



Volatile components of ethyl acetate extracts from the leaves and rhizomes of *Curcuma sahuynhensis* and their cytotoxic activity: *In vitro* and *in silico* studies

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Abstract

Curcuma sahuynhensis Škorničk. & N.S. Lý is an indigenous plant in Vietnam that is used as spices and medicine to treat diseases related to digestive disorders. This study was designed to evaluate, for the first time, the *in vitro* and *in silico* anticancer effect of the ethyl acetate extracts from *C. sahuynhensis* leaves and rhizomes against different cancer cell lines and their chemical constituents. The volatile constituents of the ethyl acetate extracts were analyzed by gas chromatography-mass spectrometry (GC-MS). The cytotoxic effect was tested against five cancer cell lines by the Sulforhodamine B and MTT methods, as well as a molecular docking study. The volatile components analysis indicated that the leaf and rhizome extracts contained twelve and eight components, respectively. The predominant volatile constituents, including diisooctyl phthalate; neophytadiene; 3,7,11,15-tetramethyl-2-hexadecen-1-ol; acetic acid, 3,7,11,15-tetramethyl-hexadecyl ester; isoborneol; methyl palmitate, were found in the leaf extract. Meanwhile, eucalyptol; ambrial; (*E*)-labda-8(17),12-diene-15,16-dial; α -copaene; *trans*- β -ionone; isolongifolol, acetate; and isobornyl formate were the main components in the rhizome extract. On five human cancer cell lines (MFC-7, SK-LU-1, Hela, MKN-7, and HL-60), the leaf and rhizome extracts showed concentration-dependent manner cytotoxic activity, with IC₅₀ values ranging from 126.11±8.27 to 254.82±10.25 μ g/mL for leaf extract and IC₅₀ values ranging from 109.30±6.75 to 184.59±10.24 μ g/mL for rhizome extract. Compounds (*E*)-labda-8(17),12-diene-15,16-dial (**19**), isolongifolol, acetate (**10**) and diisooctyl phthalate (**20**) were identified as potential inhibitors of epidermal growth factor receptors based on computational analysis. The findings of this work might offer an experimental foundation for further research on the isolation of metabolites and their *in vivo* cytotoxicity related to "vegetable turmeric" resources.

Keywords: *Curcuma sahuynhensis*; anticancer; cytotoxicity; GC-MS; molecular docking

1. Introduction

Among the leading causes of death worldwide, cancer stands as the second most significant disease, imposing a substantial burden on both health and the economy [1]. Characterized by the escape of cells from normal control mechanisms, the uncontrolled division of cells in cancer leads to the formation of

tumors. Malignant tumors, upon entering the bloodstream or lymphatic systems, can spread throughout the body and form new tumors at various places, posing great threats to vital tissues and organs [2]. Cancer is a major global health issue, responsible for one in every six deaths worldwide. Differences in cancer incidence and fatality rates among nations are

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influenced by factors such as population age demographics, the presence of risk factors, and the accessibility and utilization of preventive measures and early screening examinations. The most prevalent cancers in men include lung cancer, prostate cancer, colorectal cancer, and liver cancer. In women, the most frequently diagnosed cancers are breast cancer, colorectal cancer, and cervical cancer [3].

As our understanding of the underlying mechanisms of cancer continues to advance, various approaches have emerged. These include surgery, chemotherapy, radiation therapy, and more. Among these, chemotherapy is a systemic approach aimed at inhibiting cell proliferation and the multiplication of tumors, thereby preventing invasion and metastasis. Chemotherapeutic agents can be classified by their mechanism of action, some of which are the alkylating agents, the antimetabolites, the antimicrotubule agents, the antibiotics, and the miscellaneous. Each drug group has its own specific mechanisms and associated toxicities [4]. Chemotherapy often comes with side effects due to its cytotoxic mechanisms, impacting both cancer and healthy cells. Their toxicity mainly affects rapidly dividing cells, such as those in the bone marrow, the GI tract, and hair follicles. Common side effects include myelosuppression, mucositis, nausea, vomiting, diarrhea, hair loss, fatigue, sterility, infertility, and infusion reactions, as well as an elevated risk of infections due to immunosuppression [4].

Due to the negative impacts of current chemotherapy treatments, extensive research is being conducted to develop novel anti-cancer drugs that are more efficient and have fewer side effects to overcome the drawbacks of conventional cancer therapy, which also includes the prevention of cancer recurrence and drug resistance. Utilizing phytochemicals and their plant-based derivatives offers a promising strategy to boost treatment effectiveness in cancer patients while minimizing side effects.

Numerous phytochemicals exhibit physiological activity and possess significant potential for countering cancer [5]. Until now, thorough scientific investigations have explored the potential anti-cancer properties found in numerous plants and compounds derived from plants. Among these, certain plants and their components have shown impressive effectiveness in combating various types of cancer. Some of these are sweet wormwood (*Artemisia annua* L., having a semisynthetic derivative of Artemisinin-

Artesunate), Chinese goldthread (*Coptis chinensis* Franch., including Berberine alkaloids), Chinese bellflower (*Platycodon grandiflorus* (Jacq.) A.DC., containing Platycodin D compound), and many more [6]. The initial stage in creating a safe and efficient anticancer therapy based on phytochemicals involves evaluating natural extracts derived from plant material for potential biological anticancer properties. This is followed by the refinement of active phytochemicals and the identification of active compounds using bioassay-guided fractionation. Ultimately, these compounds are assessed for their effects in both *in vitro* and *in vivo* settings [5]. Research so far has tested the anticancer activity of a plethora of plants and plant-based compounds. Some of these plants and their compounds prove to be very effective against one or more types of cancer.

Among the compounds with the potential for treating various cancer types, curcumin and volatile compounds (e.g., (*E*)-labda-8(17),12-diene-15,16-dial, β -pinene, *n*-hexadecanoic acid, etc.) have exhibited both anti-cancer and anti-inflammatory properties, according to numerous studies. These compounds are regarded as primary components in many *Curcuma* genus species and play a crucial role in their therapeutic effects. Several *Curcuma* species, including *Curcuma longa* L., *Curcuma zedoaria* Roxb., *Curcuma rubescens* Roxb., *Curcuma aeruginosa* Roxb., *Curcuma attenuate* Wall., and *Curcuma aromatica* Salisb., etc., have been examined in the laboratory, and the curcumin, as well as volatile components of these plants, have been shown to have anti-cancer properties in leukemia, breast, cervical, and ovarian malignancies [6]-[8].

Curcuma sahuynhensis Škorničk. & N.S. Lý, commonly known as Nghe sa huynh (Sa huynh turmeric) or Rau Nghe (Vegetable turmeric), is another remarkable species of the *Curcuma* genus. Indigenous to Vietnam, the plant is a rhizomatous perennial herb that can reach up to a height of 75 ± 5.0 cm, and produce up to 10 leaves per pseudostem. It exhibits an inflorescence comprising 10 to 24 bracts and a cylindrical to oval rhizome that features a light brown surface. The rhizome includes tuberous roots and branching. Its bloom is yellow with a warm yellow midrib band, with a 0.6 cm long apex labellum incision. The anthers are L-shaped, stalked, pale yellow-orange, and covered with glandular hairs, lacking knobs beneath the thecae. The ovary consists of three chambers with numerous ovules. The fruit is

spherical, divided into three cells, and develops brown bulges as it ages. Each fruit typically contains about 18 seeds [9]. For everyday practice, *C. sahuynhensis* is used as a spice and vegetable in cooking [9].

Nonetheless, there has been limited research conducted on the chemical composition and pharmacological effects of this medicinal plant, particularly regarding its potential to inhibit the development of cancer cells. Consequently, our research is centered on examining the chemical constituents and *in vitro* and *in silico* cytotoxic properties of the EA extract derived from the leaves and rhizomes of *C. sahuynhensis*, an indigenous plant to Vietnam.

2. Experimental

2.1. Plant material

Leaves and rhizomes of *C. sahuynhensis* were gathered in August 2022 in Sa Huynh, Duc Pho ward, Quang Ngai Province, Vietnam. This study was a continuation of a previous one [9]. The plant materials were cleaned and dried in the shade before being crushed into a coarse powder and prepared for testing extracts. Figure 1 depicts the morphological features of the *C. sahuynhensis* study sample.

2.2. Chemicals and reagents

Chemicals: DMEM (Dulbecco's Modified Eagle Medium), MEME (Minimum Essential Medium with Eagle salt), L-glutamine, penicillin G, streptomycin, TCA (trichloroacetic acid), SRB (sulforhodamine B), Tris-base, PBS (phosphate buffered saline), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), FBS (10% Fetal Bovine Serum), sodium pyruvate ($C_3H_5NaO_3$), Trypsin-EDTA (0.05%), Ellipticine (Sigma, USA); ethanol (OPC, Vietnam); *n*-hexane (Chemsol, Vietnam); DMSO (dimethylsulfoxide) (Merck, German); acetic acid, and sodium bicarbonate (China).

Testing cells: The cell lines were provided by Prof. JM Pezzuto (Long-Island University, US) and Prof. Jeanette Maier (University of Milan, Italy).

2.3. Preparation of plant extract

The herbal material was exhaustively extracted using 96% ethanol (at a ratio of 1:10). The total ethanolic extract was then evaporated to obtain a concentrated form before being partitioned to liquid-liquid distribution with ethyl acetate (EA) solvent [10]. The ethyl acetate fraction was evaporated and used for the analysis of phytochemical composition by gas

chromatography-mass spectrometry (GC-MS) and cytotoxicity assays.

2.4. Analysis of the volatile compounds

The volatile components of *C. sahuynhensis* leaves and rhizomes were examined using gas chromatography (Agilent GC-7980) in conjunction with a mass spectrometry detector (Agilent MS 5977C). An HP-5MS UI column (30 m \times 0.25 mm \times 0.25 μ m, Agilent) was employed, and Helium served as the carrier gas with a flow rate of 1.5 mL/min to separate the samples. The column temperature program began at 80 °C (held for 1 minute), then climbed at a rate of 20 °C/min before ramping linearly to 300 °C (held for 15 minutes). The temperatures of the injector, MS Quad, and transfer line were set to 300, 150, and 300 °C, respectively. The temperature of the MS source was tuned to 230 °C, the ionization voltage to 70 eV, and the mass range was set at *m/z* 50–550 amu (2.0 scans/s). A 1.0 μ L aliquot of the sample was injected at a split ratio of 1:25, obtained by carefully dissolving 20.0 mg of EA extract in 1.0 mL of *n*-hexane solvent. The volatile components were identified by comparing the values of their mass spectra to those in the NIST17 database [10].

2.5. In vitro cytotoxic activity assay

MCF-7, SK-LU-1, HeLa, and MKN-7 cytotoxicity effects: The cytotoxicity of the EA extract obtained from *C. sahuynhensis* leaves and rhizomes was assessed using the procedure reported by Skehan *et al.* with minor changes [11]. The cell lines MCF-7, SK-LU-1, HeLa, and MKN-7 were grown in MEME media supplemented with 2.0 mL of L-glutamine, 1.0 mM sodium pyruvate, penicillin G (100 IU/mL), streptomycin (100 μ g/mL), and 10% FBS. The cells were incubated at 37 °C with 5% CO₂. The cells were trypsinized to remove them for the experiment and then counted using a counting chamber.

Diluting EA extract in 100% DMSO to a concentration of 0.020 μ g/mL yielded the stock solution. This solution was diluted in FBS-free cell culture media to concentrations of 500, 100, 20.0, 4.0, and 0.8 μ g/mL. In a 96-well plate, a mixture of 10 μ L of each sample and 190 μ L of cells was cultured for 72 hours in a warm incubator. The cells were fixed with 20% TCA and stained with 0.2% SRB for 30 minutes at 37 °C after incubation, washed three times with acetic acid, and then dried at room temperature. To dissolve the SRB, 10 mM Tris-base buffer was added.

The optical density (OD) of the samples was measured at 540 nm using an ELISA Plate Reader (Biotek, USA) after shaking the mixture for 10 minutes. Cancer cells (190 μ L) and 1% DMSO (10 μ L) were utilized for the blank wells, which served as controls. The blank wells were repaired with 20% TCA after 1 hour. Ellipticine was produced at quantities of 10, 2.0, 0.4, and 0.08 μ g/mL, serving as a positive control [11].

The inhibition rate of cancer cells was calculated by the following formula:

$$\% I = [1 - ((OD_{Sp} - OD_{Bi}) / (OD_{DMSO} - OD_{Bi}))] \times 100\%$$

Where I: inhibition rate of cancer cells, OD_{Sp} : average optical density value of testing sample; OD_{Bi} : average optical density value of blank sample; OD_{DMSO} : average optical density value of DMSO.

HL60 cytotoxicity effect: The cytotoxicity of the EA extract of turmeric leaves and rhizomes on the HL-60 cell line was assessed using the technique published by Lakshmi Priya et al. with minor changes [12]. The HL-60 cells were grown in DMEM using the same protocol as described before. After 72 hours of culture, each well received 10 μ L of MTT solution with a final concentration of 500 μ g/mL. The culture media was withdrawn after 4 hours of incubation, and the formazan crystals produced were dissolved in 50 L of 100% DMSO. The optical density (OD) was then measured at 540 nm using a BioTek spectrophotometer from the United States [12].

The inhibition rate of cancer cells was calculated by the following formula:

$$\% I = [1 - ((OD_{Sp} - OD_{Bi}) / (OD_{DMSO} - OD_{Bi}))] \times 100\%$$

Where I: inhibition rate of cancer cells, OD_{Sp} : average optical density value of testing sample; OD_{Bi} : average optical density value of blank sample; OD_{DMSO} : average optical density value of DMSO.

2.6. Molecular docking study

In this study, we also investigated the binding ability of phytochemical compounds from *C. sahuynhensis* leaves and rhizomes to the epidermal growth factor receptor (EGFR) - the target of a series of anti-cancer drugs such as gefitinib, erlotinib, afatinib, dacomitinib, and osimertinib [13]. The structure of EGFR in complex with erlotinib (PDB ID: 1M17) at 2.6 \AA resolution was used for molecular docking studies. We used Autodock Vina 1.2.3 software [14] and AutoDock4 forcefield [15], with the preparation steps according to the basic protocol [16]. The grid box was designed around the center of the co-crystallized ligand ($x = 21.697, y = 0.303, z = 52.093$), with dimensions of $15.75 \times 15 \times 15 \text{\AA}$. 3D conformers of ligands were downloaded from IMPPAT (Indian Medicinal Plants, Phytochemistry And Therapeutics) database (<https://cb.imsc.res.in/imppat/>) as *.pdbqt format. Before investigating the binding of phytochemical compounds to EGFR, the docking model parameters were evaluated using the re-docking procedure. Accordingly, the configurations of the initial co-crystallized ligand and after docking differ slightly with an RMSD of 1.78 \AA ($< 2 \text{\AA}$).

2.7. Data analysis

The experimental data from the investigation were assessed and documented. All findings were obtained in triplicate and provided as the mean value standard deviation (S.D). Microsoft Excel 2023 software was used for statistical computations and data processing. The IC_{50} value (μ g/mL) (i.e., 50% inhibition concentration) was determined using TableCurve 2Dv4 software.

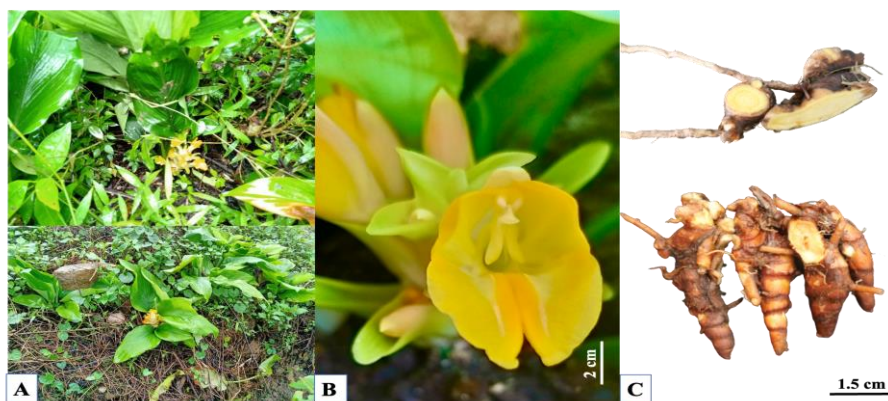


Fig. 1. Photographs of *C. sahuynhensis* (A. Whole plants; B. Inflorescence and flower; C. Rhizomes).

3. Results and discussion

3.1. Phytochemical evaluation

Gas chromatography-mass spectrometry (GC-MS) analysis indicated twelve and eight compounds in the leaf and rhizome extracts, accounting for 74.50% and 64.09% of the compositions, respectively (Table 1). Overall, the main phytochemical constituents in the leaf extract were diisooctyl phthalate (21.52%); neophytadiene (19.36%); 3,7,11,15-tetramethyl-2-hexadecen-1-ol (9.16%); acetic acid, 3,7,11,15-tetramethyl-hexadecyl ester (5.86%); isoborneol (5.46%), and methyl palmitate (3.90%) (Fig. 2A). Meanwhile, eucalyptol (17.36%); ambrial (13.81%); (*E*)-labda-8(17),12-diene-15,16-dial (12.28%); α -copaene (5.51%); *trans*- β -ionone (4.97%); isolongifolol, acetate (4.68%); and isobornyl formate (3.71%) were the major chemical compounds of the

rhizome extract (Fig. 2B). EA extracts from the leaves and rhizomes of *C. sahuynhensis* also showed differences in their compositions. Phytol (2.76%), *n*-hexadecanoic acid (1.59%), safranal (1.54%), borneol (1.43%), 2-bornanone (1.09%), and viridiflorol (0.83%) were found in small amounts in the leaf extract; however, they were not present in the rhizomes' EA extract. Similarly, camphor was only detected in the rhizomes' EA extract.

These results are similar to previous studies showing that the main chemical components from the leaves and rhizomes of some species of the genus *Curcuma* (e.g., *C. amada* Roxb., *C. cotuana* Luu, Škorničk. & H.Đ.Trần, *C. thorelii* Gagnep., *C. mangga* Valeton & Zijp, etc.) were also found, such as eucalyptol; ambrial; isoborneol; neophytadiene; methyl palmitate; (*E*)-labda-8(17),12-diene-15,16-dial, etc.[17], [18].

Table 1. Compounds identified in the EA extracts from *C. sahuynhensis* leaves and rhizomes.

No.	RT (min)	Compounds	MF	MW (g/mol)	Area (%)	
					Leaves	Rhizomes
1	5.857	Eucalyptol	C ₁₀ H ₁₈ O	154.25	-	17.36
2	8.708	2-Bornanone	C ₁₀ H ₁₆ O	152.23	1.09	-
3	8.722	Camphor	C ₁₀ H ₁₆ O	152.23	-	1.77
4	8.980	Isoborneol	C ₁₀ H ₁₈ O	154.25	5.46	-
5	8.987	Isobornyl formate	C ₁₁ H ₁₈ O ₂	182.26	-	3.71
6	9.190	Borneol	C ₁₀ H ₁₈ O	154.25	1.43	-
7	13.06	<i>trans</i> - β -Ionone	C ₁₃ H ₂₀ O	192.30	-	4.97
8	13.44	α -Copaene	C ₁₅ H ₂₄	204.35	-	5.51
9	15.171	Safranal	C ₁₀ H ₁₄ O	150.22	1.54	-
10	17.907	Isolongifolol, acetate	C ₁₇ H ₂₈ O ₂	264.40	-	4.68
11	17.914	Viridiflorol	C ₁₅ H ₂₆ O	222.37	0.83	-
12	19.944	Ambrial	C ₁₆ H ₂₆ O	234.38	-	13.81
13	20.256	Neophytadiene	C ₂₀ H ₃₈	278.50	19.36	-
14	20.337	Acetic acid, 3,7,11,15-tetramethyl-hexadecyl ester	C ₂₂ H ₄₄ O ₂	340.60	5.86	-
15	20.799	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296.50	9.16	-
16	21.342	Methyl palmitate	C ₁₇ H ₃₄ O ₂	270.50	3.90	-
17	21.729	<i>n</i> -Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.42	1.59	-
18	23.568	Phytol	C ₂₀ H ₄₀ O	296.50	2.76	-
19	26.673	(<i>E</i>)-Labda-8(17),12-diene-15,16-dial	C ₂₀ H ₃₀ O ₂	302.45	-	12.28
20	29.543	Diisooctyl phthalate	C ₂₄ H ₃₈ O ₄	390.60	21.52	-
Total (%)					74.50	64.09

Note: MW: Molecular Weight; MF: Molecular Formula; RT: Retention time (min); Area (%) in "Bold" denotes predominant volatile compounds (> 3.0%); "-" Not identified.

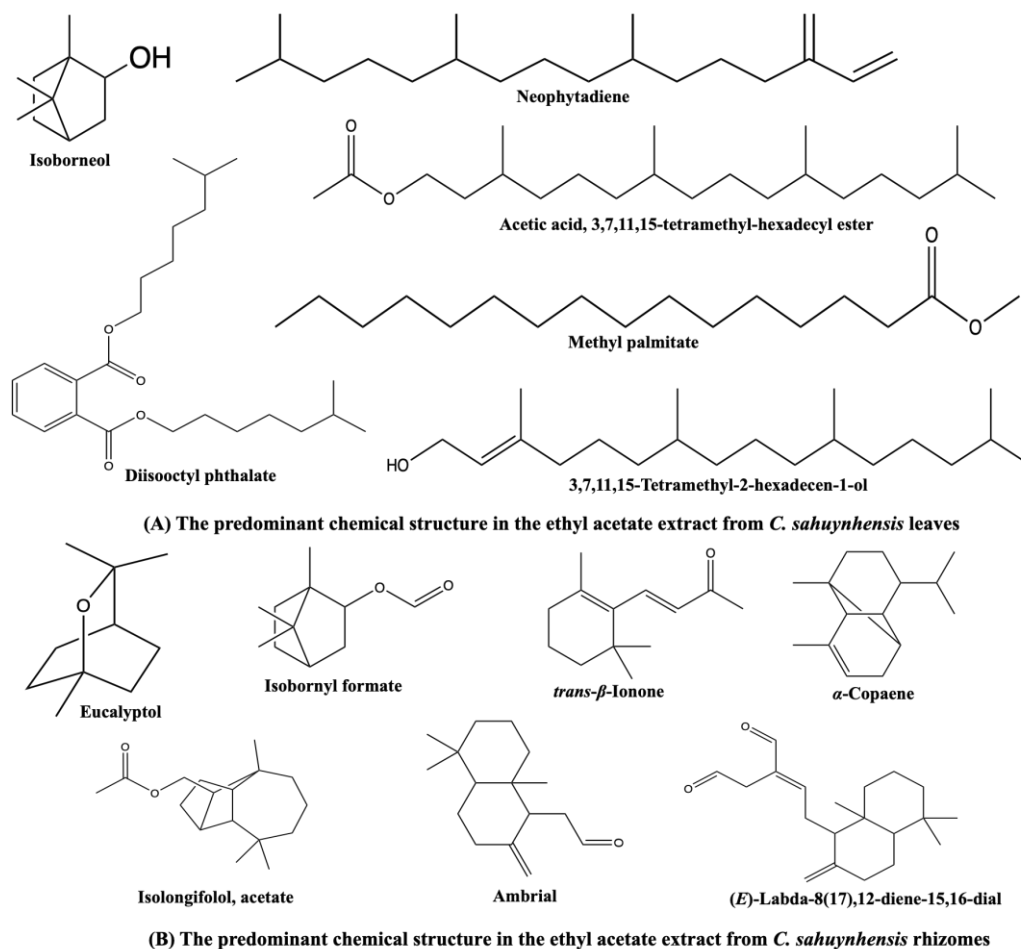


Fig. 2. Chemical structure of the predominant compounds identified in the EA extracts from *C. sahuynhensis* leaves (A) and rhizomes (B)

3.2. Cytotoxic effect evaluation

In vitro anticancer activity study, both EA extracts from the leaves (EAL) and rhizomes (EAR) of *C. sahuynhensis* showed a spectrum of anticancer activity against the proliferation of five human cancer cells, with IC_{50} values in the range of $126.11 \pm 8.27 \sim 254.82 \pm 10.25$ $\mu\text{g/mL}$ for EAL and $109.30 \pm 6.75 \sim 184.59 \pm 10.24$ $\mu\text{g/mL}$ for EAR. As shown in Table 2, the cytotoxic activity of EA extracts from *C. sahuynhensis* leaves and rhizomes has IC_{50} values against human breast carcinoma (MCF-7, 126.11 ± 8.27 $\mu\text{g/mL}$ for EAL, 184.59 ± 10.24 $\mu\text{g/mL}$

for EAR), followed by human lung carcinoma (SK-LU-1, 254.82 ± 10.25 $\mu\text{g/mL}$ for EAL, 109.30 ± 6.75 $\mu\text{g/mL}$ for EAR), human cervical carcinoma (Hela, 231.49 ± 7.82 $\mu\text{g/mL}$ for EAL, 155.49 ± 6.77 $\mu\text{g/mL}$ for EAR), human gastric carcinoma (MKN-7, 211.91 ± 10.62 $\mu\text{g/mL}$ for EAL, 147.24 ± 5.46 $\mu\text{g/mL}$ for EAR), and human acute leukemia (HL-60, 236.64 ± 5.09 $\mu\text{g/mL}$ for EAL, 118.27 ± 4.21 $\mu\text{g/mL}$ for EAR), respectively. Similarly, the positive control medication in this trial, ellipticine, was examined for cytotoxicity on experimental cancer cell lines, with IC_{50} values ($\mu\text{g/mL}$) ranging from 0.31 ± 0.02 to 0.51 ± 0.04 $\mu\text{g/mL}$ (Table 2).

Table 2. Cytotoxic potential of the EA extracts from *C. sahuynhensis* leaves and rhizomes and Ellipticine.

% inhibition of cell growth of leaf extract					
Concentration ($\mu\text{g/mL}$)	MCF-7	SK-LU-1	Hela	MKN-7	HL-60
500	97.39 ± 2.00	86.07 ± 2.04	98.41 ± 2.13	77.63 ± 1.74	87.01 ± 1.61
100	47.64 ± 2.35	23.31 ± 1.08	24.99 ± 1.48	36.12 ± 1.22	27.14 ± 0.92
20	13.35 ± 1.20	7.08 ± 0.70	5.35 ± 0.55	7.64 ± 0.75	3.46 ± 0.37
4.0	8.66 ± 0.50	1.41 ± 0.13	4.22 ± 0.27	5.35 ± 0.35	2.06 ± 0.17
0.8	4.18 ± 0.14	0.02 ± 0.001	1.58 ± 0.13	2.40 ± 0.19	0.97 ± 0.03
IC_{50} ($\mu\text{g/mL}$)	126.11 ± 8.27	254.82 ± 10.25	231.49 ± 7.82	211.91 ± 10.62	236.64 ± 5.09
% inhibition of cell growth of rhizome extract					

Concentration ($\mu\text{g/mL}$)	MCF-7	SK-LU-1	Hela	MKN-7	HL-60
500	95.74 \pm 1.27	99.05 \pm 2.46	98.86 \pm 2.31	99.64 \pm 2.17	97.80 \pm 1.63
100	33.87 \pm 1.86	52.86 \pm 1.90	39.85 \pm 1.61	33.97 \pm 1.01	50.86 \pm 1.03
20	10.82 \pm 1.22	10.69 \pm 0.83	12.08 \pm 0.35	12.75 \pm 0.24	10.52 \pm 1.08
4.0	4.39 \pm 1.04	4.65 \pm 0.33	6.52 \pm 0.11	1.78 \pm 0.14	7.22 \pm 0.72
0.8	1.22 \pm 0.20	2.67 \pm 0.22	1.46 \pm 0.12	1.09 \pm 0.11	3.66 \pm 0.44
IC₅₀ ($\mu\text{g/mL}$)	184.59\pm10.24	109.30\pm6.75	155.49\pm6.77	147.24\pm5.46	118.27\pm4.21
Ellipticine (IC ₅₀ , $\mu\text{g/mL}$)	0.47 \pm 0.03	0.51 \pm 0.04	0.41 \pm 0.02	0.44 \pm 0.02	0.31 \pm 0.02

Plants serve as reservoirs of various biologically active compounds with therapeutic potential for various ailments. Chemical profiling of volatile compounds and the bioactivity of plants should be further investigated, as scientific evidence supports traditional use practices. The discovery of different medicinal plant extracts could pave the way for novel alternative drug treatments for cancer cells and address the growing challenge of anticancer resistance. Plant-based natural substances offer a safe, effective, and non-toxic alternative to chemical drug treatments [19]. Scientists are increasingly interested in discovering products in all parts of the plant rather than focusing solely on the main portion that is traditionally utilized because of the rising demand for plant-based products.

In the current study, the chemical profile and *in vitro* cytotoxic effect of the EA extracts of *C. sahuynhensis* leaves and rhizome were performed. The volatile compounds were identified by using the GC-MS method. Gas chromatography separates the mixture's constituent parts, and mass spectroscopy examines each component separately. Previous reports have shown that the chemical components (e.g., essential oils, fatty acids, less-polar or non-polar compounds) in the whole plant ethanolic extract of *Evolvulus alsinoides* (L.) L. [20], the EA extract of *Cymodocea serrulata* (R.Br.) Asch. & Magnus [21], etc., were identified by the GC-MS method. Hence, the GC-MS technique is a useful, quick-to-use, and reliable approach for testing the amount of active ingredients in herbs [22]. Particularly, the GC-MS technique is used in this study as a preliminary step towards comprehending the nature of *in vitro* cytotoxic effects. The predominant volatile compounds identified have some significant biological potential, suggesting the possibility of developing this medicinal plant into new functional products to support disease treatment in the future, especially in relation to cancer disease.

In previous studies, the volatile components in *C. sahuynhensis* played an important role. The major constituents in the essential oil of *C. sahuynhensis* rhizomes were β -pinene, β -caryophyllene, α -pinene, cineole, (*Z*)- β -farnesene, caryophyllene oxide, camphor, caryophyllene, humulene, and humulene

epoxide II, etc.[23]. Similarly, volatile compounds as well as fatty acids (with an area > 3.0%), including methyl palmitate, methyl linoleate, ethyl palmitate, ethyl linoleate, humulene oxide II, and ambrial, were also found in the *n*-hexane extract of *C. sahuynhensis* rhizomes [24]. Moreover, *C. sahuynhensis* essential oil showed good activity against *Enterococcus faecalis*, *Staphylococcus aureus*, *Bacillus cereus*, and *Candida albicans*, with minimum inhibitory concentrations (MIC) of 64 $\mu\text{g/mL}$ for each organism [23].

In another study, Quy *et al.* reported that the volatile constituents identified in the *n*-hexane extract of *C. sahuynhensis* rhizomes showed significant activity against influenza A hemagglutinin, SARS-CoV-2 main protease, and Omicron-variant spike protein *in silico* [24].

In terms of the pharmacological effects of the predominant volatile compounds in the EA extract from *C. sahuynhensis* leaves, diisooctyl phthalate is predominant, with the highest peak area (21.52%). Diisooctyl phthalate was also found in the extracts of *Allium fistulosum* L., *Lilium brownii* Lemoinier, *Cirsium japonicum* DC., *Brassica oleracea* L., *Campanula colorata* Wall., *Dalbergia odorifera* T.C. Chen, *Myrica rubra* (Lour.) Siebold & Zucc., *Eichhornia crassipes* (Mart.) Solms, *Solanum lycopersicum* L., *Nepheleium lappaceum* L. [25], and also found in essential oils from the leaves of *Kaempferia champasakensis* Pichens. & Koonterm. [26], etc. Diisooctyl phthalate has previously been reported as having cytotoxic potential, antibacterial, anti-melanogenetic, anti-algal activities [27], etc.

Several bioactive metabolites were found in the leaf extracts of various plants, including *C. sahuynhensis*, *Crateva nurvala* Buch.-Ham., *Blumea lacera* (Burm.f.) DC., etc. One of the identified compounds was neophytadiene [28]. Neophytadiene exhibits anxiolytic, anti-convulsant, antioxidant, anti-inflammatory, cytotoxic, and cardioprotective properties, which were reported in earlier studies [28], [29]. Methyl palmitate, a fatty acid methyl ester with potential anti-inflammatory and anti-fibrotic effects

[30], was reported to cause growth inhibition in hepatocellular carcinoma cells [31].

Additionally, compound 3,7,11,15-tetramethyl-2-hexadecen-1-ol is one of the twelve compounds in *C. sahuynhensis* leaves, which were also found in the leaf extracts of *Leucaena leucocephala* (Lam.) de Wit [32], *Hugonia mystax* L. [33], etc. The identified 3,7,11,15-tetramethyl-2-hexadecen-1-ol has antimicrobial, anti-inflammatory, anticancer, antidiuretic, anti-oxidant, and hypocholesterolemic effects [32]. In addition to its antimicrobial and antifungal activities, the acetic acid, 3,7,11,15-tetramethyl-hexadecyl ester was also reported to have mucolytic, antioxidant, anti-tumor, and anticancer effects [34]. Isoborneol is a monoterpene alcohol present in the essential oils or extracts of several medicinal plants (e.g., *C. sahuynhensis*, *Valeriana officinalis* L., *Matricaria chamomilla* L., *Lavandula officinalis* Chaix, etc.) [35]. Noticeably, biological effects, including antioxidant, anti-anxiety, anti-insomnia, anti-sporulating, anti-neurodegenerative, and antiviral activities, were observed for the compound isoborneol [35], [36].

Among the identified phytochemicals from the EA extracts in *C. sahuynhensis* rhizomes, the predominant volatile compounds, including eucalyptol (17.36%), ambrial (13.81%), (*E*)-labda-8(17),12-diene-15,16-dial (12.28%), α -copaene (5.51%), trans- β -ionone (4.97%), and isobornyl formate (3.71%), have many biological properties. Previous studies have shown that various plant-derived eucalyptols (with the major ingredient being 1,8-cineole), such as *Eucalyptus smithii* R.T.Baker, *E. globulus* Labill., *E. bicostata* Maiden, Blakely & Simmonds, *E. sideroxylon* A.Cunn. ex Woolls, *E. cinerea* F.Muell. ex Benth., etc., have the properties of vasorelaxant, hypotensive, anti-viral, antimicrobial, antioxidant, antiseptic, anti-inflammatory, anticancer, and antitumor effects, as well as other biological activities [37], [38].

Interestingly, (*E*)-labda-8(17),12-diene-15,16-dial was present in the EA extract from *C. sahuynhensis* rhizomes but absent in the EA leaf extract. This compound was also commonly found in various species belonging to the family Zingiberaceae, such as *Curcuma amada* Roxb. [39], *Costus comosus* var. *bakeri* (K.Schum.) Maas, *Curcuma rubescens* Roxb., *Curcuma aeruginosa* Roxb., *Curcuma attenuata* Wall., *Zingiber zerumbet* (L.) Smith, *Hedychium brevicaulis* D.Fang, *Alpinia oxyphylla* Miq., and *Alpinia pumila* Hook.F. [8], etc. The literature indicates that (*E*)-labda-8(17),12-diene-15,16-dial particularly has anti-biotic, anti-fungal, antibacterial, anti-inflammatory, and anticancer activities [8], as

well as α -glucosidase and pancreatic lipase inhibitory effects [39].

Additionally, α -copaene, derived from different plants (e.g., *Annona reticulata* L., *Cedrelopsis grevei* Baill. & Curchet, *Xylopi laevigata* (Mart.) R.E.Fr., etc.), is a tricyclic sesquiterpene [40]. Previous study has reported that α -copaene possesses a strong cytotoxicity effect as well as antioxidant, hepatoprotective, and anti-inflammatory activities [40].

In the review by Paparella *et al.*, trans- β -ionone has been demonstrated to possess anti-inflammatory, anti-tumor, anti-cancer, antimicrobial, and anti-leishmanial properties [41]. Meanwhile, pure ingredients such as ambrial and isolongifolol, acetate have not been reported in the literature on their biological effects recently. To the best of our knowledge, isolongifolol, acetate has been identified for the first time only in *C. sahuynhensis* rhizome extract.

Different extraction techniques, extraction solvents, and analysis methods may result in different volatile component analysis results [8]. Thus, the current study has been found useful in the identification of several volatile components present in the EA extracts of *C. sahuynhensis* leaves and rhizomes by GC-MS analysis. The presence of various bioactive compounds (identified as diisooctyl phthalate; neophytadiene; 3,7,11,15-tetramethyl-2-hexadecen-1-ol; acetic acid, 3,7,11,15-tetramethyl-hexadecyl ester; isoborneol; methyl palmitate, etc., in the leaf extract and eucalyptol; ambrial; (*E*)-labda-8(17),12-diene-15,16-dial; α -copaene; trans- β -ionone; isolongifolol, acetate; isobornyl formate, etc., in the rhizome extract) justifies the use of the leaves and rhizomes from *C. sahuynhensis* for different diseases by folk practitioners.

As in our previous report, the aerial part extract of *C. sahuynhensis* contained phytochemicals like polyphenols and flavonoids [9], which exerted an inhibitory effect on cancer cell growth, as well as anti-inflammatory, antithrombotic, anti-atherosclerotic, and antioxidant activities [42].

In this context, *C. sahuynhensis*'s cytotoxic property against a number of cancer cell lines can be considered noticeable. The EA extracts of *C. sahuynhensis* leaves and rhizomes have a cytotoxic effect on the growth of experimental cancer cell lines in a concentration-dependent manner. At a concentration of 100 μ g/mL, our experimental showed that the EA extract from *C. sahuynhensis* leaves and rhizome inhibited the growth of human cancer cell lines like MFC-7, SK-LU-1, HeLa, MKN-7, and HL-60, with percentages of inhibition of cell growth being 47.64 \pm 2.35%, 23.31 \pm 1.08%, 24.99 \pm 1.48%, 36.12 \pm 1.22%, 27.14 \pm 0.92% for leaf extract and

33.87±1.86%, 52.86±1.90%, 39.85±1.61%, 33.97±1.01%, 50.86±1.03% for rhizome extract, respectively. Remarkably, the IC₅₀ value varies depending on the specific test cell line. Specifically, as shown in Table 2, the cytotoxic effect of the EA extract from *C. sahuynhensis* leaves and rhizomes per human cancer cell line was clearly shown against MCF-7 (IC₅₀ = 126.11±8.27 µg/mL) and SK-LU-1 (IC₅₀ = 109.30±6.75 µg/mL), respectively. This finding illustrates the varying molecular properties of cancer cell lines and their susceptibilities to phytochemicals found in the leaves and rhizomes of *C. sahuynhensis*. This argument is consistent with a previous study by Kumar *et al.* [43].

Particularly, the interesting point of the present study is that the anti-cancer activity of the rhizome extract is stronger than that of the leaf extract on all tested cell lines. A study by Ahmed Hamdi *et al.* [44] showed that compound (*E*)-labda-8(17),12 diene-15,16 dial (IC₅₀ = 16.3 ±0.2 µg/mL) has the ability to inhibit the MFC-7 cancer lines. Moreover, eugenol has exhibited anticancer effects against several cancer types, including leukemia, lung cancer, colon cancer, colorectal cancer, skin cancer, gastric cancer, breast cancer, cervical cancer, and prostate cancer. For example, eugenol had a cytotoxic influence on HeLa cells at concentrations of 50–200 µM, etc. [45]. This may explain the potential anti-cancer ability of *C. sahuynhensis* rhizomes. However, in-depth isolation studies and evaluations of their cytotoxic effects are necessary.

A study by Hou *et al.* indicated as an example that the petroleum ether and EA extracts of *Curcuma phaeocaulis* Val. rhizomes exhibited medium-intensity antitumor activity towards the proliferation of four cancer cells (SMMC-7721, HepG-2, A549, and Hela cell lines) [46]. The cytotoxic activity of EA extract was the lowest, with an IC₅₀ value against the Hela cell line of 255.2688 µg/mL. Meanwhile, the IC₅₀ value of petroleum ether against the HepG-2 cell line was the lowest, at 132.6822 µg/mL [46]. For the Hela cell line, the cytotoxic activity of the EA extract from *C. phaeocaulis* rhizomes (IC₅₀ = 255.2688 µg/mL) was weaker than that of the EA extract from *C. sahuynhensis* rhizomes (IC₅₀ = 155.499±6.77 µg/mL) and leaves (IC₅₀ = 231.49±7.82 µg/mL). In another study by Dwira *et al.*, also based on the results of the MTT assay against the HeLa cell line, the EA extract of white turmeric (*Kaempferia rotunda* L.) rhizomes exhibited a Hela cytotoxic effect that was stronger than the EA extract of *C. sahuynhensis* rhizomes and leaves, with IC₅₀ values (µg/mL) of 127.9, 155.499±6.77, and 231.49±7.82 µg/mL, respectively [47]. These studies lead to the hypothesis that the chemical compositions and cytotoxic effects will vary

depending on the extraction techniques and solvents used.

According to the classification of cytotoxicity based on IC₅₀ values by Dwira *et al.*, the extract from the plant has strong cytotoxic activity (IC₅₀ < 50.0 µg/mL), moderate cytotoxic activity (IC₅₀ = 101–250 µg/mL), weak cytotoxic activity (IC₅₀ = 251–500 µg/mL), and inactivity (IC₅₀ > 501 µg/mL) [47]. Therefore, the EA extracts of *C. sahuynhensis* leaves and rhizomes have moderate cytotoxic activity against tested cell lines, with IC₅₀ values ranging from 109.30±6.75 µg/mL to 236.64±5.09 µg/mL, while the leaf extract has weak cytotoxic activity for the SK-LU-1 cell line (IC₅₀ = 254.82±10.25 µg/mL).

In conclusion, the relationship between the phytochemical components and their biological effects is currently the subject of interest. An endemic plant called *C. sahuynhensis* has been forcefully used to treat digestive disorders and as food in Vietnam. However, there have been no reports on the chromatographic analysis of the vegetable turmeric's EA extract so far. Herein, this study reports for the first time the existence of certain significant chemical components in *C. sahuynhensis* leaves and rhizomes that were discovered using GC-MS analysis. Therefore, this study may provide information about the nature of the active principles of chemical compounds found in medicinal plants and help demonstrate the cytotoxic effects of EA extracts from *C. sahuynhensis* leaves and rhizomes. In other words, these detected phytoconstituents are believed to be the agents responsible for the biological effects of this species, *C. sahuynhensis*.

3.3. Molecular docking study

High epidermal growth factor receptor (EGFR) expression has been known to be associated with the development of breast cancer, non-small-cell lung cancer, metastatic colorectal cancer, human cervical cancer, gastric cancer, pancreatic cancer, etc [48]. In this study, we investigated the ability of the compounds to bind and interact with EGFR. A total of twenty phytochemical compounds from *C. sahuynhensis* were successfully docked into the EGFR pocket with docking scores ranging from -5,324 to -8,006 kcal/mol. Among them, (*E*)-labda-8(17),12-diene-15,16-dial (**19**) and isolongifolol, acetate (**10**) from *C. sahuynhensis* rhizomes, and diisooctyl phthalate (**20**) from *C. sahuynhensis* leaves, exhibited a strong affinity for the binding site of EGFR, with docking scores of -8.006, -7.446, and -7.118 kcal/mol, respectively (Table 3).

Table 3. Docking score of 20 phytochemical compounds from *C. sahyunhensis*.

No.	Compounds	Docking score (kcal/mol)
1	Eucalyptol	-5.324
2	2-Bornanone	-5.456
3	Camphor	-5.456
4	Isoborneol	-5.563
5	Isobornyl formate	-5.951
6	Borneol	-5.563
7	<i>trans</i> - β -Ionone	-6.506
8	α -Copaene	-6.605
9	Safranal	-5.506
10	Isolongifolol, acetate	-7.446
11	Viridiflorol	-7.091
12	Ambrial	-7.075
13	Neophytadiene	-7.002
14	Acetic acid, 3,7,11,15-tetramethyl-hexadecyl ester	-6.658
15	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	-6.812
16	Methyl palmitate	-5.480
17	<i>n</i> -Hexadecanoic acid	-6.113
18	Phytol	-6.644
19	(<i>E</i>)-Labda-8(17),12-diene-15,16-dial	-8.006
20	Diisooctyl phthalate	-7.118

In our molecular docking simulation model, (*E*)-Labda-8(17),12-diene-15,16-dial (**19**) showed good binding to EGFR with a docking score of -8,006 kcal/mol. The two ketone groups of the butanedial skeleton act as hydrogen bond acceptor groups to bind to the NH backbone of Met769 and Gly772. Meanwhile, the decalin framework plays a role in hydrophobic interactions with Val702, Ala719, Lys721, and Leu820 (Figures 3A, B).

Isolongifolol, acetate (**10**) also showed good binding to EGFR, although to a less extent than (*E*)-Labda-8(17),12-diene-15,16-dial (**19**). The methanoazulene framework of this compound plays an important role in forming hydrophobic interactions with Val702, Ala719 and Leu820. The O atom of the acetate group receives electrons from Thr766 to form a single hydrogen bond (Figures 3C, D).

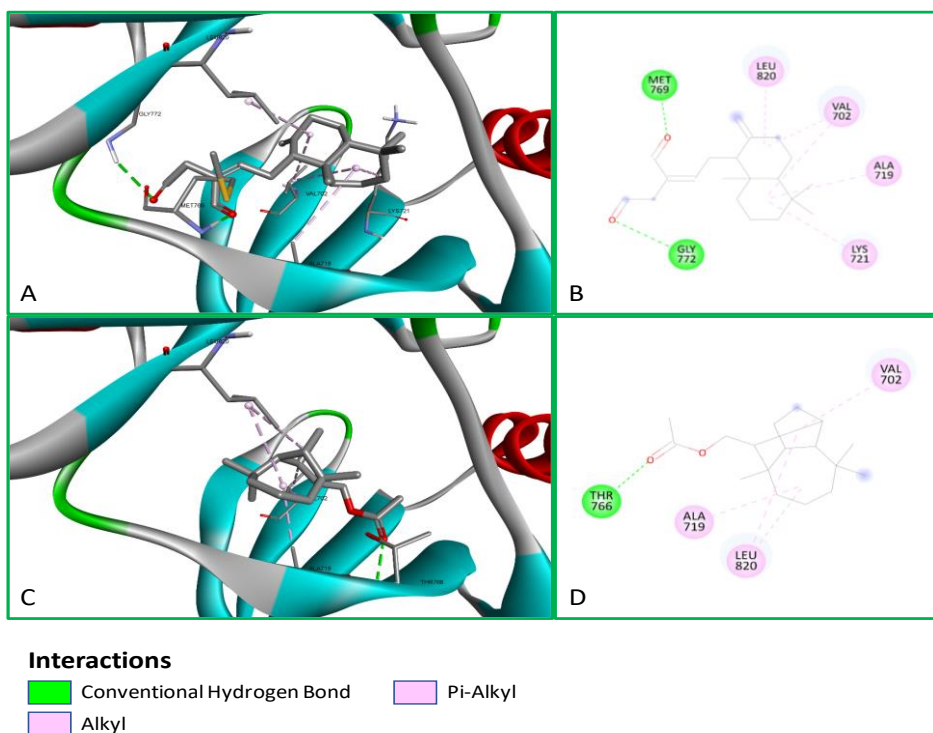


Fig. 3. (A), (B) 3D and 2D interaction of (19) with EGFR; (C), (D) 3D and 2D interaction of (10) with EGFR.

Among the compounds from *C. sahuynhensis* leaves, we have confirmed that diisooctyl phthalate (**20**) has the ability to bind strongly to EGFR through molecular docking simulation model. The binding energy of diisooctyl phthalate primarily comes from mostly hydrophobic interactions with residues in the active site such as alkyl interactions with Leu694, Ala719, Leu764, Leu768, and Leu820, pi-anion

interactions with Asp831. Additionally, there are interactions with some neighboring amino acids such as Pi-Pi T-shaped interactions with Phe699, alkyl interactions with Val702, Lys721, and Met742 (Figure 4).

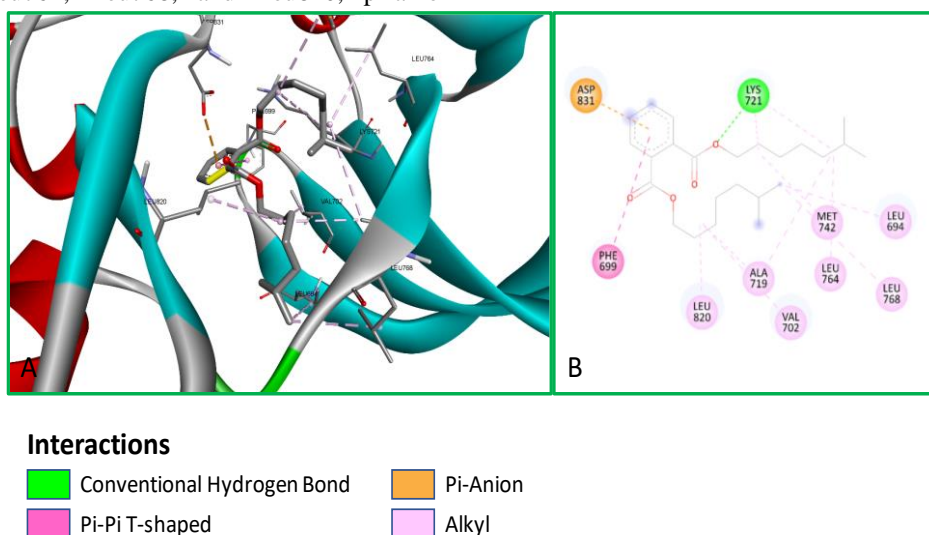


Fig. 4. (A) 3D interaction of diisooctyl phthalate with EGFR and (B) 2D interaction of (20) with EGFR.

The anticancer kinase inhibitor called erlotinib is regularly approved for use as monotherapy in the management of non-small cell lung cancer [48]. Erlotinib stabilizes the active conformation, leading to EGFR dimerization [49]. Our molecular docking model showed that (*E*)-labda-8(17),12-diene-15,16-dial (**19**) and isolongifolol, acetate (**10**) from *C. sahuynhensis* rhizomes and diisooctyl phthalate (**20**) from *C. sahuynhensis* leaves can bind to the erlotinib binding site of EGFR (ATP site). These results suggest the inhibitory potential of these compounds against EGFR overexpression.

4. Conclusion

In conclusion, the present study describes for the first time the anticancer activities of the EA extracts of leaves and rhizome from *C. sahuynhensis* and their chemical composition. The phytochemical constituents, including diisooctyl phthalate; neophytadiene; 3,7,11,15-tetramethyl-2-hexadecen-1-

ol; acetic acid, 3,7,11,15-tetramethyl-hexadecyl ester; isoborneol; methyl palmitate; eucalyptol; ambrial; (*E*)-labda-8(17),12-diene-15,16-dial; α -copaene; *trans*- β -ionone; isolongifolol, acetate; and isobornyl formate were found in the rhizome and leaf extracts. The EA extracts of *C. sahuynhensis* rhizome and leaves exhibit moderate cytotoxic activity against most of the tested cell lines, with the exception of the leaf extract against the SK-LU-1 cell line, which demonstrates weak cytotoxic activity. In molecular docking *in silico*, compounds (**19**), (**10**), and (**20**) are substances with a strong affinity for the binding site of EGFR, with docking scores of -8.006, -7.446 and -7.118 kcal/mol, respectively. These results show that *C. sahuynhensis* has significant *in vitro* and *in silico* cytotoxic effects against breast carcinoma, lung carcinoma, cervical carcinoma, gastric carcinoma, and acute leukemia. Further research will be conducted to clarify the mechanisms of action and identify potential molecules with anti-cancer properties.

5. Conflicts of interest

There are no conflicts to declare.

6. Acknowledgments

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