract against Breast Cancer Cell. Cancers. 15: 2785.
46. **Le, H.L., Nguyen, V.H., Nguyen, T.D., Nguyen, T.V.A., Le, D.H. (2023).** Potential antiaggregatory and anticoagulant activity of *Kaempferia parviflora* extract and its methoxyflavones. Ind. Crops Prod. 192: 116030.