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Chemical characterization, antioxidant, antimicrobial, enzyme inhibitory and cytotoxic activities of *Illicium lanceolatum* essential oilsTianming Zhao^{*}, Guang Fan, You Tai, Xinhe Shu, Fu Tian, Shuliang Zou, Qin Wu

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ABSTRACT

Illicium lanceolatum is a medicinal and aromatic plant widely distributed in the south of China. The reports on chemical composition and biological activities of its essential oils (EOs) were very limited. In this study, *Illicium lanceolatum* EOs were extracted by hydro distillation, and analyzed by GC-MS and GC-FID. DPPH radical scavenging assay, ABTS cation radical scavenging assay and ferric reducing/antioxidant power (FRAP) assay were used for antioxidant activity evaluation. Minimum inhibitory concentrations (MICs) and minimum microbiocidal concentrations (MMCs) against 9 microorganisms were determined. The inhibitory effects on tyrosinase, α -glucosidase and cholinesterases were evaluated and cytotoxic activities were evaluated using MTT assay. The results revealed 110 identified compounds, with asaricin, eucalyptol, linalool and caryophyllene oxide as major compounds. Eucalyptol was the most abundant compound in the stem, leaf and fruit EOs while asaricin accounted for $50.52 \pm 0.33\%$ in the root EO. Very weak radical scavenging capacities were noticed for all EOs, but the root EO showed moderate antioxidant activity (176.33 ± 4.52 mg TE/g of EO) in the FRAP assay, which could be attributed to asaricin. The root EO displayed better antimicrobial activities than other three EOs, with MIC values as 3.13 mg/mL against three bacteria including *Staphylococcus aureus* BNCC 186335, *Bacillus cereus* BNCC 103930 and *Listeria monocytogenes* BNCC 336877. Camphor and borneol were found to be important antimicrobial compounds. No inhibitory effect on α -glucosidase was found. The leaf EO displayed better acetylcholinesterase inhibitory activity (17.79 ± 0.32 mg GE/g of EO) while the root EO showed better tyrosinase (30.34 ± 0.40 mg KAE/g of EO) and butyrylcholinesterase (43.25 ± 1.50 mg GE/g of EO) inhibitory activities. Molecular docking between active compounds and enzymes revealed the main interactions as hydrophobic interaction, hydrogen bond and π -stacking. All EOs displayed weak cytotoxicity to HK-2 cells of normal kidney at six tested concentrations. The leaf EO showed strong anticancer activities to HepG2 cells at the concentration of 500 μ g/mL. *I. lanceolatum* EOs showed promising prospects with possible applications in pharmaceutical and cosmetic industries.

1. Introduction

The genus *Illicium* consists of approximately 40 species which are

represented by evergreen trees and shrubs, and 28 of them are distributed in the south, southwest, and east of China (Kubo et al., 2022). The plants of this genus are rich in monoterpenoids, sesquiterpenoids,

Abbreviations: ABTS, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); AChE, acetylcholinesterase; AE, acarbose equivalent; ATCI, acetylthiocholine iodide; *B. cereus*, *Bacillus cereus*; BChE, butyrylcholinesterase; BHT, butylated hydroxytoluene; BTCl, butyryl thiocholine iodide; *C. albicans*, *Candida albicans*; DPPH, 2,2-diphenyl-1-picrylhydrazyl; DTNB, 5,5'-dithiobis-(2-nitrobenzoic acid); *E. coli*, *Escherichia coli*; EO, essential oil; FRAP, ferric reducing/antioxidant power; GC-FID, gas chromatography-flame ionization detector; GC-MS, gas chromatography-mass spectrometry; GE, galanthamine equivalent; *I. lanceolatum*, *Illicium lanceolatum*; KAE, kojic acid equivalent; *L. monocytogenes*, *Listeria monocytogenes*; MIC, minimum inhibitory concentration; MMC, minimum microbiocidal concentration; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; *P. aeruginosa*, *Pseudomonas aeruginosa*; pNPG, *p*-nitrophenyl- α -D-glucopyranoside; RI, retention index; *S. aureus*, *Staphylococcus aureus*; *S. epidermidis*, *Staphylococcus epidermidis*; *S. lentus*, *Staphylococcus lentus*; *S. typhimurium*, *Salmonella typhimurium*; TE, trolox equivalent; TIC, total ion chromatogram; TPTZ, 2,4,6-tripyridyl-s-triazine; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

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phenylpropanoids and lignans, and were found to have various biological activities, such as insecticidal, antioxidative, antibacterial, neurotrophic, anti-inflammatory and enzyme inhibitory activities (Liu et al., 2009). Some species of the genus *Illicium* have been used in traditional Chinese medicine for the treatment of traumatic injury, rheumatism and skin inflammation. The fruit of *Illicium verum* (*I. verum*) and stem bark of *Illicium difengpi* are listed officially in the Chinese Pharmacopoeia. The fruit of *I. verum*, known as star anise, could be used for extraction of shikimic acid to produce oseltamivir (Estevez, A.M. and Estevez, R.J., 2012) and is also a commonly-used spice in Chinese and Southeast-Asian food.

Illicium lanceolatum A. C. Smith (*I. lanceolatum*) is a medicinal and aromatic plant, commonly known as 'Mangcao' or 'Hongduhui' in Chinese. Its star shaped fruit closely resembles that of *I. verum*, but it is highly toxic due to the presence of neurotoxic sesquiterpene named anisatin (Mathon et al., 2013). Some analytical methods were established to distinguish these two fruits, such as Vis/NIR hyperspectral imaging combined with chemometrics (Lu et al., 2020) and thermal desorption-GC-MS (Howes et al., 2009). However, its roots and leaves have been used to treat bruises, internal injuries and back pain in folk medicines (Liang et al., 2009). The sterilized aqueous solution produced from the root or branch of *I. lanceolatum* could be used as injection to relieve pain (Gao et al., 2020). The past decade has seen the extensive studies on the chemical compounds from the fruits, leaves and roots of *I. lanceolatum*. In particular, sesquiterpenoids have drawn a lot of interest, including seco-prezizaane-type (Liu et al., 2019, 2020; Nie et al., 2021, 2022), germacrane-type (Kubo et al., 2022; Zhao et al., 2014), tetranor and santalane-type sesquiterpenoids (Kubo et al., 2015). Essential oils (EOs) (Huang et al., 2012; Liang et al., 2012), monoterpenes (Liu et al., 2013; Zhao et al., 2014), flavonoids (Li et al., 2014) and phenylpropanoids (Gui et al., 2014; Nie et al., 2022) were also reported in different parts of *I. lanceolatum*. In previous studies, the compounds of *I. lanceolatum* have shown various biological activities, including anti-inflammatory (Gui et al., 2014; Liang et al., 2012), antimicrobial (Kubo et al., 2015) and neuroprotection activities (Liu et al., 2019). In addition to medicinal use, *I. lanceolatum* can also be used as an ornamental plant. As the demand for this plant is increasing, *I. lanceolatum* has been cultivated in some provinces in China.

Essential oils are natural oily liquids characterized by unique odors. Due to their many beneficial effects, including antioxidant, antimicrobial, anti-inflammatory, anticancer, and enzyme inhibitory properties, essential oils have found increasing uses in pharmaceutical, food and cosmetic industries. Because oxidation can damage various biological molecules and subsequently lead to many diseases, antioxidant activity is one of the mostly studied topics in essential oil research (Shaaban et al., 2012). Antioxidants have been used for the treatment of many diseases to prevent oxidative damage. Antioxidant activities of numerous essential oils have been evaluated and the results have shown that essential oils are good natural sources of antioxidants, such as thyme essential oil (Wei and Shibamoto, 2010). Pathogenic microorganisms cause a lot of diseases in human and lead to natural spoilage in foods. Antibiotics and other synthetic antimicrobial chemicals have been used to control the growth of these microorganisms. However, overuse of these antimicrobial agents could lead to drug resistance. Natural antimicrobial agents including essential oils from medicinal and aromatic plants as safe alternatives have received much attention. For example, oregano essential oil has been used as food additive in food products due to its obvious antimicrobial and antioxidant activity (Rodríguez-García et al., 2016). Enzymes are important targets for developing drugs and evaluation of enzyme inhibition activity could contribute to discovery of potential lead compounds for related diseases. Essential oils have shown various enzyme inhibitory effects in previous studies, such as tyrosinase (Salem et al., 2022), α -glucosidase (Ahmad, 2021) and cholinesterases (Burcul et al., 2020). As one of the biggest causes of mortality in humans, cancer has received much more attention from researchers. Many essential oils have been reported to play an

important role in cancer prevention and treatment, and various mechanisms have been unveiled, such as antioxidant, antiproliferative, enhancement of immune function, and synergistic mechanism of volatile constituents (Bhalla et al., 2013).

The different parts of *I. lanceolatum*, including roots, stems, leaves and fruits, are rich in essential oils. However, the studies on the chemical composition and biological activities of *I. lanceolatum* EOs are quite limited up to date. The chemical compositions of essential oils from the roots, leaves and fruits of *I. lanceolatum* originated in Jiangxi province of China were reported in previous studies (Huang et al., 2012; Liang et al., 2012), antinociceptive and anti-inflammatory properties of the root EO were evaluated. Cineole (24.5 %) and D-limonene (18.3 %) were found to be the major components of the *I. lanceolatum* fruit EO. The leaf EO mainly contained linalool (16.2 %), elemicin (14.9 %) and cineole (14.8 %) while myristicin (17.6 %), α -asarone (17.2 %) and methyl isoeugenol (11.2 %) were the major components of the root EO (Huang et al., 2012). To the best of our knowledge, there is no report on the antioxidant, antimicrobial, enzyme inhibitory and cytotoxic activities of *I. lanceolatum* EOs.

As second metabolites produced in the plants, chemical composition of essential oils could be affected by environmental conditions, geographic variations, physiological variations and other factors (Figueiredo et al., 2008). The chemical components of essential oils of the same species from different geographical areas may differ significantly. *I. lanceolatum* collected in the previous studies was from Jiangxi province. The investigation on chemical composition of *I. lanceolatum* EOs from other locations could contribute to a better understanding of the chemical variety of this species' essential oils.

The current research has been carried out to investigate the chemical composition of essential oils from the roots, stems, leaves and fruits of *I. lanceolatum* and to evaluate their antioxidant, antimicrobial, enzyme inhibitory and cytotoxic activities. Nine major compounds in the root EO were further evaluated for their antioxidant, antimicrobial and enzyme inhibitory activities. The chemical composition of the stem EO, antioxidant, antimicrobial, enzyme inhibitory and cytotoxic activities of *I. lanceolatum* EOs were firstly reported in this study. Our results could bring some insights for applications of *I. lanceolatum* EOs into pharmaceutical and cosmetic industries.

2. Materials and methods

2.1. Plant materials

The roots, stems, leaves and fruits of *I. lanceolatum* were collected in August 2022 in a village near Guiyang (Latitude 2641'5N, longitude 10630'41E, altitude 1241 m a.s.l.) and naturally dried at room temperature in the shade for about three weeks before extraction of essential oils. The moisture contents in the roots, stems, leaves and fruits of *I. lanceolatum* were determined as 7.04 ± 0.15 %, 6.58 ± 0.09 %, 5.42 ± 0.12 % and 6.33 ± 0.08 %, respectively. Associate Prof. Yazhou Zhang from Guizhou University of Traditional Chinese Medicine made the identification of this species according to the Flora of China. Voucher specimens (MC015) of this plant were placed at Guizhou Institute of Technology.

2.2. Reagents

DPPH (2,2-diphenyl-1-picrylhydrazyl) and a mixed standard of n-alkanes C7-C30 were purchased from Sigma-Aldrich. ABTS [2,2'-Azinobis (3-ethylbenzothiazoline-6-sulfonic Acid)], TPTZ (2,4,6-Tripyridyl-s-triazine) and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were from Shanghai Yuanye Biotechnology company. Amphotericin B, gentamycin sulfate, tyrosinase from mushroom, levodopa, kojic acid, *p*-nitrophenyl- α -D-glucopyranoside (pNGP), acarbose, α -glucosidase from yeast, acetylthiocholine iodide (ATCI), butyryl thiocholine iodide (BTCI), 5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB),

acetylcholinesterase (AChE) from electric eel, butyrylcholinesterase (BChE) from horse serum and galanthamine were purchased from Shanghai Yuanye. For identification and activity evaluation of nine compounds in the root EO, terpinen-4-ol (95 %), linalool (98 %), α -terpineol (96 %) and borneol (98 %) were from Shanghai Yuanye; eucalyptol (99 %), camphene (96 %) and camphor (96 %) were from Shanghai Rhawn; estragole (98 %) was from Shanghai Aladdin; asaricin (98 %) was from Sichuan Weikeqi Biotech.

2.3. Extraction of essential oils

The dried roots, stems and leaves were firstly chopped into small pieces and then ground using a grinder. The dried fruits were directly ground. The powder of each organ was divided into three parts. The hydrodistillations of dry roots (300 g), stems (400 g), leaves (400 g) and fruits (150 g) of *I. lanceolatum* were performed in Clevenger-type apparatus for 5 h, according to the method listed in the Chinese Pharmacopoeia. The experiments were carried out in triplicates for each organ of *I. lanceolatum* to obtain three essential oils. 12 essential oils obtained in total were stored at $-20\text{ }^{\circ}\text{C}$ for further GC-MS and GC-FID analysis. For biological activity evaluation, three essential oils obtained from each organ were combined together to obtain one essential oil for each organ.

2.4. GC-MS and GC-FID analysis

The 12 essential oils from different parts of *I. lanceolatum* were diluted in n-hexane (1:50, v/v) and analyzed using a TG-5MS capillary column (30 m \times 0.25 mm \times 0.25 μm) on GC-MS (Shimadzu, TQ8040 NX) and GC (Thermo Scientific, Trace 1310) systems, according to the previous method (Zhao et al., 2021) with slight modifications. For GC-MS analysis, column temperature was initially maintained at $50\text{ }^{\circ}\text{C}$ for 3 min, and raised to $140\text{ }^{\circ}\text{C}$ at $3\text{ }^{\circ}\text{C}/\text{min}$ and kept for 2 min; then heated to $190\text{ }^{\circ}\text{C}$ at $2\text{ }^{\circ}\text{C}/\text{min}$ and maintained for 2 min; finally raised up to $220\text{ }^{\circ}\text{C}$ at $10\text{ }^{\circ}\text{C}/\text{min}$. The carrier gas was helium with a flow rate of 1 mL/min and the injection volume was 1 μL in split mode (1:20). The temperature of injection port, interface and ion source was all set at $250\text{ }^{\circ}\text{C}$. The mass range was 45–550. A mixed standard of n-alkanes (C7–C30) was analyzed under the same conditions. Identification of each component in the *I. lanceolatum* EOs was based on the comparison of its calculated retention index and experimental mass spectra with those published in the literature (Adams, 2007) and NIST libraries. The quantification of essential oil components was carried out on GC with FID detector. The column and temperature programming conditions were the same as the GC-MS system. Nitrogen was used as the carrier gas with a flow rate of 1 mL/min. 1 μL of sample solution was injected with the split ratio as 1:20. The temperature of injection port and detector was $250\text{ }^{\circ}\text{C}$. The identification of asaricin, borneol, camphor, eucalyptol, camphene, estragole, terpinen-4-ol, linalool and α -terpineol were further confirmed by injection of authentic compounds on GC. The relative percentage (%) of each component in essential oils was based on peak area normalization of GC chromatogram.

2.5. Antioxidant activity assays

Three different methods, including DPPH radical scavenging assay, ABTS cation radical scavenging assay and ferric reducing/antioxidant power (FRAP) assay, were employed to evaluate the antioxidant activities of *I. lanceolatum* EOs. The experiments were conducted according to the previously reported method (Zhao et al., 2021). In brief, essential oil solution of 20 mg/mL in methanol was firstly prepared. For DPPH assay, 6 mL of DPPH in methanol (6×10^{-5} mol/L) was mixed with 150 μL of essential oil solution. The mixed solution was put in the darkness for 30 min and then measured at 515 nm on the UV-vis spectrophotometer (UH5300, HITACHI). For ABTS assay, 50 μL of essential oil solution was mixed with 4 mL of diluted ABTS solution. After 6 min of reaction, the

absorbance of mixture was recorded at 734 nm. For FRAP assay, 150 μL of essential oil solution was mixed with 450 μL of methanol, and then 4.5 mL of FRAP reagent. The mixture was incubated for 30 min at $37\text{ }^{\circ}\text{C}$ and the absorbance was recorded at 593 nm. The antioxidant activities of nine major compounds in the root EO, including asaricin, borneol, camphor, eucalyptol, camphene, estragole, terpinen-4-ol, linalool and α -terpineol, were further evaluated using FRAP assay. The methanol solutions of Trolox with known concentrations ranging from 25 to 250 mg/L were used for calibration. The antioxidant activity was expressed in mg Trolox equivalent (TE)/g of EO. BHT (butylated hydroxytoluene), a synthetic antioxidant, was used as positive control.

2.6. Antimicrobial activity assays

The pathogenic microorganisms including 5 gram-positive bacteria, 3 gram-negative bacteria and 1 yeast, were purchased from BeNa Culture Collection company (Beijing, China). *Bacillus cereus* BNCC 103930 (*B. cereus*), *Listeria monocytogenes* BNCC 336877 (*L. monocytogenes*), *Staphylococcus aureus* BNCC 186335 (*S. aureus*), *Staphylococcus epidermidis* BNCC 102555 (*S. epidermidis*), *Staphylococcus lentus* BNCC 336683 (*S. lentus*), *Escherichia coli* BNCC 133264 (*E. coli*), *Pseudomonas aeruginosa* BNCC 337005 (*P. aeruginosa*) and *Salmonella typhimurium* BNCC 108207 (*S. typhimurium*) were cultured in Mueller-Hinton Broth medium. *Candida albicans* BNCC 186382 (*C. albicans*) was grown in yeast maltose medium.

The minimum inhibitory concentrations (MICs) and minimum microbiocidal concentrations (MMCs) of *I. lanceolatum* EOs were determined by the previous method with some modifications (Zhao et al., 2021). The concentration of microbial strain suspension was diluted to 10^5 CFU/mL for bacteria or 10^3 CFU/mL for yeast. 120 μL of essential oil solutions was firstly added and then serial concentrations were obtained by 2-fold dilution method. After that, 60 μL of diluted strain suspension was added. The plates were incubated at $37\text{ }^{\circ}\text{C}$ for 16–24 h for the bacteria and at $28\text{ }^{\circ}\text{C}$ for 48 h for the yeast. The lowest concentrations of essential oils corresponding to the wells in which the mixture remained clear were the MICs. After obtaining the MIC results, 60 μL of the suspension from the wells in which there was no visible growth was spread on nutrient agar plates. For the bacteria, the Petri dishes were incubated at $37\text{ }^{\circ}\text{C}$ for 16–24 h, and for the yeast, they were at $28\text{ }^{\circ}\text{C}$ for 48 h. The lowest essential oil concentrations of the MIC wells in which strains failed to grow were recorded as MMCs. The gentamycin sulfate and amphotericin B were used as positive controls for antibacterial and antifungal tests, respectively. The MICs and MMCs of nine compounds against *S. aureus*, *B. cereus* and *L. monocytogenes*, including asaricin, borneol, camphor, eucalyptol, camphene, estragole, terpinen-4-ol, linalool and α -terpineol, were further determined.

2.7. Enzyme inhibitory activity assays

Tyrosinase, α -glucosidase, acetylcholinesterase and butyrylcholinesterase inhibitory activity assays of *I. lanceolatum* EOs were conducted to evaluate their whitening effect, anti-diabetes effect and improvement effect on neurodegenerative diseases. Essential oil solutions in ethanol of 50 mg/mL were firstly prepared and diluted with buffer solution to the concentrations of 4, 2 and 1 mg/mL before the tests. All the enzymes and substrates were prepared daily, and kept in the ice bath for subsequent experiments. The inhibition effects on tyrosinase and butyrylcholinesterase of nine compounds including asaricin, borneol, camphor, eucalyptol, camphene, estragole, terpinen-4-ol, linalool and α -terpineol were further evaluated.

Tyrosinase inhibition activity assay was conducted according to the previous method (Sharmeen Jugreet et al., 2021) with some modifications. In brief, 110 μL of phosphate buffer (pH6.8) was mixed with 40 μL of tyrosinase solution (110 U/mL) and 10 μL of essential oil solution in a 96-well microplate. After 15 min of incubation at $37\text{ }^{\circ}\text{C}$, 40 μL of levodopa (5 mM) was added to start the reaction. After an additional 10 min

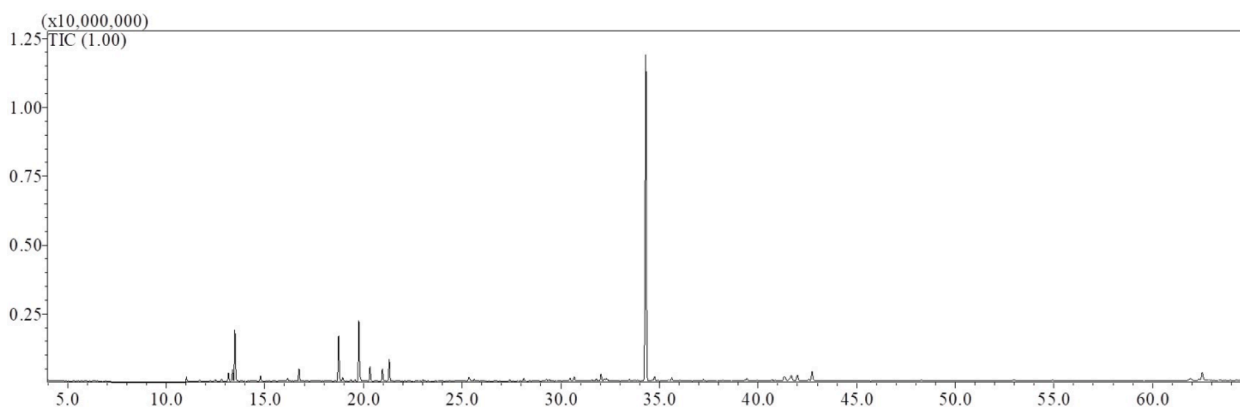


Fig. 1. Total Ion Chromatogram of the root EO of *I. lanceolatum*.

of incubation at 37 °C, absorbance was immediately measured at 492 nm using microplate reader (DNM-9606, Perlong, Beijing). A blank solution without essential oil was tested using the same procedure and then inhibition percentages of essential oils were calculated. Kojic acid was used as positive control and the tyrosinase inhibitory activities of essential oils were expressed as mg kojic acid equivalents (KAE)/g of EO.

α -glucosidase inhibitory activity assay was based on the previous method (Hong et al., 2021) with some modifications. 80 μ L of phosphate buffer (pH6.8) was mixed with 20 μ L of α -glucosidase solution (1 U/mL) and 10 μ L of essential oil solution in a 96-well microplate. After 15 min of incubation at 37 °C, 10 μ L of p-NPG (1 mM) was added to initiate the reaction. The reaction lasted for 20 min and stopped by adding 80 μ L of sodium carbonate solution (0.2 M). The absorbances were recorded at 405 nm using microplate reader. A blank solution without essential oil was tested using the same method. Acarbose was used as positive control and the results were expressed as mg acarbose equivalents (AE)/g of EO.

Acetylcholinesterase and butyrylcholinesterase inhibitory activity assays were carried out following the previous method (Hong et al., 2021) with modifications. 140 μ L or 120 μ L of Tris-HCl buffer (pH8.0) was firstly added to a 96-well microplate, followed by the addition of 20 μ L of DTNB solution (1 mM), 10 μ L of essential oil solution and 10 μ L of acetylcholinesterase solution (2 U/mL) or 30 μ L of butyrylcholinesterase solution (2 U/mL). After 15 min of incubation at 37 °C, 20 μ L of ATCI (1 mM) or BTCI (1 mM) was added to start the reaction. After an additional 15 min of incubation at 37 °C, absorbance was immediately measured at 405 nm. A blank solution without essential oil was tested using the same method. Galanthamine was used as positive control and the results were expressed as mg galanthamine equivalents (GE)/g of EO.

2.8. Cytotoxic activity assays

Cytotoxic activities of *I. lanceolatum* EOs were evaluated by using the MTT assay against three cell lines, including HK-2 cells (proximal tubular cell line of normal kidney), HepG2 cells (human liver carcinoma cell line) and MCF-7 cells (human breast cancer cell line). Cytotoxicity assays were conducted according to the previous method (Zhang et al., 2017) with some modifications. Essential oil solutions in DMSO of 100 mg/mL were firstly prepared and six final concentrations (500, 200, 100, 50, 20 and 2 μ g/mL) were evaluated for each essential oil. The tested cells were cultured in a humidified 5 % CO₂ atmosphere. When the cells were cultivated to the logarithmic growth stage, they were seeded at 1×10^4 cells/well in 100 μ L of growth medium and incubated at 37 °C for 24 h. The microplates were treated with the essential oil solutions and incubated for 24 h. Then, 20 μ L of MTT solution (5 g/L) was added to each well and the microplate were incubated for an additional 4 h. 100 μ L of DMSO was added to each well. The absorbance was measured at 570 nm. The percentages of cell viability were calculated by the formula: cell viability (%) = $[A_{\text{sample}}/A_{\text{control}}] \times 100$ %. Doxorubicin was used as the positive control.

2.9. Molecular docking study

Molecular docking studies of selected compounds with better enzyme inhibitory activities in the root EO were carried out according to a previous method (Sun et al., 2023) with some modifications. The interactions between these compounds and enzymes were simulated using AutoDock Vina 1.1.2 (Trott and Olson, 2010). The protein structures of tyrosinase (Mauracher et al., 2014) and butyrylcholinesterase (Knez et al., 2018) were obtained from the Protein Data Bank database, with PDB ID codes as 4OUA and 6F7Q, respectively. Docking results were

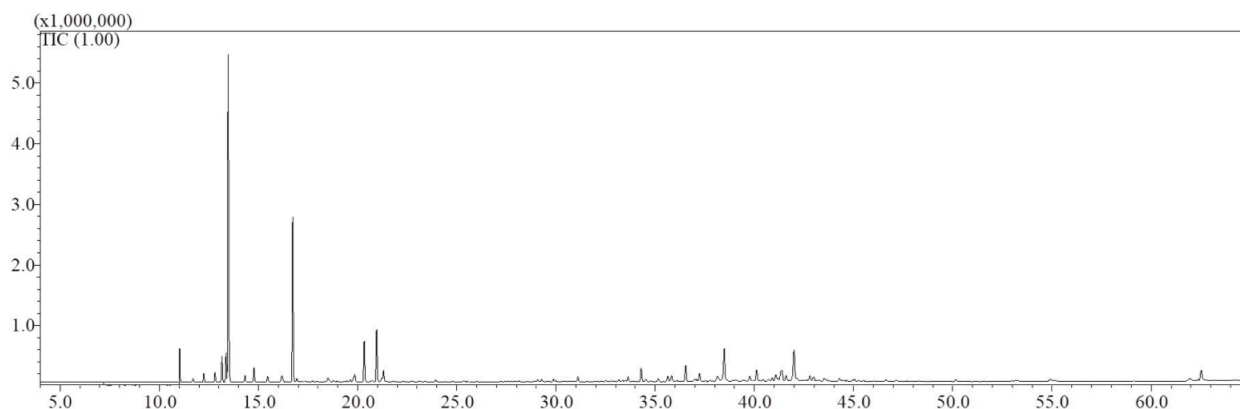


Fig. 2. Total Ion Chromatogram of the stem EO of *I. lanceolatum*.

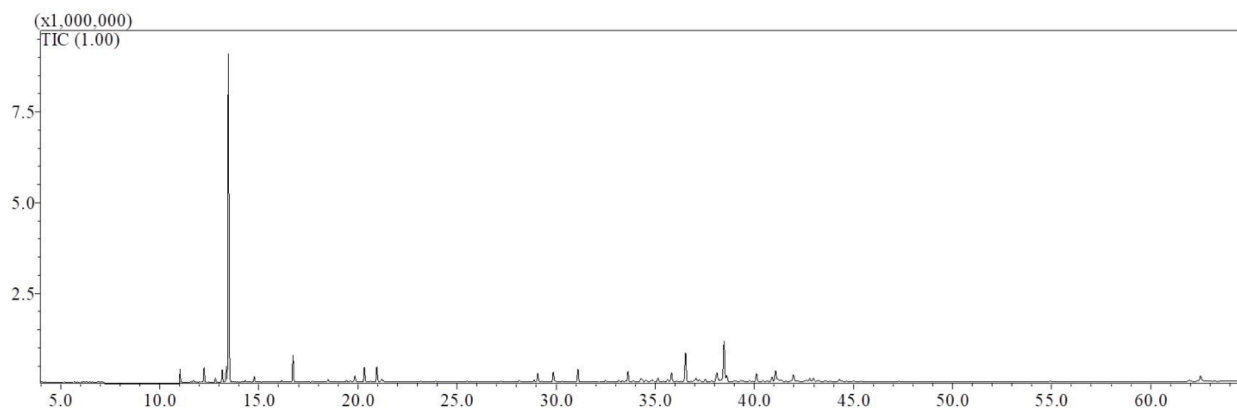


Fig. 3. Total Ion Chromatogram of the leaf EO of *I. lanceolatum*.

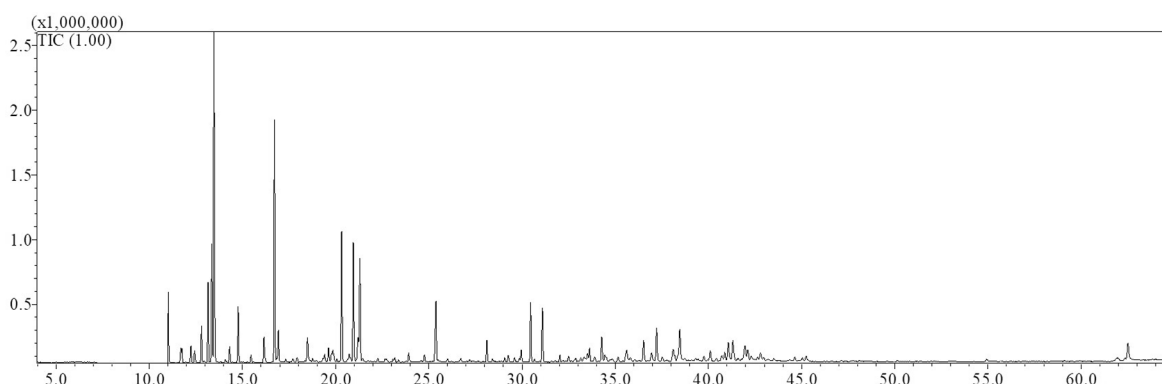


Fig. 4. Total Ion Chromatogram of the fruit EO of *I. lanceolatum*.

given as binding affinity values (kcal/mol). PyMOL and online tool named Protein-Ligand Interaction Profiler (PLIP) (Salentin et al., 2015) were used to analyze the interactions between the ligands and the active sites of enzymes.

2.10. Statistical analysis

Hydrodistillation, GC-MS and GC-FID analysis of essential oils were performed in triplicates. For antioxidant, antimicrobial and cytotoxic activity assays, three parallel experiments were performed. For enzyme inhibitory activity assays, four parallel experiments were performed in a 96-well plate. The data were expressed as the mean \pm SD. Statistical analysis was performed by use of SPSS 25.0 software. One-way ANOVA analysis was used to evaluate the differences among samples and statistical significance is indicated by a value of $p < 0.05$.

3. Results and discussion

3.1. Chemical composition of *I. lanceolatum* EOs

I. lanceolatum EOs were light yellow oily liquids. Essential oil yields in different parts of *I. lanceolatum* ranged from 0.09 % to 1.02 %. The leaves showed the highest essential oil yield (1.02 ± 0.05 %), followed by the fruits (0.30 ± 0.02 %), stems (0.21 ± 0.01 %) and roots (0.09 ± 0.01 %). The results seemed to be much lower than the previous studies (Huang et al., 2012; Liang et al., 2012) in which essential oil yields in the roots and fruits were 0.31 ± 0.03 % and 1.37 ± 0.11 %, respectively.

Total ion chromatograms of the root EO, stem EO, leaf EO and fruit EO could be seen in Figs. 1-4. Chemical composition of these four EOs were presented in Table 1. In the essential oils from the roots, stems, leaves and fruits, 45, 63, 57 and 82 chemical compounds were

identified, accounting for 91.85 %, 93.19 %, 94.14 % and 92.62 % of the total composition of the essential oils, respectively. The major compounds in the root EO were asaricin (50.52 %), borneol (7.01 %), camphor (5.71 %), eucalyptol (5.44 %) and camphene (3.29 %). The stem EO had eucalyptol (27.63 %), linalool (14.42 %), caryophyllene oxide (6.02 %), α -cadinol (5.30 %) and α -terpineol (4.11 %) as the major compounds. The major compounds in the leaf EO included eucalyptol (42.42 %), caryophyllene oxide (9.77 %), α -calacorene (3.77 %), linalool (3.35 %) and caryophylla-4(12),8(13)-dien-5-ol (3.08 %), while the dominant compounds in the fruit EO were eucalyptol (12.23 %), linalool (10.22 %), terpinen-4-ol (5.02 %), α -terpineol (4.41 %) and α -pinene (4.32 %). In terms of chemical structure, oxygenated monoterpenes dominated in the stem, leaf and fruit EOs, representing 51.84 %, 51.06 % and 40.93 % of the total composition, respectively. However, the most abundant compound in the root EO was asaricin, a phenylpropanoid compound which accounted for 50.52 %.

A total of 110 chemical compounds were identified, but only 18 compounds existed in all four essential oils. These 18 compounds accounted for 19.49 %, 67.54 %, 59.79 % and 54.06 % in the root, stem, leaf and fruit EOs, respectively. Five compounds, including eucalyptol, linalool, terpinen-4-ol, α -terpineol and α -cadinol, had the relative percentages of more than 1 % in all four essential oils and could be regarded as representative compounds in *I. lanceolatum* EOs. In particular, eucalyptol was the most abundant compound in the stem, leaf and fruit EOs. As the most abundant compound in the root EO, asaricin accounted for less than 1 % in the stem and leaf EOs, and even not detected in the fruit EO. Therefore, there were more differences between the root EO and other three EOs.

Of four EOs of *I. lanceolatum*, the chemical composition of the stem EO was firstly reported. The previous study (Huang et al., 2012) reported myristicin (17.6 %), α -asarone (17.2 %) and methyl isoeugenol

Table 1
Chemical composition of essential oils from roots, stems, leaves and fruits of *I. lanceolatum*.

No.	Compound	RI.c	RI.l	Relative percentage (%)				Identification
				Root EO	Stem EO	Leaf EO	Fruit EO	
1	tricyclene	921	921	0.43 ± 0.01	nd	nd	nd	MS, RI
2	α -thujene	926	924	0.03 ± 0.00	0.10 ± 0.00	0.14 ± 0.01	nd	MS, RI
3	α -pinene	932	932	0.94 ± 0.01	2.64 ± 0.04	2.18 ± 0.03	4.32 ± 0.02	MS, RI
4	camphene	947	946	3.29 ± 0.05	0.16 ± 0.00	0.11 ± 0.00	0.21 ± 0.01	MS, RI, S
5	sabinene	972	969	0.15 ± 0.00	0.62 ± 0.01	1.23 ± 0.02	0.36 ± 0.01	MS, RI
6	β -pinene	975	974	0.49 ± 0.01	2.83 ± 0.05	1.53 ± 0.02	3.43 ± 0.13	MS, RI
7	myrcene	991	988	0.17 ± 0.00	0.29 ± 0.01	0.26 ± 0.00	0.54 ± 0.01	MS, RI
8	dehydroxy-trans-linalool oxide	992	991	nd	nd	nd	0.38 ± 0.01	MS, RI
9	α -phellandrene	1003	1002	nd	0.53 ± 0.01	1.28 ± 0.02	0.49 ± 0.02	MS, RI
10	dehydroxy-cis-linalool oxide	1007	1006	nd	nd	nd	0.40 ± 0.01	MS, RI
11	α -terpinene	1016	1014	0.18 ± 0.01	0.55 ± 0.01	0.31 ± 0.01	0.94 ± 0.03	MS, RI
12	<i>p</i> -cymene	1023	1020	0.63 ± 0.01	1.44 ± 0.03	1.01 ± 0.01	2.16 ± 0.07	MS, RI
13	β -phellandrene	1027	1025	0.92 ± 0.03	1.34 ± 0.01	0.64 ± 0.03	3.60 ± 0.09	MS, RI
14	eucalyptol	1030	1026	5.44 ± 0.09	27.63 ± 0.49	42.42 ± 0.66	12.23 ± 0.19	MS, RI, S
15	2-heptyl acetate	1043	1038	nd	nd	nd	0.10 ± 0.01	MS, RI
16	(<i>E</i>)- β -ocimene	1048	1044	nd	0.38 ± 0.01	0.13 ± 0.00	0.46 ± 0.02	MS, RI
17	γ -terpinene	1058	1054	0.48 ± 0.01	0.93 ± 0.01	0.56 ± 0.01	1.76 ± 0.05	MS, RI
18	cis-linalool oxide	1072	1067	nd	0.29 ± 0.01	0.04 ± 0.01	0.19 ± 0.01	MS, RI
19	terpinolene	1087	1086	0.26 ± 0.00	0.57 ± 0.01	0.18 ± 0.00	0.95 ± 0.02	MS, RI
20	linalool	1100	1095	1.47 ± 0.01	14.42 ± 0.20	3.35 ± 0.04	10.22 ± 0.25	MS, RI, S
21	hotrienol	1104	1101	nd	0.24 ± 0.01	nd	1.32 ± 0.04	MS, RI
22	α -fenchol	1112	1114	nd	nd	nd	0.09 ± 0.01	MS, RI
23	cis- <i>p</i> -menth-2-en-1-ol	1121	1118	nd	0.10 ± 0.00	nd	0.15 ± 0.01	MS, RI
24	α -campholenal	1125	1122	nd	nd	nd	0.14 ± 0.00	MS, RI
25	trans-pinocarveol	1137	1135	nd	0.39 ± 0.01	0.35 ± 0.01	1.09 ± 0.02	MS, RI
26	camphor	1142	1141	5.71 ± 0.03	0.07 ± 0.00	nd	0.14 ± 0.00	MS, RI, S
27	camphene hydrate	1147	1145	0.37 ± 0.01	nd	nd	nd	MS, RI
28	nerol oxide	1155	1154	nd	nd	nd	0.07 ± 0.01	MS, RI
29	isoborneol	1156	1155	0.12 ± 0.00	nd	nd	nd	MS, RI
30	sabina ketone	1157	1154	nd	0.06 ± 0.00	0.18 ± 0.02	0.37 ± 0.01	MS, RI
31	pinocarvone	1161	1160	nd	0.13 ± 0.01	0.13 ± 0.01	0.51 ± 0.01	MS, RI
32	borneol	1164	1165	7.01 ± 0.02	0.26 ± 0.01	nd	0.24 ± 0.01	MS, RI, S
33	δ -terpineol	1166	1162	nd	0.45 ± 0.01	0.75 ± 0.02	0.50 ± 0.01	MS, RI
34	terpinen-4-ol	1176	1174	1.59 ± 0.01	3.10 ± 0.04	1.63 ± 0.02	5.02 ± 0.06	MS, RI, S
35	4-methylacetophenone	1183	1181	nd	0.06 ± 0.01	0.14 ± 0.00	0.09 ± 0.01	MS, RI
36	cryptone	1185	1183	nd	0.11 ± 0.01	nd	0.29 ± 0.01	MS, RI
37	trans-isocarveol	1187	1187	nd	nd	0.04 ± 0.00	0.09 ± 0.00	MS, RI
38	α -terpineol	1190	1186	1.34 ± 0.01	4.11 ± 0.03	1.70 ± 0.02	4.41 ± 0.06	MS, RI, S
39	myrtenal	1195	1195	nd	0.37 ± 0.03	0.40 ± 0.01	1.08 ± 0.03	MS, RI
40	estragole	1197	1195	2.04 ± 0.01	0.69 ± 0.03	nd	3.20 ± 0.04	MS, RI, S
41	trans-carveol	1218	1215	nd	0.05 ± 0.00	nd	0.14 ± 0.00	MS, RI
42	cis- <i>p</i> -mentha-1(7),8-dien-2-ol	1227	1227	nd	nd	nd	0.26 ± 0.01	MS, RI
43	thymol methyl ether	1235	1232	0.09 ± 0.00	nd	nd	nd	MS, RI
44	cuminaldehyde	1239	1238	nd	nd	0.10 ± 0.00	0.16 ± 0.01	MS, RI
45	geraniol	1255	1249	nd	0.19 ± 0.01	nd	0.55 ± 0.01	MS, RI
46	phellandral	1273	1272	nd	nd	nd	0.26 ± 0.00	MS, RI
47	bornyl acetate	1286	1284	0.38 ± 0.00	0.04 ± 0.00	0.04 ± 0.01	nd	MS, RI
48	safrole	1287	1285	nd	nd	nd	2.01 ± 0.03	MS, RI
49	<i>p</i> -cymen-7-ol	1290	1289	nd	nd	0.11 ± 0.01	nd	MS, RI
50	thymol	1292	1289	0.10 ± 0.00	nd	nd	nd	MS, RI
51	carvacrol	1301	1298	nd	nd	nd	0.06 ± 0.00	MS, RI
52	δ -terpinyl acetate	1317	1316	nd	nd	nd	0.16 ± 0.00	MS, RI
53	α -terpinyl acetate	1349	1346	0.29 ± 0.01	nd	nd	0.86 ± 0.02	MS, RI
54	α -longipinene	1350	1350	nd	0.07 ± 0.00	0.19 ± 0.01	nd	MS, RI
55	cyclosativene	1368	1369	nd	nd	0.15 ± 0.01	nd	MS, RI
56	α -ylangene	1372	1373	nd	0.13 ± 0.00	0.89 ± 0.01	0.13 ± 0.01	MS, RI
57	α -copaene	1376	1374	0.17 ± 0.01	0.15 ± 0.00	0.05 ± 0.00	0.23 ± 0.01	MS, RI
58	geranyl acetate	1384	1379	nd	nd	nd	0.21 ± 0.00	MS, RI
59	sativene	1390	1390	nd	0.25 ± 0.01	1.16 ± 0.02	0.14 ± 0.01	MS, RI
60	β -elemene	1392	1389	nd	nd	nd	0.47 ± 0.01	MS, RI
61	methyleugenol	1404	1403	0.27 ± 0.01	nd	nd	1.88 ± 0.05	MS, RI
62	α -barbatene	1409	1407	0.56 ± 0.00	nd	nd	nd	MS, RI
63	β -caryophyllene	1419	1418	nd	0.43 ± 0.01	1.48 ± 0.02	2.28 ± 0.06	MS, RI
64	cis-thujopsene	1431	1429	0.14 ± 0.00	nd	nd	nd	MS, RI
65	γ -elemene	1436	1434	0.24 ± 0.01	nd	nd	nd	MS, RI
66	β -barbatene	1442	1440	0.91 ± 0.01	nd	nd	0.31 ± 0.01	MS, RI
67	prezizaene	1446	1444	0.27 ± 0.01	nd	nd	nd	MS, RI
68	α -guaiene	1450	1445	0.11 ± 0.01	nd	nd	nd	MS, RI
69	α -humulene	1453	1452	nd	0.07 ± 0.01	0.13 ± 0.01	0.36 ± 0.01	MS, RI
70	9- <i>epi</i> -(<i>E</i>)-caryophyllene	1460	1464	nd	0.09 ± 0.01	nd	nd	MS, RI
71	cis-muurolo-4(14),5-diene	1462	1465	nd	nd	nd	0.23 ± 0.01	MS, RI
72	cadina-1(6),4-diene	1472	1475	nd	nd	nd	0.27 ± 0.01	MS, RI
73	eudesma-2,4,11-triene	1473	1479	nd	0.14 ± 0.01	0.11 ± 0.02	nd	MS, RI

(continued on next page)

Table 1 (continued)

No.	Compound	RI.c	RI.l	Relative percentage (%)				Identification
				Root EO	Stem EO	Leaf EO	Fruit EO	
74	β -chamigrene	1476	1476	0.16 ± 0.01	nd	nd	0.12 ± 0.01	MS, RI
75	α -amorphene	1480	1483	nd	0.42 ± 0.02	1.39 ± 0.07	0.55 ± 0.02	MS, RI
76	β -selinene	1486	1489	nd	nd	0.10 ± 0.00	0.28 ± 0.01	MS, RI
77	α -selinene	1495	1498	nd	nd	nd	1.16 ± 0.05	MS, RI
78	asaricin or sarisan	1496	1495	50.52 ± 0.33	0.16 ± 0.01	0.46 ± 0.01	nd	MS, RI, S
79	epizonarene	1499	1501	nd	nd	nd	0.47 ± 0.02	MS, RI
80	(Z)- α -bisabolene	1503	1506	0.12 ± 0.00	nd	nd	nd	MS, RI
81	cuparene	1506	1504	0.51 ± 0.01	nd	nd	nd	MS, RI
82	δ -amorphene	1507	1511	nd	nd	0.27 ± 0.00	0.18 ± 0.02	MS, RI
83	β -bisabolene	1509	1511	0.12 ± 0.01	nd	nd	nd	MS, RI
84	γ -cadinene	1513	1513	nd	nd	nd	0.25 ± 0.01	MS, RI
85	α -dehydro- <i>ar</i> -himachalene	1514	1516	nd	0.33 ± 0.01	0.53 ± 0.03	nd	MS, RI
86	myristicin	1520	1517	0.15 ± 0.00	nd	nd	nd	MS, RI
87	δ -cadinene	1523	1522	0.36 ± 0.00	0.54 ± 0.00	0.29 ± 0.02	0.92 ± 0.04	MS, RI
88	γ -dehydro- <i>ar</i> -himachalene	1527	1530	nd	0.56 ± 0.00	1.12 ± 0.00	0.18 ± 0.01	MS, RI
89	<i>cis</i> -calamenene	1532	1528	nd	nd	nd	0.08 ± 0.01	MS, RI
90	α -calacorene	1542	1544	nd	1.59 ± 0.01	3.77 ± 0.05	0.93 ± 0.05	MS, RI
91	elemicin	1556	1555	0.20 ± 0.01	0.92 ± 0.03	0.97 ± 0.01	1.47 ± 0.07	MS, RI
92	β -calacorene	1563	1564	nd	0.11 ± 0.02	0.32 ± 0.01	0.27 ± 0.02	MS, RI
93	himachalene epoxide	1575	1578	nd	1.15 ± 0.02	1.99 ± 0.02	1.01 ± 0.05	MS, RI
94	spathulenol	1576	1577	0.18 ± 0.01	nd	nd	0.15 ± 0.02	MS, RI
95	caryophyllene oxide	1582	1582	nd	6.02 ± 0.04	9.77 ± 0.07	2.85 ± 0.14	MS, RI
96	β -oplophenone	1608	1607	nd	0.75 ± 0.03	nd	0.30 ± 0.03	MS, RI
97	muurola-4,10(14)-dien-1-ol	1615	1615	nd	1.74 ± 0.03	1.31 ± 0.01	0.96 ± 0.10	MS, RI
98	α -corocalene	1622	1622	nd	0.19 ± 0.01	0.17 ± 0.01	0.21 ± 0.01	MS, RI
99	1- <i>epi</i> -cubenol	1627	1627	0.18 ± 0.01	0.25 ± 0.01	nd	0.40 ± 0.04	MS, RI
100	caryophylla-4(12),8(13)-dien-5-ol	1634	1639	nd	1.57 ± 0.01	3.08 ± 0.02	2.08 ± 0.11	MS, RI
101	6-methyl-6-(3-methylphenyl)-heptan-2-one	1637	1639	nd	0.30 ± 0.01	0.36 ± 0.01	nd	MS, RI
102	τ -cadinol	1639	1638	1.35 ± 0.08	nd	nd	1.74 ± 0.10	MS, RI
103	τ -muurolol	1640	1640	nd	2.27 ± 0.04	nd	nd	MS, RI
104	α -muurolol	1645	1644	nd	0.87 ± 0.02	nd	nd	MS, RI
105	α -cadinol	1653	1652	1.41 ± 0.07	5.30 ± 0.02	1.37 ± 0.01	1.29 ± 0.06	MS, RI
106	alloaromadendrene oxide	1655	1650	nd	0.60 ± 0.02	0.33 ± 0.01	1.47 ± 0.10	MS, RI
107	alohimachalol	1659	1661	nd	nd	nd	0.57 ± 0.03	MS, RI
108	<i>cis</i> -calamenen-10-ol	1666	1660	nd	nd	0.38 ± 0.00	nd	MS, RI
109	cadalene	1673	1675	nd	0.58 ± 0.00	0.61 ± 0.01	0.22 ± 0.01	MS, RI
110	10- <i>nor</i> -calamenen-10-one	1699	1702	nd	0.50 ± 0.03	0.45 ± 0.04	nd	MS, RI
	Compounds identified			45	63	57	82	
	Total identified (%)			91.85	93.19	94.14	92.62	
	Monoterpene hydrocarbons (%)			7.97	12.38	9.56	19.22	
	Oxygenated monoterpenes (%)			23.91	51.84	51.06	40.93	
	Sesquiterpene hydrocarbons (%)			3.67	5.65	12.73	10.24	
	Oxygenated sesquiterpenes (%)			3.12	20.82	18.59	12.82	
	Others (%)			53.18	2.50	2.20	9.41	

RI.c.: retention index **calculated** against n-alkane series; RI.l: retention index from **literature**; MS: mass spectrum; S: identification of chemical compounds by injection their **standards** on GC; EO: essential oil; nd: not detected.

(11.2 %) as the major compound in the root EO, which was quite different from our results. α -asarone was not detected and myristicin accounted for only 0.15 % in the current research. However, eucalyptol or 1,8-cineole (24.5 %) was previously reported as the most abundant compound in the fruit EO (Huang et al., 2012), which were in accordance with our results. For leaf EO, linalool (16.2 %), elemicin (14.9 %) and eucalyptol (14.8 %) were major compound in the previous study (Huang et al., 2012) while our study gave eucalyptol (42.42 %), caryophyllene oxide (9.77 %), α -calacorene (3.77 %) and linalool (3.35 %). The geographic location of *I. lanceolatum* could be the reason for the significant differences in chemical composition of essential oils.

In comparison with other species in the genus *Illicium*, many compounds in common could be found, such as eucalyptol, estragole, pinene and linalool in the essential oils from *I. verum* (Li et al., 2020), safrole, myristicin and eucalyptol in the essential oils from *I. henryi* (Liu et al., 2015). However, significant differences were also observed. Anethole, the most important compound in the *I. verum* essential oil was not detected in our study. Safrole (54.09 %) and myristicin (22.24 %), which were the major compounds in the *I. henryi* essential oil, accounted for only 2.01 % and 0.15 % in this study, respectively.

Of chemical compounds of *I. lanceolatum* EOs, attention should be paid to several compounds with similar structure, including asaricin,

myristicin, elemicin and safrole. Myristicin, elemicin and safrole have been extensively studied. Myristicin could be found in nutmeg and parsley, and has shown several promising activities, such as anti-inflammatory, anticancer and insecticidal activities (Seneme et al., 2021). The toxicities of myristicin and elemicin have been reported in some studies, and safrole has been classified as genotoxic carcinogens based on extensive toxicological evidence (Götz et al., 2022). In this work, safrole was found in the fruit EO with relative percentage of 2.01 % and its existence could limit the application of the fruit EO in food industry. Asaricin accounted for 50.52 % in the root EO and has been previously reported in the essential oils of some plants, such as *Beilschmiedia miersii* (Carvajal et al., 2016) and several Piper species (Soares et al., 2022). Due to its insecticidal activity, this compound has been structurally modified to obtain a series of asaricin analogues (Guo et al., 2016). Many biological activities of this compound remained unknown. As asaricin has a similar structure with myristicin, it is likely to have some degree of toxicity. The root EO of *I. lanceolatum* could be a natural source of asaricin for further studies. Due to the existence of these toxic compounds, the application of *I. lanceolatum* essential oils in food industry may be restricted.

Table 2
Antioxidant activities of *I. lanceolatum* EOs by three methods.

Sample	DPPH assay mg TE/g of EO	ABTS assay mg TE/g of EO	FRAP assay mg TE/g of EO
Root EO	3.18 ± 0.15	10.24 ± 0.11	176.33 ± 4.52
Stem EO	1.31 ± 0.06	4.46 ± 0.20	2.48 ± 0.02
Leaf EO	1.06 ± 0.07	4.01 ± 0.16	4.16 ± 0.06
Fruit EO	6.36 ± 0.10	8.97 ± 0.05	14.26 ± 0.29
BHT *	631.56 ± 8.42	1169.81 ± 8.04	642.37 ± 13.35

EO: essential oil; *: the unit for its antioxidant activities is mg TE/g of BHT.

Table 3
Antioxidant activities of nine compounds in the root EO by FRAP assay.

Component	Relative percentage/%	FRAP assay (mg TE/g of TC)
asaricin	50.52 ± 0.33	311.76 ± 3.64
borneol	7.01 ± 0.02	No activity
camphor	5.71 ± 0.03	No activity
eucalyptol	5.44 ± 0.09	No activity
camphene	3.29 ± 0.05	3.21 ± 0.04
estragole	2.04 ± 0.01	No activity
terpinen-4-ol	1.59 ± 0.01	No activity
linalool	1.47 ± 0.01	No activity
α -terpineol	1.34 ± 0.01	No activity
BHT	/	642.37 ± 13.35

TE: Trolox equivalent; TC: tested compound.

3.2. Antioxidant activity of *I. lanceolatum* EOs

In order to give a comprehensive evaluation on antioxidant activities of *I. lanceolatum* EOs, three methods with different mechanisms were employed. The antioxidant activity results were displayed in Table 2. In the DPPH assay, the fruit EO had the highest Trolox equivalent value (6.36 ± 0.10 mg TE/g of EO), while the highest Trolox equivalent value (10.24 ± 0.11 mg TE/g of EO) was observed for the root EO in the ABTS assay. In the DPPH and ABTS assays, *I. lanceolatum* EOs showed very weak radical scavenging capacity when they were compared with BHT. However, the root EO showed moderate antioxidant activity in the FRAP assay, with the Trolox equivalent value as 176.33 ± 4.52 mg TE/g of EO. Other three essential oils displayed very weak antioxidant activity in the FRAP assay.

Strong radical scavenging capacity of essential oil is closely connected with phenolic compounds, like thymol, carvacrol and eugenol, which can react with radicals by donating hydrogen atom (Zeb, 2020). Thymol and carvacrol existed in the root and fruit EOs, however, their relative percentages were only 0.10 % and 0.06 %, respectively. Very low relative percentages of phenolic compounds in the *I. lanceolatum* EOs could be the reason for their low radical scavenging capacities in the DPPH and ABTS assays.

As the root EO showed moderate antioxidant activity in the FRAP assay, its major compounds were further studied. There were 11 compounds which had the relative percentages of more than 1 % in the root EO. 9 purchased compounds could be in Table 3 and 2 compounds including τ -cadinol (1.35 %) and α -cadinol (1.41 %) were not available.

Table 4
MICs and MMCs of *I. lanceolatum* EOs against 5 gram-positive bacteria (mg/mL).

Samples	<i>S. aureus</i>		<i>S. lentus</i>		<i>S. epidermidis</i>		<i>B. cereus</i>		<i>L. monocytogenes</i>	
	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC
Root EO	3.13	6.25	12.5	12.5	12.5	25	3.13	3.13	3.13	12.5
Stem EO	6.25	12.5	12.5	25	12.5	25	6.25	6.25	6.25	25
Leaf EO	6.25	12.5	25	>25	12.5	25	6.25	6.25	6.25	25
Fruit EO	3.13	3.13	12.5	12.5	12.5	25	6.25	6.25	6.25	12.5
Gentamycin sulfate	0.0005	0.0001	0.0004	0.0008	0.0004	0.0008	0.0001	0.0001	0.0004	0.0008

MIC: minimum inhibitory concentration; MMC: minimum microbiocidal concentration; EO: essential oil;

Table 5
MICs and MMCs of *I. lanceolatum* EOs against 3 gram-negative bacteria (mg/mL).

Samples	<i>E. coli</i>		<i>P. aeruginosa</i>		<i>S. typhimurium</i>	
	MIC	MMC	MIC	MMC	MIC	MMC
Root EO	6.25	12.5	25	> 25	6.25	12.5
Stem EO	12.5	25	25	> 25	12.5	25
Leaf EO	25	> 25	25	> 25	12.5	25
Fruit EO	12.5	25	25	25	12.5	12.5
Gentamycin sulfate	0.013	0.025	0.0002	0.0008	0.0008	0.0016

Table 6
MICs and MMCs of *I. lanceolatum* EOs against one yeast (mg/mL).

Samples	<i>C. albicans</i>	
	MIC	MMC
Root EO	12.5	25
Stem EO	12.5	25
Leaf EO	25	>25
Fruit EO	12.5	25
Amphotericin B	0.003	0.006

These nine compounds were further evaluated by FRAP assay and results were given in Table 3. Seven compounds, such as borneol and eucalyptol, showed no activities. Camphene had very weak antioxidant activity in the FRAP assay. However, antioxidant activity with 311.76 ± 3.64 mg TE/g of TC was noticed for asaricin which had higher Trolox equivalent value than the root EO. Considering its high relative percentage (50.52 %), this molecule could be the main antioxidant compound in the root EO. Certainly, some other compounds which had lower relative percentages in the root EO could contribute together to the antioxidant activity. Synergistic and antagonistic effects between these compounds could exist.

The antioxidant activity of asaricin in the FRAP assay was firstly reported in this research, however, antioxidant mechanism of asaricin was still unknown. Subsequent investigations could be performed to find out how asaricin react with ferric and if asaricin could exert real antioxidant protection in the application experiments.

3.3. Antimicrobial activity of *I. lanceolatum* EOs

Antimicrobial activities of essential oils from the roots, stems, leaves and fruits of *I. lanceolatum* were evaluated against 5 gram-positive bacteria, 3 gram-negative bacteria and 1 yeast. The MICs and MMCs of four essential oils were determined and presented in Tables 4-6. *I. lanceolatum* EOs showed some degree of antimicrobial activities against all tested microorganisms, with MIC values ranging from 3.13 to 25 mg/mL. According to the MIC criteria of essential oils (Van Vuuren and Holl, 2017), if the essential oil displayed a MIC of less than 1000 μ g/ml, the antimicrobial activity could be considered to be noteworthy; from 101 to 500 μ g/mL, the antimicrobial activity was strong; from 500 to 1000 μ g/ml, the antimicrobial activity was moderate. In this work, *I. lanceolatum* EOs showed very weak antibacterial or antifungal

Table 7

MICs and MMCs of nine compounds in the root EO against three bacteria.

Compound	Relative percentages %	<i>S. aureus</i>		<i>B. cereus</i>		<i>L. monocytogenes</i>	
		MIC	MBC	MIC	MBC	MIC	MBC
asaricin	50.52 ± 0.33	12.5	25	> 12.5	> 12.5	> 12.5	> 12.5
borneol	7.01 ± 0.02	3.13	3.13	1.56	3.13	3.13	12.5
camphor	5.71 ± 0.03	0.78	1.56	1.56	1.56	3.13	3.13
eucalyptol	5.44 ± 0.09	>25	>25	>25	>25	>25	>25
camphene	3.29 ± 0.05	6.25	12.5	3.13	6.25	12.5	12.5
estragole	2.04 ± 0.01	>25	>25	25	>25	>25	>25
terpinen-4-ol	1.59 ± 0.01	12.5	25	12.5	12.5	12.5	25
linalool	1.47 ± 0.01	12.5	25	12.5	25	25	>25
α -terpineol	1.34 ± 0.01	12.5	12.5	6.25	6.25	12.5	12.5
gentamycin sulfate	/	0.00005	0.0001	0.0001	0.0001	0.0004	0.0008

MIC: minimum inhibitory concentration; MMC: minimum microbiocidal concentration;

Table 8Enzyme inhibitory activities of the *I. lanceolatum* EOs.

Sample	Tyrosinase mg KAE/g of EO	α -glucosidase mg AE/g of EO	Acetylcholinesterase mg GE/g of EO	Butyrylcholinesterase mg GE/g of EO
Root EO	30.34 ± 0.40	No activity	4.83 ± 0.16	43.25 ± 1.50
Stem EO	5.39 ± 0.36	No activity	8.39 ± 0.40	28.78 ± 0.84
Leaf EO	9.11 ± 0.17	No activity	17.79 ± 0.32	28.19 ± 1.03
Fruit EO	10.59 ± 0.53	No activity	4.35 ± 0.32	31.16 ± 0.84

KAE: kojic acid equivalent; AE: acarbose equivalent; GE: galanthamine equivalent; EO: essential oil.

activities, which was also confirmed by comparing their MICs with those of gentamycin sulfate or amphotericin B.

The root EO showed better antimicrobial activities than other three EOs, with MIC values as 3.13 mg/mL against three gram-positive bacteria including *S. aureus*, *B. cereus* and *L. monocytogenes*. MICs and MMCs of nine major compounds in the root EO against *S. aureus*, *B. cereus* and *L. monocytogenes* were further determined and displayed in Table 7. Two compounds (eucalyptol and estragole) showed no antimicrobial activities against three bacteria. MIC of asaricin against *S. aureus* was 12.5 mg/mL, which was higher than the MIC of the root EO. Of nine compounds, camphor showed the best antimicrobial activities, followed by borneol. MICs of camphor against three bacteria ranged from 0.78 to 3.13 mg/mL while MICs of borneol ranged from 1.56 to 3.13 mg/mL. These two compounds accounted for 7.01 % and 5.71 % in the root EO, while their highest relative percentage in other three EOs was only 0.26 %. Therefore, camphor and borneol could be the reason for better antibacterial activities of the root EO than other three EOs, and were two important antimicrobial compounds in the root EO. Besides the compounds listed in Table 7, many other compounds in the root EO, such as *p*-cymene (Marchese et al., 2017), α -pinene, β -pinene (Rivas da Silva et al., 2012) and thymol (Escobar et al., 2020) have been reported with antimicrobial activities. In general, essential oils with high content of thymol and other phenolic compounds have strong antimicrobial activities, as in the case of oregano essential oil. The relative percentage of thymol in the root EO was only 0.10 %, which means little contribution to antimicrobial activity. Only by looking at the antimicrobial activities of these compounds in the root EO, it was difficult to fully explain antimicrobial activity of this essential oil as synergistic and antagonistic effects existed in the essential oils. In previous studies, camphor has been reported to have synergistic antimicrobial effects with *Lavandula latifolia* essential oil (Karaca et al., 2021), and three essential oils from cinnamon, manuka, and winter savory have demonstrated synergistic antimicrobial activities (Fratini et al., 2019). Synergistic effects could be another factor for better antimicrobial activities of the root EO.

3.4. Enzyme inhibitory activities of *I. lanceolatum* EOs

Tyrosinase, α -glucosidase, acetylcholinesterase and butyrylcholinesterase inhibitory activities of *I. lanceolatum* EOs were determined to evaluate their whitening effect, anti-diabetes effect and

Table 9

Tyrosinase and butyrylcholinesterase inhibitory activities of nine compounds in the root EO.

Compound	Relative percentages %	Tyrosinase mg KAE/g of TC	Butyrylcholinesterase mg GE/g of TC
asaricin	50.52 ± 0.33	33.42 ± 2.11	29.01 ± 2.60
borneol	7.01 ± 0.02	7.09 ± 0.24	No activity
camphor	5.71 ± 0.03	6.99 ± 0.55	20.68 ± 0.50
eucalyptol	5.44 ± 0.09	7.12 ± 0.37	7.47 ± 0.29
camphene	3.29 ± 0.05	4.69 ± 0.30	No activity
estragole	2.04 ± 0.01	14.68 ± 0.17	No activity
terpinen-4-ol	1.59 ± 0.01	8.77 ± 0.68	55.58 ± 3.41
linalool	1.47 ± 0.01	7.21 ± 0.85	No activity
α -terpineol	1.34 ± 0.01	9.19 ± 0.94	128.14 ± 2.85

KAE: kojic acid equivalent; GE: galanthamine equivalent; TC: tested compound.

improvement effect on neurodegenerative disease, and the results expressed as mg kojic acid equivalent (KAE)/g of EO, mg acarbose equivalent (AE)/g of EO and mg galanthamine equivalent (GE)/g of EO were presented in Table 8. In the antidiabetic drugs, one important category is α -glucosidase inhibitor, like acarbose and miglitol. Four essential oils showed no inhibitory activity on α -glucosidase, which indicated no potential of *I. lanceolatum* EOs as antidiabetic agents. However, *I. lanceolatum* EOs displayed inhibitory activities on other three enzymes. For tyrosinase and butyrylcholinesterase, the highest KAE value (30.34 ± 0.40 mg KAE/g of EO) and GE value (43.25 ± 1.50 mg GE/g of EO) were observed for the root EO, followed by the fruit EO.

The root EO showed better tyrosinase and butyrylcholinesterase inhibitory activities than other three EOs, its nine major compounds were further evaluated and the results could be seen in Table 9. Tyrosinase play an important role in the biogenesis of melanin and tyrosinase inhibitors could find their use in skin-whitening. All nine tested compounds showed tyrosinase inhibitory activities. Asaricin showed the highest KAE value (33.42 ± 2.11 mg KAE/g of TC), followed by estragole (14.68 ± 0.17 KAE/g of TC). Considering its high relative percentage (50.52 %), asaricin could be main contributor to tyrosinase inhibitory activity of the root EO. Estragole has been reported to have skin-whitening effect and compounds synthesized from this molecule showed good tyrosinase inhibitory activity (Motoki et al., 2003). In the

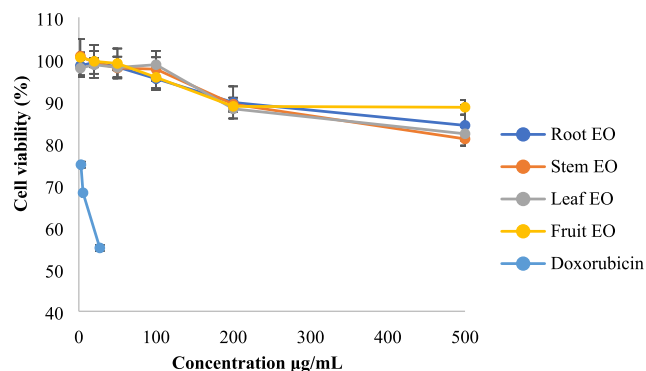


Fig. 5. Cytotoxic effects on HK-2 treated with root, stem, leaf and fruit EOs at six concentrations.

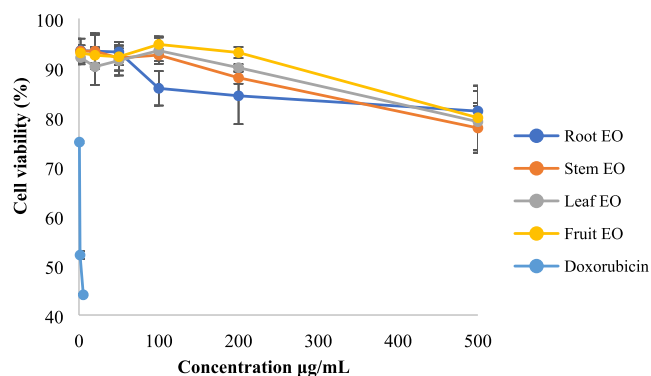


Fig. 6. Cytotoxic effects on MCF-7 treated with root, stem, leaf and fruit EOs at six concentrations.

previous studies, polyphenols like flavonoids and phenolic acids as natural tyrosinase inhibitor (Zolghadri et al., 2019) have attracted a lot of attention. There were also numerous studies on the tyrosinase inhibitory activities of different essential oils, however, the reports on the tyrosinase inhibitory activities of individual compounds in the essential oils were limited. In this study, tyrosinase inhibitory activities of some compounds like asaricin were firstly reported. Synergistic strategy is often used to improve the inhibitory activities of tyrosinase inhibitors (Zolghadri et al., 2019), the synergistic effect could be one reason for the better tyrosinase inhibitor activity of the root EO.

For butyrylcholinesterase, only five tested compounds showed inhibitory activities. α -terpineol showed the best butyrylcholinesterase inhibitory activity (128.14 ± 2.85 mg GE/g of TC), followed by terpinen-4-ol (55.58 ± 3.41 mg GE/g of TC) and asaricin (29.01 ± 2.60 mg GE/g of TC). Eucalyptol (Costa et al., 2012a), camphor (Costa et al., 2012b) and terpinen-4-ol (Bonesi et al., 2010) have been reported to have butyrylcholinesterase inhibitory activities in previous studies. Though α -terpineol and terpinen-4-ol showed higher GE values than the root EO (43.25 ± 1.50 mg GE/g of EO), their relative percentages were only 1.34 % and 1.59 %, respectively, counterbalancing their contribution to the butyrylcholinesterase inhibitory activity of the essential oil. Asaricin, with relative percentage of 50.52 %, was still the most important contributor to the butyrylcholinesterase inhibitory activity of the root EO. Certainly, the activity of the root EO could not be explained by these five compounds. There were other active compounds in the root EOs, like α -pinene and β -pinene (Orhan et al., 2008), which have been reported with activities. On the other hand, there were also synergistic and antagonistic effects between these compounds.

For acetylcholinesterase, the leaf EO displayed better inhibitory activity (17.79 ± 0.32 mg GE/g of EO) than other three EOs. Chemical compounds of leaf EO were represented by eucalyptol (42.42 %) and

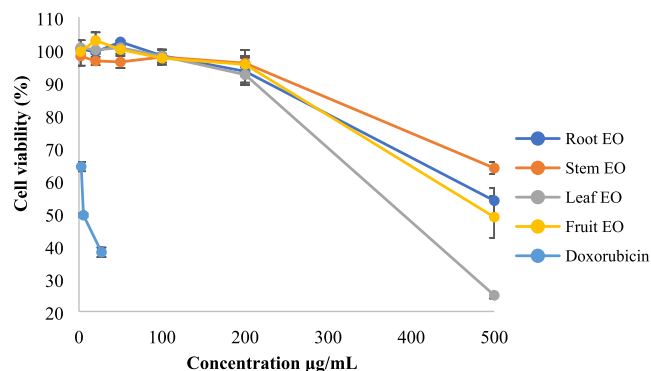


Fig. 7. Cytotoxic effects on HepG2 treated with root, stem, leaf and fruit EOs at six concentrations.

caryophyllene oxide (9.77 %). The acetylcholinesterase inhibitory activity of eucalyptol (or 1,8-cineole) has been reported in many studies (Burcul et al., 2020) and that of caryophyllene oxide was also displayed in previous study (Savelev et al., 2004). In this study, eucalyptol was further evaluated for its acetylcholinesterase inhibition activity, with GE value as 32.59 ± 0.57 mg GE/g of TC. It could be concluded that eucalyptol was important active compound in the leaf EO. If considering both acetylcholinesterase and butyrylcholinesterase inhibitory activities values, the leaf EO seemed to be a better one which could exert improvement on neurodegenerative diseases.

3.5. Cytotoxic activity of *I. lanceolatum* EOs

Cytotoxic effects of *I. lanceolatum* EOs on HK-2 (proximal tubular cell line of normal kidney), MCF-7 (human breast cancer cell line) and HepG2 (human liver carcinoma cell line) cells could be seen in Figs. 5-7, respectively. Doxorubicin was used as the positive control. At concentrations of 2, 20, 50 and 100 μ g/mL, the cell viabilities of HK-2 were found to be more than 95 %, which means that *I. lanceolatum* EOs were nontoxic to HK-2 at these concentrations. When the concentrations reached to 200 μ g/mL, the cell viabilities of HK-2 were nearly 90 %. At the concentration of 500 μ g/mL, the cell viabilities of HK-2 treated with the fruit EO, were still 88.57 %. The fruit was highly toxic due to the existence of anisatin, however, only weak toxicity to normal kidney cells was observed for the fruit EO at the tested concentrations.

As shown in Fig. 6, the cell viabilities of MCF-7 cells ranged from 77.84 % to 94.74 % at six tested concentrations. At the concentration of 500 μ g/mL, *I. lanceolatum* EOs showed some degree of anticancer activities to MCF-7. Compared with doxorubicin which can inhibit almost 50 % of MCF-7 at the concentration of 1.36 μ g/mL, anticancer activities to MCF-7 of *I. lanceolatum* EOs were very weak, indicating no potential as anticancer drugs.

For HepG2 cells treated with the *I. lanceolatum* EOs at concentrations of 200 μ g/mL and below 200 μ g/mL, the cell viabilities were more than 92 %. However, when the concentration reached up to 500 μ g/mL, anticancer activities to HepG2 cells were observed for all four essential oils. The root and fruit EOs almost inhibited 50 % of HepG2 cells. The leaf EO inhibited even 75 % of HepG2 cells, indicating potential use in anticancer therapy.

A number of studies have been performed on the anticancer activities of the essential oils (Bhalla et al., 2013) and their constituents (Silva et al., 2021). Many compounds in the *I. lanceolatum* EOs have been reported with anticancer activities to HepG2 cells, such as eucalyptol (Rodenak-Kladniew et al., 2020), linalool (Rodenak-Kladniew et al., 2018), α -pinene (Xu et al., 2018) and β -elemene (Dai et al., 2013). The leaf EO was represented by high percentage of eucalyptol (42.42 %), which could be the reason for its better anticancer activity. Caryophyllene oxide, which accounted for 6.02 %, 9.77 % and 2.85 % in the stem, leaf and fruit EOs, respectively, were found to have no inhibition

Table 10

Binding affinities of four compounds against tyrosinase and butyrylcholinesterase.

No.	Compound	PubChem ID	Binding Affinities (kcal/mol)	
			Tyrosinase	Butyrylcholinesterase
1	asaricin	95289	-6.3 ± 0.0	-6.0 ± 0.1
2	estragole	8815	-5.7 ± 0.1	/
3	terpinen-4-ol	11230	/	-5.9 ± 0.1
4	α -terpineol	17100	-6.0 ± 0.0	-6.1 ± 0.0

effects on HepG2 in previous study (Xiu et al., 2022). The synergistic effects between anticancer compounds may exist in the inhibition of HepG2 cells.

3.6. Molecular docking studies of selected compounds in the root EO

In the root EO, asaricin, estragole and α -terpineol showed better tyrosinase inhibitory activities while α -terpineol, terpinen-4-ol and asaricin showed better butyrylcholinesterase inhibitory activities. These compounds were selected for molecular docking to investigate the interactions between ligands and enzymes. The binding affinities (kcal/mol) of these compounds against tyrosinase and butyrylcholinesterase were presented in Table 10. Asaricin showed the lowest binding affinities (-6.3 kcal/mol) with tyrosinase, indicating the strongest inhibitory activity of these three compounds, which was in accordance with the experimental results. The butyrylcholinesterase inhibitory activity of α -terpineol (128.14 ± 2.85 mg GE/g TC) was much higher than those of terpinen-4-ol (55.58 ± 3.41 mg GE/g TC) and asaricin (29.01 ± 2.60 mg GE/g TC), however, no significant differences could be observed among binding affinities of these three compounds ($p < 0.05$). It is difficult to accurately predict the activity of one molecule only by using binding affinity. Generally, different molecular docking software should be employed together for screening or comparison of active compounds.

The interactions of asaricin, estragole and α -terpineol with amino acid residues in the binding pocket of tyrosinase could be seen in Fig. 8. The main interactions of asaricin and estragole with tyrosinase were hydrophobic interaction, hydrogen bond and π -stacking. α -terpineol formed two hydrogen bonds with ASP-344 and THR-345, and had hydrophobic interactions with three residues including THR-345, PHE-355 and VAL-366. The interactions of α -terpineol, terpinen-4-ol and asaricin with amino acid residues of butyrylcholinesterase were shown in Fig. 9. α -terpineol formed one hydrogen bond with TRP-82 and had hydrophobic interactions with three residues including PRO-285, TYR-332 and TRP-430. Terpinen-4-ol mainly interacted with butyrylcholinesterase by hydrophobic interaction. Asaricin had hydrophobic and π -stacking interactions with TRP-82. From the interactions of these three compounds, it can be seen that TRP-82 was one of important amino acid residues in the active site of butyrylcholinesterase.

4. Conclusion

In the present study, chemical compounds and biological activities of essential oils from the roots, stems, leaves and fruits of *I. lanceolatum* were comprehensively investigated. 110 compounds were identified in these essential oils, representing 91.85 % to 94.84 % of the total compositions. Asaricin was found to be the most abundant compound in the root EO while eucalyptol had the highest relative percentages in other three EOs. *I. lanceolatum* EOs showed weak radical scavenging capacities, but the root EO displayed moderate reducing ability which was caused by asaricin. *I. lanceolatum* EOs showed antimicrobial activities against all tested microorganisms. Especially, the root EO showed better antimicrobial activities than other three EOs, with MIC values as 3.13 mg/mL against three bacteria including *S. aureus*, *B. cereus* and *L. monocytogenes*. Camphor and borneol were found to be important antimicrobial compounds in the root EO. For enzyme inhibitory activities, no inhibitory activity on α -glucosidase was observed. The root EO

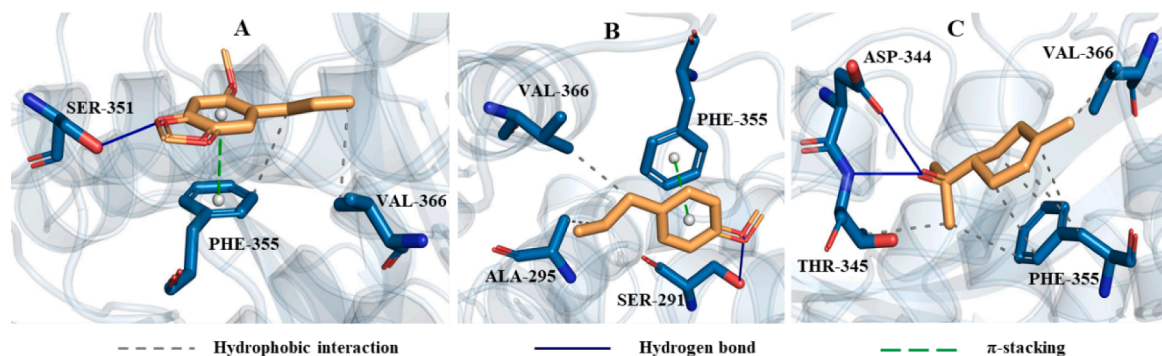


Fig. 8. The interactions of tyrosinase with asaricin (A), estragole (B) and α -terpineol (C).

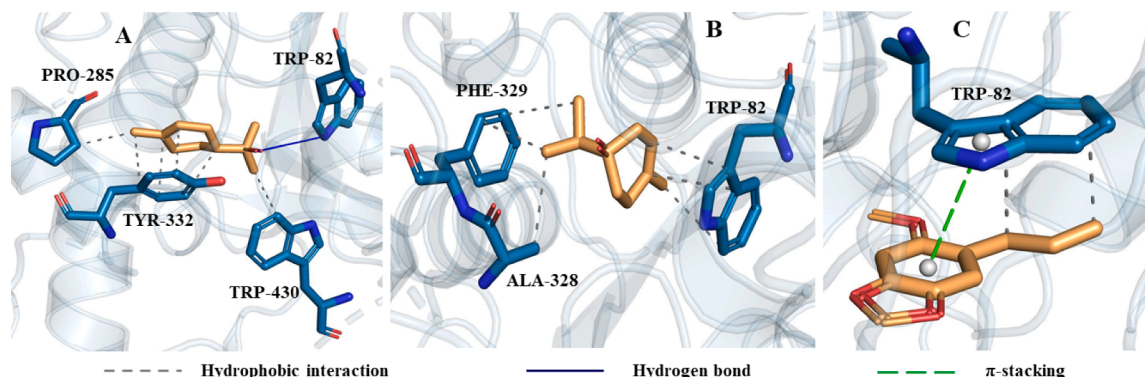


Fig. 9. The interactions of butyrylcholinesterase with α -terpineol (A), terpinen-4-ol (B) and asaricin (C).

showed better tyrosinase and butyrylcholinesterase inhibitory activities while the leaf EO displayed better acetylcholinesterase inhibitory activity. Molecular docking revealed that the main interactions between active compounds (asaricin, estragole, terpinen-4-ol and α -terpineol) and enzymes (tyrosinase and butyrylcholinesterase) were hydrophobic interaction, hydrogen bond and π -stacking. At six tested concentrations, only weak cytotoxicity was observed to HK-2 cells of normal kidney. *I. lanceolatum* EOs showed very weak anticancer activities to MCF-2 cells but significant anticancer activities to HepG2 cells at the concentration of 500 $\mu\text{g/mL}$. In this study, antioxidant, antimicrobial, enzyme inhibitory and cytotoxic activities of *I. lanceolatum* EOs and some compounds were firstly reported. *I. lanceolatum* EOs was found to be promising with possible applications in pharmaceutical and cosmetic industries.

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CRediT authorship contribution statement

Tianming Zhao: Conceptualization, Funding acquisition, Methodology, Investigation, Formal analysis, Supervision, Writing – original draft, Writing – review & editing. **Guang Fan:** Investigation, Formal analysis. **You Tai:** Investigation. **Xinhe Shu:** Investigation. **Fu Tian:** Investigation, Formal analysis. **Shuliang Zou:** Software, Investigation. **Qin Wu:** Software, Investigation, Funding acquisition.

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.

References

- Adams, R.P., 2007. Identification of essential oil components by gas chromatography/mass spectrometry, 4th Edition. Allured Publishing Corporation, Carol Stream.
- Ahamad, J., 2021. Aroma profile and α -glucosidase inhibitory activity of essential oil of *Mentha spicata* leaves. J. Essent. Oil Bear. Plants 24 (5), 1042. <https://doi.org/10.1080/0972060X.2021.2003257>.
- Bhalla, Y., Gupta, V.K., Jaitak, V., 2013. Anticancer activity of essential oils: a review. J. Sci. Food Agric. 93 (15), 3643–3653. <https://doi.org/10.1002/jsfa.6267>.
- Bonesi, M., Menichini, F., Tundis, R., Loizzo, M.R., Conforti, F., Passalacqua, N.G., Statti, G.A., Menichini, F., 2010. Acetylcholinesterase and butyrylcholinesterase inhibitory activity of *Pinus* species essential oils and their constituents. J. Enzyme Inhib. Med. Chem. 25 (5), 622–628. <https://doi.org/10.3109/14756360903389856>.
- Burcul, F., Blazevic, I., Radan, M., Politeo, O., 2020. Terpenes, phenylpropanoids, sulfur and other essential oil constituents as inhibitors of cholinesterases. Curr. Med. Chem. 27 (26), 4297–4343. <https://doi.org/10.2174/0929867325666180330992607>.
- Carvajal, M.A., Vergara, A.P., Santander, R., Osorio, M.E., 2016. Chemical composition and anti-phytopathogenic activity of the essential oil of *Beilschmiedia miersii*. Nat Prod Commun. 11(9), 1367–1372. <https://www.ncbi.nlm.nih.gov/pubmed/30807044>.
- Costa, P., Grosso, C., Gonçalves, S., Andrade, P.B., Valentão, P., Gabriela Bernardo-Gil, M., Romano, A., 2012a. Supercritical fluid extraction and hydrodistillation for the recovery of bioactive compounds from *Lavandula viridis* L'Hér. Food Chem. 135 (1), 112–121. <https://doi.org/10.1016/j.foodchem.2012.04.108>.
- Costa, P., Gonçalves, S., Grosso, C., Andrade, P.B., Valentão, P., Gabriela Bernardo-Gil, M., Romano, A., 2012b. Chemical profiling and biological screening of *Thymus lotocephalus* extracts obtained by supercritical fluid extraction and hydrodistillation. Ind. Crop. Prod. 36 (1), 246–256. <https://doi.org/10.1016/j.indcrop.2011.09.014>.
- Dai, Z.J., Tang, W., Lu, W.F., Gao, J., Kang, H.F., Ma, X.B., Min, W.L., Wang, X.J., Wu, W. Y., 2013. Antiproliferative and apoptotic effects of beta-elemene on human hepatoma HepG2 cells. Cancer Cell Int. 13 (1), 27. <https://doi.org/10.1186/1475-2867-13-27>.
- Escobar, A., Pérez, M., Romanelli, G., Blustein, G., 2020. Thymol bioactivity: a review focusing on practical applications. Arab. J. Chem. 13 (12), 9243–9269. <https://doi.org/10.1016/j.arabj.2020.11.009>.

- Estevez, A.M., Estevez, R.J., 2012. A short overview on the medicinal chemistry of (-)-shikimic acid. Mini Rev. Med. Chem. 12 (14), 1443–1454. <https://doi.org/10.2174/13895712803832735>.
- Figueiredo, A.C., Barroso, J.G., Pedro, L.G., Scheffer, J.J.C., 2008. Factors affecting secondary metabolite production in plants: volatile components and essential oils. Flavour Fragr. J. 23 (4), 213–226. <https://doi.org/10.1002/ffj.1875>.
- Fratini, F., Mancini, S., Turchi, B., Sparagni, D., Al-Gwad, A.A., Najjar, B., Pistelli, L., Cerri, D., Pedonesi, F., 2019. Antimicrobial activity of three essential oils (cinnamon, manuka, and winter savory), and their synergic interaction, against *Listeria monocytogenes*. Flavour Fragr. J. 34 (5), 339–348. <https://doi.org/10.1002/ffj.3514>.
- Gao, W., Wang, D., Yang, X., Pan, T., Chai, X., Zhang, Z., 2020. Hong-Hui-Xiang alleviates pain hypersensitivity in a mouse model of monoarthritis. Pain Res. Manag. 2020, 5626948. <https://doi.org/10.1155/2020/5626948>.
- Götz, M.E., Sachse, B., Schäfer, B., Eisenreich, A., 2022. Myristicin and elemicin: potentially toxic alkenylbenzenes in food. Foods 11, 1988. <https://doi.org/10.3390/foods11131988>.
- Gui, X., Wang, G., Zhang, N., Huang, B., 2014. New phenylpropanoid and other compounds from *Illicium lanceolatum* with inhibitory activities against LPS-induced NO production in RAW 264.7 macrophages. Fitoterapia 95, 51–57. <https://doi.org/10.1016/j.fitote.2014.02.015>.
- Guo, Y., Qu, L., Wang, X., Huang, M., Jia, L., Zhang, Y., 2016. Iodine-catalyzed oxidative cyclisation for the synthesis of asaricin analogues containing 1,3,4-oxadiazole as insecticidal agents. RSC Adv. 6, 93505–93510. <https://doi.org/10.1039/C6RA22343E>.
- Hong, Y., Liu, X.L., Wang, H.J., Zhang, M., Tian, M.Y., 2021. Chemical composition, antibacterial, enzyme-inhibitory, and anti-inflammatory activities of essential oil from *Hedychium puerense* Rhizome. Agronomy-Basel 11 (12), 2506. <https://doi.org/10.3390/agronomy11122506>.
- Howes, M.J.R., Kite, G.C., Simmonds, M.S.J., 2009. Distinguishing Chinese star anise from Japanese star anise using thermal desorption-gas chromatography-mass spectrometry. J. Agric. Food Chem. 57 (13), 5783–5789. <https://doi.org/10.1021/jf9009153>.
- Huang, B., Liang, J., Wang, G., Qin, L., 2012. Comparison of the volatile components of *Illicium verum* and *I. lanceolatum* from East China. J. Essent. Oil Bear. Plants 15 (3), 467–475. <https://doi.org/10.1080/0972060X.2012.10644074>.
- Karaca, N., Sener, G., Demirci, B., Demirci, F., 2021. Synergistic antibacterial combination of *Lavandula latifolia* Medik. essential oil with camphor. Z Naturforsch C. J. Biosci. 76 (3–4), 169–173. <https://doi.org/10.1515/znc-2020-0051>.
- Knez, D., Coquelle, N., Pislar, A., Zakej, S., Jukic, M., Sova, M., Mravljak, J., Nachon, F., Brazzolotto, X., Kos, J., Colletier, J.P., Gobec, S., 2018. Multi-target-directed ligands for treating Alzheimer's disease: Butyrylcholinesterase inhibitors displaying antioxidant and neuroprotective activities. Eur. J. Med. Chem. 156, 598–617. <https://doi.org/10.1016/j.ejmech.2018.07.033>.
- Kubo, M., Nishikawa, Y., Harada, K., Oda, M., Huang, J.M., Domon, H., Terao, Y., Fukuyama, Y., 2015. Tetranorsesquiterpenoids and santalane-type sesquiterpenoids from *Illicium lanceolatum* and their antimicrobial activity against the oral pathogen *Porphyromonas gingivalis*. J. Nat. Prod. 78 (6), 1466–1469. <https://doi.org/10.1021/acs.jnatprod.5b00237>.
- Kubo, M., Nishikawa, Y., Nakagawa, H., Shimada, S., Mori, A., Harada, K., Huang, J.-M., Ohsaki, A., Fukuyama, Y., 2022. Germacrane-type sesquiterpenoids from *Illicium lanceolatum*. Tetrahedron 109, 132673. <https://doi.org/10.1016/j.tet.2022.132673>.
- Li, H., Wang, L., Liu, M., Zheng, Y., Bi, Y., Liu, J., 2014. Chemical constituents from the fruits of *Illicium lanceolatum*. Chin. Pharm. J. 49 (08), 636–639.
- Li, Y., Wang, Y., Kong, W., Yang, S., Luo, J., Yang, M., 2020. *Illicium verum* essential oil, a potential natural fumigant in preservation of lotus seeds from fungal contamination. Food Chem. Toxicol. 141, 111347. <https://doi.org/10.1016/j.fct.2020.111347>.
- Liang, J., Huang, B., Wang, G., 2012. Chemical composition, antinociceptive and anti-inflammatory properties of essential oil from the roots of *Illicium lanceolatum*. Nat. Prod. Res. 26 (18), 1712–1714. <https://doi.org/10.1080/14786419.2011.603318>.
- Liu, J.F., Li, H.J., Wang, L.X., Liu, M.Q., Bi, Y.F., Zhang, Y.B., 2013. Two new monoterpenes from the fruits of *Illicium lanceolatum*. Molecules 18 (10), 11866–11872. <https://doi.org/10.3390/molecules181011866>.
- Liu, Y.L., Li, W.R., Zhang, J.P., Yong, J.Y., Zhang, D., Ma, S.G., 2019. Investigation on seco-prezizaane sesquiterpenes from fruits of *Illicium lanceolatum* and their neuroprotection activity. China J. Chinese Mater. Med. 44 (19), 4207–4211. <https://doi.org/10.19540/j.cnki.cjcm.20190520.201>.
- Liu, Y.L., Li, W.R., Wang, X.J., Wang, R.B., Li, M., Zhang, J.P., Yong, J.Y., Bao, X.Q., Zhang, D., Ma, S.G., 2020. Highly oxidized sesquiterpenes from the fruits of *Illicium lanceolatum* A. C. Smith. Phytochemistry 172, 112281. <https://doi.org/10.1016/j.phytochem.2020.112281>.
- Liu, X.C., Liu, Q.Y., Zhou, L., Liu, Z.L., 2015. Larvicidal activity of essential oil derived from *Illicium henryi* Diels (Illiciaceae) leaf. Trop. J. Pharm. Res. 14 (1), 111–116. <https://doi.org/10.4314/tjpr.v14i1.16>.
- Liu, Y.N., Su, X.H., Huo, C.H., Zhang, X.P., Shi, Q.W., Gu, Y.C., 2009. Chemical constituents of plants from the genus *Illicium*. Chem. Biodivers. 6 (7), 963–989. <https://doi.org/10.1002/cbdv.200700433>.
- Lu, Y., Wang, W., Ni, X., Zhuang, H., 2020. Non-destructive discrimination of *Illicium verum* from poisonous adulterant using Vis/NIR hyperspectral imaging combined with chemometrics. Infrared Phys. Technol. 111, 103509. <https://doi.org/10.1016/j.infrared.2020.103509>.
- Marchese, A., Arciola, C.R., Barbieri, R., Silva, A.S., Nabavi, S.F., Tsetegho Sokeng, A.J., Izadi, M., Jafari, N.J., Suntar, I., Daglia, M., Nabavi, S.M., 2017. Update on monoterpenes as antimicrobial agents: a particular focus on p-cymene. Materials (Basel) 10 (8), 0947. <https://doi.org/10.3390/ma10080947>.

- Mathon, C., Bongard, B., Duret, M., Ortelli, D., Christen, P., Bieri, S., 2013. Analysis of the neurotoxin anisatin in star anise by LC-MS/MS. *Food Addit. Contamin.: Part A* 30 (9), 1598–1605. <https://doi.org/10.1080/19440049.2013.804955>.
- Mauracher, S.G., Molitor, C., Al-Oweini, R., Kortz, U., Rompel, A., 2014. Latent and active abPPO4 mushroom tyrosinase cocrystallized with hexatungstotellurate(VI) in a single crystal. *Acta Crystallogr. D Biol. Crystallogr.* 70 (9), 2301–2315. <https://doi.org/10.1107/S1399004714013777>.
- Motoki, Y., Fujita, S., Fujihara, Y., Okada, Y., Nomura, M., 2003. Skin whitening effects of estragole derivatives. *J. Oleo Sci.* 52 (9), 495–498. <https://doi.org/10.5650/jos.52.495>.
- Nie, W., Ding, L.F., Lei, T., Pan, Z.H., Song, L.D., Wu, X.D., Zhao, Q.S., 2021. Illilanceolide A, a unique seco-prezizaane sesquiterpenoid with 5/5/6 tricyclic scaffold from the fruits of *Illicium lanceolatum* A. C. Smith. *Tetrahedron Lett.* 70, 153022. <https://doi.org/10.1016/j.tetlet.2021.153022>.
- Nie, W., Ding, L.F., Tie, L., Zhou, H.F., Bao, Y., Song, L.D., Pan, Z.H., Wu, X.D., Zhao, Q. S., 2022. seco-Prezizane sesquiterpenes and prenylated C6–C3 compounds from the fruits of *Illicium lanceolatum* A. C. Smith. *Chem. Biodivers.* 19 (1), 202100868. <https://doi.org/10.1002/cbdv.202100868>.
- Orhan, I., Kartal, M., Kan, Y., Sener, B., 2008. Activity of essential oils and individual components against acetyl- and butyrylcholinesterase. *Z. Naturforsch., C: J. Biosci.* 63 (7–8), 547–553. <https://doi.org/10.1515/znc-2008-7-813>.
- Rivas da Silva, A.C., Lopes, P.M., Barros de Azevedo, M.M., Costa, D.C., Alviano, C.S., Alviano, D.S., 2012. Biological activities of alpha-pinene and beta-pinene enantiomers. *Molecules* 17 (6), 6305–6316. <https://doi.org/10.3390/molecules17066305>.
- Rodenak-Kladniew, B., Castro, A., Starkel, P., De Saeger, C., Garcia de Bravo, M., Crespo, R., 2018. Linalool induces cell cycle arrest and apoptosis in HepG2 cells through oxidative stress generation and modulation of Ras/MAPK and Akt/mTOR pathways. *Life Sci.* 199, 48–59. <https://doi.org/10.1016/j.lfs.2018.03.006>.
- Rodenak-Kladniew, B., Castro, A., Starkel, P., Galle, M., Crespo, R., 2020. 1,8-Cineole promotes G0/G1 cell cycle arrest and oxidative stress-induced senescence in HepG2 cells and sensitizes cells to anti-senescence drugs. *Life Sci.* 243, 117271. <https://doi.org/10.1016/j.lfs.2020.117271>.
- Rodriguez-Garcia, I., Silva-Espinoza, B.A., Ortega-Ramirez, L.A., Leyva, J.M., Siddiqui, M.W., Cruz-Valenzuela, M.R., Gonzalez-Aguilar, G.A., Ayala-Zavala, J.F., 2016. Oregano essential oil as an antimicrobial and antioxidant additive in food products. *Crit. Rev. Food Sci. Nutr.* 56 (10), 1717–1727. <https://doi.org/10.1080/10408398.2013.800832>.
- Salem, M.A., Manaa, E.G., Osama, N., Aborehab, N.M., Ragab, M.F., Haggag, Y.A., Ibrahim, M.T., Hamdan, D.I., 2022. Coriander (*Coriandrum sativum* L.) essential oil and oil-loaded nano-formulations as an anti-aging potentiality via TGFβ/SMAD pathway. *Sci. Rep.* 12 (1), 6578. <https://doi.org/10.1038/s41598-022-10494-4>.
- Salentin, S., Schreiber, S., Haupt, V.J., Adasme, M.F., Schroeder, M., 2015. PLIP: fully automated protein-ligand interaction profiler. *Nucleic Acids Res.* 43 (1), 443–447. <https://doi.org/10.1093/nar/gkv315>.
- Savelev, S.U., Okello, E.J., Perry, E.K., 2004. Butyryl- and acetyl-cholinesterase inhibitory activities in essential oils of *Salvia* species and their constituents. *Phytother. Res.* 18 (4), 315–324. <https://doi.org/10.1002/ptr.1451>.
- Seneme, E.F., dos Santos, D.C., Silva, E.M.R., Franco, Y.E.M., Longato, G.B., 2021. Pharmacological and therapeutic potential of myristicin: A literature review. *Molecules* 26, 5914. <https://doi.org/10.3390/molecules26195914>.
- Shaaban, H.A.E., El-Ghorab, A.H., Shibamoto, T., 2012. Bioactivity of essential oils and their volatile aroma components: Review. *J. Essent. Oil Res.* 24 (2), 203–212. <https://doi.org/10.1080/10412905.2012.659528>.
- Sharmeen Jugreet, B., Kouadio Ibrahime, S., Zengin, G., Abdallah, H.H., Fawzi Mahomoodally, M., 2021. GC/MS profiling, in vitro and in silico pharmacological screening and principal component analysis of essential oils from three exotic and two endemic plants from Mauritius. *Chem. Biodivers.* 18 (3), 2000921. <https://doi.org/10.1002/cbdv.202000921>.
- Silva, B.I.M., Nascimento, E.A., Silva, C.J., Silva, T.G., Aguiar, J.S., 2021. Anticancer activity of monoterpenes: a systematic review. *Mol. Biol. Rep.* 48 (7), 5775–5785. <https://doi.org/10.1007/s11033-021-06578-5>.
- Soares, N.R., Correa, C.T.R., da Silva, J.C., da Silva Negreiros, J.R., Techio, V.H., Torres, G.A., 2022. Comparative cytogenetics of three economically important Piper L. species from the Brazilian Amazon. *Protoplasma* 259 (4), 1099–1108. <https://doi.org/10.1007/s00709-021-01721-2>.
- Sun, Z., Su, X., Lin, Y., Long, C., Zhang, Y., Zhao, T., 2023. Chemical composition, and antioxidant and cholinesterase inhibitory activities of *Lindera glauca* fruit essential oil and molecular docking studies of six selected compounds. *Horticulturae* 9 (2), 289. <https://doi.org/10.3390/horticulturae9020289>.
- Trott, O., Olson, A.J., 2010. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* 31 (2), 455–461. <https://doi.org/10.1002/jcc.21334>.
- Van Vuuren, S., Holl, D., 2017. Antimicrobial natural product research: A review from a South African perspective for the years 2009–2016. *J. Ethnopharmacol.* 208, 236–252. <https://doi.org/10.1016/j.jep.2017.07.011>.
- Wei, A., Shibamoto, T., 2010. Antioxidant/lipoxygenase inhibitory activities and chemical compositions of selected essential oils. *J. Agric. Food Chem.* 58, 7218–7225. <https://doi.org/10.1021/jf101077s>.
- Xiu, Z., Zhu, Y., Han, J., Li, Y., Yang, X., Yang, G., Song, G., Li, S., Li, Y., Cheng, C., Li, Y., Fang, J., Li, X., Jin, N., 2022. Caryophyllene oxide induces Ferritinophagy by regulating the NCOA4/FTH1/LC3 pathway in Hepatocellular Carcinoma. *Front. Pharmacol.* 13, 930958. <https://doi.org/10.3389/fphar.2022.930958>.
- Xu, Q., Li, M., Yang, M., Yang, J., Xie, J., Lu, X., Wang, F., Chen, W., 2018. Alpha-pinene regulates miR-221 and induces G(2)/M phase cell cycle arrest in human hepatocellular carcinoma cells. *Biosci. Rep.* 38(6), BSR20180980. <https://doi.org/10.1042/BSR20180980>.
- Zeb, A., 2020. Concept, mechanism, and applications of phenolic antioxidants in foods. *J. Food Biochem.* 44 (9), e13394.
- Zhang, H.Y., Gao, Y., Lai, P.X., 2017. Chemical composition, antioxidant, antimicrobial and cytotoxic activities of essential oil from *Premna microphylla* Turczaninow. *Molecules* 22 (3), 0381. <https://doi.org/10.3390/molecules22030381>.
- Zhao, T., Ma, C., Zhu, G., 2021. Chemical composition and biological activities of essential oils from the leaves, stems, and roots of *Kadsura coccinea*. *Molecules* 26 (20), 6259. <https://doi.org/10.3390/molecules26206259>.
- Zhao, M., Zhang, X., Wang, Y., Huang, M., Duan, J.A., Goedecke, T., Szymulanska-Ramamurthy, K.M., Yin, Z., Che, C.T., 2014. Germacrane and m-Menthane from *Illicium lanceolatum*. *Molecules* 19 (4), 4326–4337. <https://doi.org/10.3390/molecules19044326>.
- Zolghadri, S., Bahrami, A., Hassan Khan, M.T., Munoz-Munoz, J., Garcia-Molina, F., Garcia-Canovas, F., Saboury, A.A., 2019. A comprehensive review on tyrosinase inhibitors. *J. Enzyme Inhib. Med. Chem.* 34 (1), 279–309. <https://doi.org/10.1080/14756366.2018.1545767>.