Journal of Essential Oil Bearing Plants

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

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To cite this article: Nguyen Thi Thu, Hieu Tran-Trung, Dau Xuan Duc, Nguyen Xuan Ha, Pham Ngoc Khanh, Nguyen Thanh Tung, Nguyen Van Hoa, Nguyen Hoang Tuan & Do Thi Ha (2024) Rhizome essential oil of *Curcuma zedoaroides* Chaveer. & Tanee: chemical composition, cytotoxic activities, and molecular docking approach, Journal of Essential Oil Bearing Plants, 27:1, 188-197, DOI: 10.1080/0972060X.2024.2314554

To link to this article: https://doi.org/10.1080/0972060X.2024.2314554

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JOURNAL OF ESSENTIAL OIL BEARING PLANTS

Taylor & Francis
Taylor & Francis Group

DOI: 10.1080/0972060X.2024.2314554

Research Article

Rhizome essential oil of *Curcuma zedoaroides* Chaveer. & Tanee: chemical composition, cytotoxic activities, and molecular docking approach

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Received 01 November 2022
Revised 09 January 2024
Accepted 29 January 2024

Abstract
Curcuma zedoaroides Chaveer. & Tanee (Vietnamese name: Nghệ đắng) belongs to the Zingiberaceae family which is the new *Curcuma* species discovered in Thailand and Vietnam in recent years. This study aimed to determine the chemical compositions and the cytotoxic activities of *C. zedoaroides* rhizome essential oil for the first time. The essential oil was obtained from the rhizomes through hydro-distillation and analyzed by gas chromatography-mass spectrometry (GC-MS) technique. Results showed that the majority of the rhizome essential oil of *C. zedoaroides* was dominated by oxygenated sesquiterpenes (61.54%) mainly curdione (27.45%), followed by widdrol (8.03%) and *trans*-β-elemenone (5.47%). Notably, the essential oil of *C. zedoaroides* rhizomes exhibited cytotoxic activities using the MTT assay with the IC_{50} (half-maximal effective concentration) values of 75.16 ± 2.79, 77.08 ± 1.98, 81.35 ± 1.55, 83.67 ± 2.26, 32.74 ± 1.95, 23.14 ± 1.43, 73.35 ± 2.20 µg/mL against A549, MCF-7, HepG2, HT29, HL-60, K562 and MDA-MB-231 cells, respectively. In addition, three major compounds targeting specific proteins related to essential oil's anti-cancer activity were assessed using molecular docking simulation. From the results observed, widdrol was suggested to have the highest binding affinity for both EGFR and HER2, with ΔG values of -6.246 kcal/mol and -6.916 kcal/mol, respectively. These results suggest the potential of the rhizome essential oil of *C. zedoaroides* to provide leads for the development of anti-cancer agents as well as considered a valuable source of bioactive components.

Keywords
Curcuma zedoaroides, Essential oil, GC-MS, Cytotoxic activity, Molecular docking

INTRODUCTION

The genus *Curcuma* is one of the largest in the Zingiberaceae family, encompassing about 130 species distributed in South and Southeast Asia[1]. The rhizomes of these plants have diverse applications, including their use as food, spices, sources of essential oils, and in traditional Vietnamese medicine[2]. For example, *C. longa* (yellow turmeric) rhizomes are commonly used for the treatment of stomach pain and wound healing, while *C. zedoaria* (black turmeric) is associated with digestive disorder remedies.

Curcuma zedoaroides Chaveer. & Tanee (Fig. S1), is an endemic Zingiberaceae species of Thailand, which was recently discovered in Thai Nguyen Province, Vietnam[3]. Ethnobotanical records from Ban Khok Sa-Nga (the village where native peoples feed king cobra and many kinds of snakes), Khon Kaen Province, Northeastern Thailand document the traditional use of this plant as an antidote for snake bites[4]. In 2018, seven components consisting of one diarylheptanoid and six guaiane-type sesquiterpene lactones were isolated from the

bioactive chloroform extract of *C. zedoaroides* rhizomes[5]. Moreover, their anti-inflammatory activity was also investigated on NO and TNF-α production using RAW264.7 cells[5].

As a part of the research project "Study on anticancer and immune-modulation of some Vietnamese medicinal plants", our recent research focused on uncovering potential anti-cancer agents within the rhizomes of *C. zedoaroides* led to the isolation of twelve compounds, including ten sesquiterpenes and two diterpenes[6]. The anti-cancer activity test revealed significant effects of the extracts (*n*-hexane, ethyl acetate, and water) on eight cancer cell lines (A549, MCF-7, HT-29, MB49, HepG2, MDA-MB231, JB6-C141, and K562), exhibiting IC_{50} values ranging from 5.43 to 11.96 µg/mL. Among them, eleven compounds displayed considerable activity against five cancer cell lines (A549, MCF-7, MDA-MB231, HL-60, and HepG2), with IC_{50} values ranging from 3.13 µM to 30.10 µM. Notably, the A549 cell line exhibited the most potent response, with an IC_{50} range of 3.13-13.54 µM[6]. Continuing this project, in the present study, the chemical composition and the cytotoxic activities of *C. zedoaroides*'s rhizome essential oil were investigated, which has been lacking in the literature. Furthermore, *in silico* computation applied through molecular docking studies may predict the binding mechanism and affinity of a lead molecule with proteins[7].

Material and methods

Plant materials

Rhizomes of *C. zedoaroides* were collected from Dong Hy District, Thai Nguyen Province, Vietnam (21°41'16.30"N; 105°48'30.49"E), in August 2020. The plant was identified by botanists, MSc. Nguyen Quynh Nga and MSc. Nguyen Van Hieu, from the Medicinal Material Resources Center at NIMM (National Institute of Medical Materials). A voucher specimen (DL-120820) was deposited at the Herbarium of NIMM.

Isolation of the essential oil

The essential oil of *C. zedoaroides* rhizomes was obtained after hydro-distillation that lasted for approximately 3.0 h utilizing a Clevenger-type apparatus, according to the Vietnamese Pharmacopoeia[8]. The experiment was performed in triplicate. The obtained essential oil was dried with anhydrous sodium sulfate and kept under refrigeration (4°C) until further experiments.

Gas chromatography - mass spectrometry (GC-MS) analysis of the essential oil

The chemical composition of rhizome essential oil of *C. zedoaroides* was analyzed by a Gas Chromatograph (7890B GC) coupled with a Mass Selective Detector (5977B MSD), as reported in previous studies[9,10]. The GC column used was an HP-5MS UI (30 m × 0.25 mm i.d. × 0.25 µm film thickness, Agilent Technologies). The carrier gas was helium (flow rate of 1.0 mL/min). The injection volume was 1.0 µL (split ratio of 25:1). Both Inlet-F and Aux-2 temperatures were set at 300°C, while MS Quad temperature was set at 50°C and MS source was set at 230°C. The GC oven temperature was initiated at 60°C for 1 min, increased at 4°C/min to 240°C, and kept steady at 240°C for 4 min. Mass range was 50-550 amu (2 scans/s), ionization energy was 70 eV. Identification of the essential oil components was based on comparison of the fragmentation patterns of mass spectra and retention indices with those reported in the literature (NIST17 and Adams's book)[11].

Cytotoxic activity assay

Seven human cancer cell lines, including A549 (human lung carcinoma), MCF-7 (human breast carcinoma), HepG2 (human hepatocellular carcinoma), HT-29 (human colon adenocarcinoma), HL-60 (human leukemia), K562 (human chronic myelogenous leukemia), and MDA-MB-231 (human breast carcinoma), were employed for cytotoxic evaluation. The cancer cell lines were cultured following the standard guidelines of the American Type Culture Collection. Specifically, the seven experimental cancer cell lines were grown in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum, 100 units/mL penicillin, and 100 µg/mL streptomycin, along with other necessary components, in a 5% CO_2 environment

at 37°C. Cultivation continued until the cells reached a growth density of 80 - 90%.

The cytotoxic activity of essential oils against two cancer cell lines (HL-60 and K562) was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), following a previously established method.[12] Briefly, cells were seeded at a density of 1×10^4 cells/well in 96-well plates and incubated at 37°C with 5% CO_2 for 24 hours. Subsequently, the cells were treated with essential oils at different concentrations for 72 hours under the same conditions. After the incubation period, MTT (10 µL, final concentration 5 mg/mL) was added, and the cells were further incubated for 4 hours at 37°C with 5% CO_2. Formazan crystals formed were dissolved in dimethyl sulfoxide (DMSO), and the optical density (OD) values were measured at a wavelength of 540 nm using an ELISA Plate Reader (BioTek, Winooski, VT, USA). The inhibition percentage of cell growth in the presence of the treated sample is determined by the following formula:

(%) inhibition = 100% - $[(OD_{sample} - OD_{blank})/(OD_{DMSO} - OD_{blank})]x100$

The sulforhodamine B (SRB) assay was employed to assess the cytotoxic activity of essential oils against five cancer cell lines (A549, MCF-7, HepG2, HT-29, and MDA-MB-231) following a previously described protocol[13,14]. The test aimed to determine cell density by measuring the total cellular protein content stained with SRB. In summary, trypsinized cells were seeded in a 96-well plate and incubated with the sample for 72 hours. Wells containing cells treated with a diluted solution were used as the negative control. After the incubation period, cells were fixed with trichloroacetic acid (TCA) for 1 hour and then stained with SRB for 30 minutes at 37°C. Following three washes with acetic acid to remove the nonstaining dye, the plates were air-dried at room temperature. The SRB-stained protein in the cells was dissolved in 10 mM unbuffered Tris base, and the plate was gently shaken for 10 minutes at room temperature. The optical density (OD) at 540 nm was determined using an ELISA Plate Reader (BioTek, Winooski, VT, USA). The percentage of inhibition in cell growth in the presence of the treated sample was calculated using the following formula:

(%) inhibition = 100% - $[(OD_{sample} - OD_{day\ 0})/(OD_{DMSO} - OD_{day\ 0})]x100$

The MTT and SRB assays were repeated three times to ensure accuracy. Ellipticine was used as a positive control and tested at various concentrations. The IC_{50} values were calculated based on the percent inhibition of cell growth using TableCurve 2Dv4 software.

Molecular docking study

The three-dimensional crystal structures of the EGFR tyrosine kinase domain in complex with erlotinib (PDB ID: 4HJO) and the kinase domain of human HER2 (PDB ID: 3PP0) were downloaded from the Protein Data Bank RCSB (https://www.rcsb.org/) with resolutions of 2.75 Å and 2.25 Å, respectively[15,16]. The co-crystallized ligands and water molecules were removed from the protein structure data files. AutoDock Vina v1.2.3 program was used to perform docking simulations for the current study[17]. The geometric structures of the major compounds from the essential oil of *C. zedoaroides* rhizomes including *trans*-β-elemenone, widdrol, and curdione were sketched using ChemSketch v2022.1.2 software (ChemSketch, version 2022.1.2, Advanced Chemistry Development, Inc. (ACD/Labs), Toronto, ON, Canada, www.acdlabs.com.). Subsequently, the 2D structures were converted into 3D structures and energy-optimized using the MMFF94s force field in the 3D Viewer software (Freeware)[18]. All PDBQT files for the proteins and ligands for input into the molecular docking were prepared using AutoDockTools software. Grid boxes for the selected proteins were set to 24x24x24 points with a grid spacing of 1, and the grid box center coordinates were based on the center of the ligand binding site. Default other parameters were used, with an exhaustiveness value of 400, similar to previous studies[19,20]. Experimental re-docking was performed to validate the docking protocol. Finally, the results obtained from the docking simulations were then analyzed to identify the best-clustered poses for each ligand with the

lowest binding affinity. The interactions between molecules in the docked complexes were visualized using Discovery Studio Visualizer v2021 software.

Results and Discussion

Chemical composition of the essential oil

The average yield of the essential oil from the rhizomes of *C. zedoaroides* was 0.84% (v/w), according to its dry weight. The essential oil was a pleasantly smelling, yellow liquid, and lighter than water. The GC-MS analysis (Fig. S2) revealed a total of 46 components in the essential oils of *C. zedoaroides* rhizomes (accounting for 90.76% of the content), the result of which is listed in Table 1.

As shown in Table 1, the main class of compounds in the essential oil of *C. zedoaroides* rhizomes was oxygenated sesquiterpenes (18 components, 61.54%). This essential oil was also found to have a greater amount of oxygenated monoterpenes (12 components, 10.86%) and sesquiterpene hydrocarbons (10 components, 10.62%). Besides, monoterpene hydrocarbons (5 components, 6.69%) and one norditerpenoid compound (1.05%) were identified in the rhizome essential oil of *C. zedoaroides*. The major constituents (>5%) were curdione (27.45%), widdrol (8.03%), and *trans*-β-elemenone (5.47%). In addition, ambrial (4.75%), camphor (3.99%), 13-nor-eremophil-1(10)-en-11-one (3.73%), β-pinene (3.71%), humulene (3.47%), isoborneol (2.84%), β-elemene (2.43%), and isolongifolol methyl ether (2.25%) were the other significant compounds (>2%) in the essential oil.

The chemical components of the rhizome essential oils of several other *Curcuma* species from Vietnam were reported in previous studies. For example, the main compounds of the rhizome essential oil of *C. sahuynhensis* from Quang Ngai Province were β-pinene (52.7%), β-caryophyllene (11.1%), α-pinene (8.6%), caryophyllene oxide (6.5%), and (*Z*)-β-farnesene (5.9%)[21]. The major components of the rhizome essential oil of *C. singularis* from Gia Lai Province were camphor (25.83%) and germacrone (8.00%)[22]. The major compounds in the rhizome essential oil of *C. rhabdota* from Tay Ninh Province were 3-carene (16.6%), camphene (9.8%), *α*-copaene (7.4%), γ-terpinene (7.3%), camphor (5.9%), and β-curcumene (5.7%)[23]. The predominant compounds of the rhizome essential oil of *C. thorelii* from Binh Thuan Province were xanthorrhizol (40.7%), β-curcumene (20.7%), and α-curcumene (8.9%), while camphene (17.0%), (*E*)-β-elemenone (16.8%), (*E*)-β-farnesene (13.6%), germacrone (8.9%), 1,8-cineole (7.2%), and camphor (6.0%) were the most abundant components in the essential oil of *C. petiolata* rhizomes from Tay Ninh Province[23]. The variation in these results and the present work can be explained by the difference in species used in the studies[24].

Cytotoxic activity of the essential oil

The results of cytotoxicity assessment against cancer cell lines are presented in Table 2. The rhizome essential oil of *C. zedoaroides* showed weak cytotoxic activity against different cancer cell lines A549, MCF-7, HepG2, HT29, and MDA-MB-231 cell lines, with IC_{50} values in the range of 73.35-83.67 µg/mL. Meanwhile, it exhibited moderate cytotoxicity against K562 (IC_{50} = 23.14 ± 1.43 µg/mL) and HL-60 cells (IC_{50} = 32.74 ± 1.95 µg/mL).

The essential oils obtained from different plants of the genus *Curcuma* demonstrate noteworthy cytotoxicity against various cancer cell lines. For instance, *C. longa* essential oil from Thailand has exhibited significant cytotoxic effects against human mouth epidermal carcinoma (KB) cells and mouse leukemia (P388) cells with IC_{50} values of 1.09 and 0.08 mg/mL, respectively[25]. The essential oil of *C. zedoaria* rhizomes from Malaysia was found to be cytotoxic against human breast (MCF-7), lung (SK-LU-1) and cervical (HeLa S3 and SiHa) cancer cell lines with IC_{50} values being less than 10 µg/mL[26]. The cytotoxic effects of essential oils may be related to their main components. Similarly, the chemical components accounting for a high percentage of *C. zedoaroides* rhizome essential oil have been shown to exhibit significant anti-cancer properties. It is reported that curdione shows effectiveness against breast cancer by

Table 1. Chemical composition of the rhizome essential oil of *C. zedoaroides*

No.	RT (min)	Compounds	RI[a]	RI[b]	Formula	Content (%)
1	5.547	α-Pinene	939	937	$C_{10}H_{16}$	1.45
2	5.903	Camphene	955	952	$C_{10}H_{16}$	0.97
3	6.612	β-Pinene	981	979	$C_{10}H_{16}$	3.71
4	7.899	p-Cymene	1028	1025	$C_{10}H_{14}$	0.22
5	8.025	Limonene	1033	1030	$C_{10}H_{16}$	0.34
6	8.106	1,8-Cineole	1036	1032	$C_{10}H_{18}O$	0.32
7	10.176	Linalool	1100	1099	$C_{10}H_{18}O$	0.49
8	11.458	*trans*-Pinocarveol	1143	1139	$C_{10}H_{16}O$	0.43
9	11.653	Camphor	1149	1145	$C_{10}H_{16}O$	3.99
10	11.773	Camphene hydrate	1153	1148	$C_{10}H_{18}O$	0.31
11	12.048	Isoborneol	1161	1157	$C_{10}H_{18}O$	2.84
12	12.248	Pinocarvone	1167	1164	$C_{10}H_{14}O$	0.24
13	12.334	*endo*-Borneol	1170	1167	$C_{10}H_{18}O$	0.81
14	12.717	Terpinen-4-ol	1180	1177	$C_{10}H_{18}O$	0.15
15	12.900	Myrtanal	1186	1188	$C_{10}H_{16}O$	0.10
16	13.152	α-Terpineol	1192	1189	$C_{10}H_{18}O$	0.23
17	13.350	Myrtenal	1198	1193	$C_{10}H_{14}O$	0.95
18	19.732	β-Elemene	1394	1391	$C_{15}H_{24}$	2.43
19	20.596	Caryophyllene	1423	1419	$C_{15}H_{24}$	0.53
20	21.014	γ-Elemene	1437	1433	$C_{15}H_{24}$	0.51
21	21.323	Guaia-6,9-diene	1447	1443	$C_{15}H_{24}$	0.83
22	21.649	Humulene	1458	1454	$C_{15}H_{24}$	3.47
23	22.319	γ-Muurolene	1480	1477	$C_{15}H_{24}$	0.42
24	22.645	β-Eudesmene	1489	1486	$C_{15}H_{24}$	1.19
25	22.914	Curzerene	1498	1498	$C_{15}H_{20}O$	1.29
26	23.051	α-Selinene	1502	1494	$C_{15}H_{24}$	0.22
27	23.504	Cubebol	1518	1515	$C_{15}H_{26}O$	0.16
28	23.743	δ-Cadinene	1527	1524	$C_{15}H_{24}$	0.45
29	24.745	Germacrene B	1561	1557	$C_{15}H_{24}$	0.57
30	25.506	Caryophyllene oxide	1587	1581	$C_{15}H_{24}O$	1.02
31	25.620	*epi*-Globulol	1591	1585	$C_{15}H_{26}O$	0.34
32	25.838	Viridiflorol	1597	1591	$C_{15}H_{26}O$	0.66
33	26.124	*trans*-β-Elemenone	1608	1597	$C_{15}H_{22}O$	5.47
34	26.273	Widdrol	1614	1610	$C_{15}H_{26}O$	8.03
35	26.810	13-nor-Eremophil-1(10)-en-11-one	1633	1629	$C_{14}H_{22}O$	3.73
36	26.908	Caryophylladienol II	1637	1637	$C_{15}H_{24}O$	1.60
37	27.401	β-Eudesmol	1655	1649	$C_{15}H_{26}O$	0.71
38	27.520	Pogostole	1659	1660	$C_{15}H_{26}O$	1.22
39	27.989	Isolongifolol methyl ether	1676	1672	$C_{16}H_{28}O$	2.25
40	28.653	Germacrone	1697	1693	$C_{15}H_{22}O$	1.67
41	29.008	β-Nootkatol	1712	1712	$C_{15}H_{24}O$	0.58

Table 1 ***cont***.

No.	RT (min)	Compounds	RI[a]	RI[b]	Formula	Content (%)
42	29.317	Curdione	1724	1726	$C_{15}H_{24}O_2$	27.45
43	31.405	Ambrial	1803	1809	$C_{16}H_{26}O$	4.75
44	32.412	Curcumenone	1844	1844	$C_{15}H_{22}O_2$	0.35
45	32.739	Isolongifolol acetate	1856	1850	$C_{17}H_{28}O_2$	0.26
46	35.983	(*E*)-15,16-Dinorlabda-8(17),11-dien-13-one	1988	1994	$C_{18}H_{28}O$	1.05
		Total				**90.76**
		Monoterpene hydrocarbons (Sr. No. 1-5)				6.69
		Oxygenated monoterpenes (Sr. No. 6-17)				10.86
		Sesquiterpene hydrocarbons (Sr. No. 18-24, 26, 28, 29)				10.62
		Oxygenated sesquiterpenes (Sr. No. 25, 27, 30-45)				61.54
		Norditerpenoid (Sr. No. 46)				1.05

RT: Retention time (min); [a] Retention indices on HP5-MS UI column; [b] Literature retention indices (NIST17 and Adams's book)[11]

Table 2. Cytotoxic activity of the rhizome essential oil of *C. zedoaroides*

Cell lines	Half-maximal inhibitory concentration (IC_{50}, µg/mL)	
	Essential oil[a]	Ellipticine[b]
A549	75.16 ± 2.79	0.32 ± 0.02
MCF-7	77.08 ± 1.98	0.35 ± 0.03
HepG2	81.35 ± 1.55	0.34 ± 0.04
HT29	83.67 ± 2.26	0.39 ± 0.04
HL-60	32.74 ± 1.95	0.34 ± 0.02
K562	23.14 ± 1.43	0.32 ± 0.02
MDA-MB-231	73.35 ± 2.20	0.36 ± 0.02

[a,b] Mean ± SD, n = 3

impeding cell proliferation, inducing apoptosis, and preventing migration and invasion. It targets prostaglandin E2 production, COX-2 gene expression, and protein phosphorylation. It also regulates apoptosis-associated proteins and displays potential in idiopathic pulmonary fibrosis treatment[27]. ar-Tumerone is a main compound found in the essential oil of *C. xanthorrhiza* leaves[28] and it has been demonstrated to possess anti-tumor activity and cytotoxic activity such as HL-60, human leukemia (K-562), rat leukemia (RBL-2H3), and mouse leukemia (L-1210)[29]. Other bisabolane sesquiterpenoids are turmerone and curdione from *C. aromatica* essential oil, which exhibit *in vitro* and *in vivo* inhibitory effects on laryngeal cancer (HEp-2) cells[30]. Furthermore, among the major compounds, widdrol in *C. zedoaroides*, as indicated by current research, has been reported to have the ability to induce apoptosis in various cancer cells, activate AMPK, and show anti-angiogenic effects[31-33].

Molecular docking study

Correlating the main chemical components of this essential oil to target proteins related to the observed cytotoxic activities using a molecular docking approach. Firstly, the AutoDock Vina v.1.2.3 program was used for the re-docking of the erlotinib and 2-{2-[4-({5-chloro-6-[3-(trifluoromethyl)phenoxy]pyridin-3-yl}amino)-

5H-pyrrolo[3,2-d]pyrimidin-5yl]ethoxy}ethanol (03Q) ligands into the active sites of the EGFR (PDB ID: 4HJO) and HER2 (PDB ID: 3PP0) proteins, respectively, to verify the docking process parameters. The RMSD of erlotinib was found to be 1.47 Å for EGFR (Fig. 1), while for HER2, the RMSD value of 03Q was 0.90 Å (Fig. 1), both values falling within an acceptable range (RMSD < 2 Å) when using the docking method to predict the binding affinity of small molecules[34,35]. This may demonstrate the reliability of the docking process parameters.

Both EGFR and HER2 are prominent targets found on cancer cells[36,37]. EGFR plays a crucial role in the signaling pathway necessary for regulating the development, differentiation, and survival of cells, while the typical expression of HER2 on the cell surface is essential for controlling cell growth and viability in tissue cell expression[38,39]. However, numerous aggressive cancer forms are thought to be linked to dysregulation of EGFR signaling, and invasive breast cancer and other malignant tumors are frequently found to have HER2 oncogene overexpression and amplification[40,41]. Therefore, the EGFR and HER2 targets were chosen for molecular docking study. In this study, three main compounds from the essential oil of *C. zedoaroides* rhizomes, including *trans*-β-elemenone, widdrol, and curdione, were docked into the active sites of specific cancer targets, namely, epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2 (HER2). The binding affinities (ΔG, kcal/mol) of each compound were determined and compared with known reference ligands for EGFR and HER2, including the erlotinib and 03Q ligands. The results of this analysis are described in Fig. S3. As shown in Fig. S3, the compound widdrol exhibited the best binding affinity among the three main compounds for both EGFR and HER2, with ΔG values of -6.246 kcal/mol and -6.916 kcal/mol, respectively. Additionally, prior research indicates that widdrol demonstrates cytotoxic activity on the HT29 cell line with an IC_{50} of 14.05 µg/mL[33]. Another example is that this compound inhibits the expression of MCM protein in A549 cells, which results in anticoagulation processes and G1 phase arrest[42]. These pieces of information support the hypothesis that the activity of the oil on cancer cell lines and its binding affinity in the docking model correlate. These findings suggest that widdrol may have the potential to be an effective plant compound from the essential oil of *C. zedoaroides* rhizomes for both EGFR and HER2 and warrants further research.

The molecular docking analysis was conducted

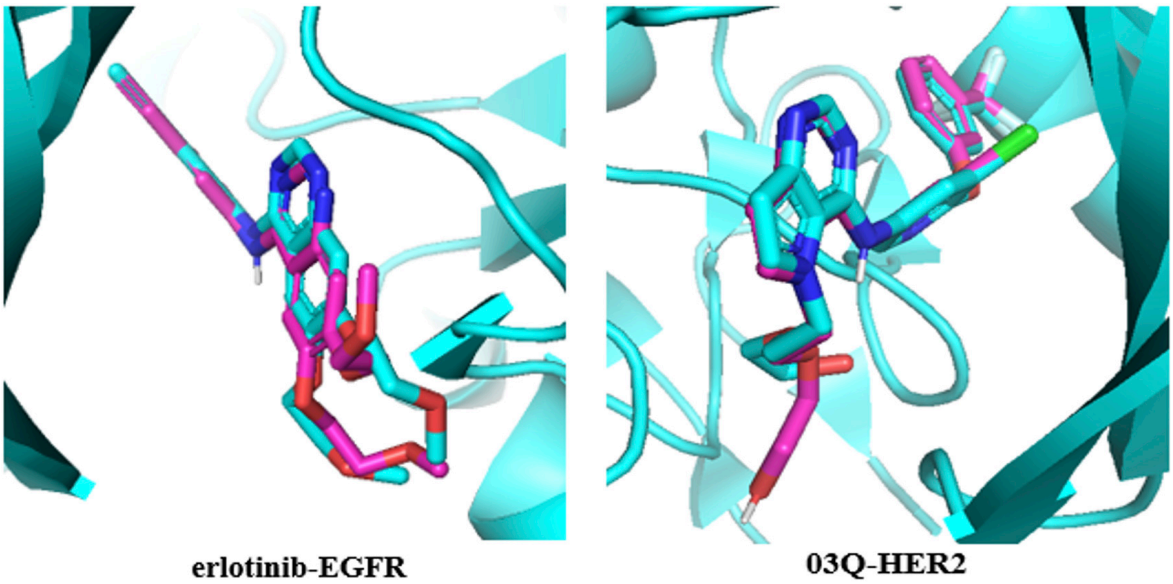

Figure 1. The redocking results of co-crystallized ligands

to compare the binding interactions of *trans*-β-elemenone, widdrol, curdione, and erlotinib with the EGFR protein. The analysis results presented in Fig. S4 provide an in-depth insight into the molecular interactions between these compounds and the EGFR protein at the active binding site. Curdione forms a hydrogen bond with Arg817 at a distance of 2.67 Å. This compound establishes alkyl and pi-alkyl interactions with amino acid residues Ala698, Lys851, and Phe699. The oxygenated sesquiterpene compound, *trans*-β-elemenone, forms alkyl interactions with amino acid residues Cys773, Leu694, Leu820, Val702, and Arg817. On the other hand, widdrol also forms similar interactions with amino acid residues as *trans*-β-elemenone but does not interact with Arg817.

For the HER2 protein, the molecular docking results suggest that the binding interactions of *trans*-β-elemenone, widdrol, curdione, and 03Q may have favorable interactions with the HER2 receptor, evidenced by the formation of hydrogen bonds, pi-sigma interactions, pi-alkyl interactions, and alkyl interactions with specific amino acid residues. These interactions may contribute to the binding affinity of these compounds to the HER2 receptor. The analysis results presented in Fig. S5 provide an in-depth insight into the molecular interactions between these compounds and the HER2 protein at the active binding site. Widdrol forms a hydrogen bond between the hydroxyl group in the molecule and the Gly865 residue. Additionally, this compound creates alkyl and pi-alkyl interactions with amino acid residues Ala763, Phe731, and Arg756. Curdione forms a pi-sigma interaction with Phe731 and alkyl and pi-alkyl interactions with residues Ala763, Leu755, Arg756, Ile767, and Leu866. Meanwhile, *trans*-β-elemenone establishes alkyl and pi-alkyl interactions with residues Ile767, Phe731, Leu755, Ala763, and Arg756.

Conclusion

The chemical composition of the essential oil from *C. zedoaroides* rhizomes was investigated for the first time. The main compounds of this essential oil were curdione, widdrol, and *trans*-β-elemenone. Furthermore, the essential oil of *C. zedoaroides* rhizomes showed potent cytotoxic effects against the tested cancer cell lines with IC_{50} values ranging from 23.14-83.67 µg/mL. In addition, molecular docking studies showed widdrol may have the potential to be an effective natural inhibitor from the essential oil of *C. zedoaroides* for both EGFR and HER2 although further research is needed.

Acknowledgment

This research was a part of the research project "Study on anticancer and immune-modulation of some Vietnamese medicinal plants", code NĐT.85.KR/20. Nguyen Thi Thu was funded by the Master, PhD Scholarship Programme of Vingroup Innovation Foundation (VINIF), code VINIF.2023.TS.123.

Competing interests

No potential conflict of interest are reported by the authors.

Supplementary Data

Figures S1 to S4 are given in supplementary file.

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