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RESEARCH ARTICLE

GC-MS analysis and cytotoxic activity of the *n*-hexane fraction from *Curcuma sahuynhensis* Škorničk. & N.S.Lý leaves collected in Vietnam

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Abstract

Curcuma sahuynhensis Škorničk. & N.S.Lý is an endemic plant in Vietnam that has been used by the Sa Huynh people as a spice and medicine to cure illnesses linked to digestive disorders. Very little information is available so far about the chemical composition and biological effects of *C. sahuynhensis*. To find new pharmaceutical ingredients, the *in vitro* cytotoxic effect and the chemical profile of *C. sahuynhensis* leaf extract were investigated. In this study, the percolation method and liquid-liquid dispersion technique were used to extract dry sample powder. The chemical composition was detected by gas chromatography-mass spectrometry (GC-MS). The Sulforhodamine B and MTT methods were used to determine the cytotoxic activity. The chemical composition analysis showed that the leaf extract contained 14 components. The major components in the *n*-hexane extract were 6,10,14-trimethylpentadecan-2-one, phytol, 1-ethylbutyl hydroperoxide, isoborneol, 1-methylpentyl hydroperoxide, and neophytadiene. On human cancer cell lines, namely MFC-7, SK-LU-1, Hela, MKN-7, and HL-60, the leaf extract showed dose-dependent cytotoxic activity, with IC_{50} values ranging from 221.70±10.24 to 369.42±10.60 µg/mL. The present study provides significant information on the chemical components and cytotoxic effects of the *n*-hexane extract from *C. sahuynhensis* leaves. The findings will continue to be crucial in future research on the evaluation of secondary metabolite compound analysis for cancer therapeutic effects.

Keywords

Curcuma sahuynhensis; anticancer; cytotoxicity; endemic plant; GC-MS

Introduction

Cancer is a malignant disease of cells in which cancer cells lose normal control, leading to uncontrolled growth, loss of differentiation, invasion of surrounding tissues, and spreading throughout the body via the blood and lymphatic systems. According to the World Health Organization and the American Cancer Society in 2018, there were 18 million people diagnosed with cancer, with lung and breast cancer being the most common, prevalent, and dangerous types. Cancer is also one of the leading causes of death in the world, second only to cardiovascular disease, and is predicted to become the leading cause of death by 2060 (1).

There are several therapeutics for treating cancer, such as surgery, radiation therapy, hormone therapy, etc. Among them, chemotherapy is a systemic cancer treatment that aims to address tumors that have spread throughout the system. Many chemotherapy drugs have been approved for cancer treatment, such as 5-fluorouracil, methotrexate, cisplatin, daunorubicin, and so on. However, as cell-toxic chemicals, cancer treatment drugs cause many adverse reactions such as constipation, nausea, vomiting, hair loss, hematologic changes, etc. (2).

An integral part of the healthcare system is the inherited traditional practice of using plants as a source of herbal medications. There are many different chemicals found in plants that are utilized in traditional medicine to treat chronic and infectious diseases (3). In addition to chemotherapy drugs, herbal medicines also play a significant role in supporting and treating cancer. In other words, a significant source of anti-cancer medications has been found to be plants. The combination of herbal medicines with chemotherapy drugs not only reduces the side effects of chemotherapy drugs but also reduces the drug resistance of cancer cells, thereby increasing the effectiveness of treatment (4). Many medicinal herbs have been shown to have anti-tumor effects, such as garlic (*Allium sativum* L. with allicin and ajoene compounds), wormwood (*Artemisia absinthium* L. with artesunate compound), yew (*Taxus baccata* L. with paclitaxel compound), etc. (5).

Another group of medicinal herbs that are believed to have the potential in supporting and treating various types of cancer are the species belonging to the *Curcuma* genus, with the group of curcumin compounds having anti-cancer effects on leukemia, breast, cervical, and ovarian cancers (5). Many species in the *Curcuma* genus have been studied for their cytotoxic and anti-tumor properties *in vitro* (6), such as *Curcuma amada* Roxb. (MCF-7 and MDA MB 231 breast cancer cell lines) (7), *Curcuma aromatica* Salisb. (HaCaT keratinocyte cells) (8), *Curcuma zedoaria* (Christm.)Roscoe (PC3 prostate cancer cell) (9), *Curcuma aeruginosa* Roxb. (A-549 and HeLa breast cancer cells) (10), *Curcuma comosa* Roxb. (K562 and HL-60 leukaemic cells) (11), etc. Among these species, *Curcuma sahuynhensis* Škorničk. & N.S.Lý is a unique species in Vietnam, known as Nghe sa huynh (Sa huynh turmeric) or Rau Nghe (Vegetable turmeric). *C. sahuynhensis* is a perennial rhizomatous herb. It can grow up to 75±5.0 cm tall and up to 10 per pseudostem of leaves. The inflorescence is composed of 10–24 bracts. The rhizome is cylindrical to oval, light brown from outside. The rhizome has branched and tuberous roots. The flower was yellow with a warm yellow midrib band and an incision up to 0.6 cm long on the apex labellum. Anthers are L-shaped, stalked 1.2 cm long, pale yellow-orange, containing glandular hair, with no knobs below the thecae. The ovary's cross-section has 3 chambers containing many ovules. The fruit is simple capsule style, globose, three-celled, with brown bulges on older fruit, each fruit has about 18 seeds. The seed is irregular oblong ovoid, light brown, and glossy (12). Moreover, *C. sahuynhensis* is used as a spice and vegetable in daily meals (12).

A previous study by Reda *et al*. reported that the result of chemical analysis inferred that there are significant differences between the five *Centaurea* species in essential oil composition depending on the variation of plant species and/or extraction method (13). Similarly, Ch *et al*. conducted the geographical discrimination of rice samples from India, China, and Vietnam by using the GC-MS technique (14). Thus, building chemical composition profiles for plant species is also a method to help compare the identification of plant species with each other. Gas chromatography-mass spectrometry (GC-MS) has solidified its position as a major technical platform for secondary metabolite profiling in both plant and non-plant species in recent years (3). However, there is little research on the chemical composition and pharmacological effects of this medicinal herb, especially its effects on inhibiting the growth of cancer cells. Therefore, the present study's purpose was to identify the chemical profile and cytotoxic activity of the *n*-hexane fraction from *C. sahuynhensis* leaves, the endemic plant of Vietnam.

Materials and Methods

Plant material

Leaves of *Curcuma sahuynhensis* were collected in August, 2022 in Sa Huynh, Duc Pho ward, Quang Ngai Province, Vietnam. This research project was a continuation of a previous study (12). The morphological characteristics of the research sample of *C. sahuynhensis* are shown in Fig. 1.

The plant materials were washed and dried in the shade, then ground into a coarse powder and prepared to obtain the extracts for testing.

Fig. 1. Photographs of the parts of *C. sahuynhensis* Škorničk. & N.S. Lý.

Chemicals and reagents

Chemcials

DMEM (Dulbecco's Modified Eagle Medium), MEME (Minimum Esental Medium with Eagle salt), L-glutamine, penicillin G, streptomycin, TCA (trichloro-acetic 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 ($C_3H_3NaO_3$), 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).

Preparation of plant extract

The plant materials were exhaustedly extracted with ethanol 96% (at a ratio of 1:10) (15). The ethanolic extract was evaporated to obtain the concentrated extract. The *n*-hexane extract, which was prepared by liquid-liquid partitioning with *n*-hexane as the solvent, was used for analyzing chemical composition by gas chromatography-mass spectrometry (GC-MS) and for testing the cytotoxic effect.

Analysis of the volatile compounds

The volatile compounds of *C. sahuynhensis* leaves were analyzed by gas chromatography (Agilent GC-7980) coupled with a mass spectrometry detector (Agilent MS 5977C). The separation was achieved by an HP-5MS UI column (30 m × 0.25 mm × 0.25 µm, Agilent) and Helium as a carrier gas with a flow rate of 1.5 mL/min. The column temperature program started at 80°C (held for 1 min), then increased at a rate of 20°C/min and linearly ramped to 300°C (held for 15 min). The injector, MS Quad, and transfer line temperatures were set at 300, 150, and 300°C, respectively. The MS source was set at 230°C, the ionization voltage was 70 eV, and the mass range was m/z 50–550 amu (2.0 scans/s). 1.0 µL aliquot of the sample which was prepared by precisely dissolving 20 mg of *n*-hexane extract with 1.0 mL of *n*-hexane was injected at a split ratio of 1:25. The volatile components were identified based on a comparison of their mass spectra values with those in the NIST17 database.

In vitro cytotoxic activity assay

MCF-7, SK-LU-1, Hela, and MKN-7 cytotoxicity effects: The cytotoxicity effects of the *n*-hexane extract from *C. sahuynhensis* leaves were conducted according to the protocol of Skehan *et al*. with some minor modifications (16). The MCF-7, SK-LU-1, Hela, and MKN-7 cell lines were cultured in MEME medium supplemented with 2.0 mL L-glutamine, 1 mM sodium pyruvate, penicillin G(100 IU/mL), streptomycin (100 µg/mL), 10% FBS, and incubated at 37°C and 5% CO_2. The experimental cells were trypsinized to detach and were counted in a counting chamber.

The stock solution was prepared by diluting *n*-hexane extract in 100% DMSO to a concentration of 20 mg/mL. This solution was diluted in the cell culture medium without FBS to a concentration range of 500, 100, 20.0, 4.0, and 0.8 µg/mL. A mixture of 10 µL of each sample and 190 µL of cells in a 96-well plate was incubated for 72 hr in a warm incubator. After incubating, the cells were fixed with 20% TCA and stained with 0.2% SRB for 30 min at 37°C, washed 3 times with acetic acid, and then dried at room temperature. 10 mM Tris-base buffer was added to dissolve the SRB. The mixture was gently shaken for 10 min and the optical density (OD) was measured at a wavelength of 540 nm using an ELISA Plate Reader (Biotek, USA). The blank wells were prepared similarly with cancer cells (190 µL) and 1% DMSO (10 µL). After 1 hr, the blank wells were fixed with 20% TCA. Ellipticine was prepared at concentrations of 10, 2.0, 0.4, and 0.08 µg/mL as the positive control.

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

$$\% I = [1- ((OD_{Sp} - OD_{Bl})/(OD_{DMSO} - OD_{Bl}))] \times 100\% \text{.....(Eqn. 1)}$$

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

HL60 cytotoxicity effect: The HL60 cytotoxicity effect of vegetable turmeric leaves *n*-hexane extract was conducted according to the method of Lakshmipriya *et al*. with some minor modifications (17). The HL-60 test cell line was cultured in DMEM with the described above procedure. However, after 72 hr of culturing, 10 µL of MTT (final concentration 500 µg/mL) was added to each well. After 4 hr, the medium was removed and the formazan crystals were dissolved in 50 µL of 100% DMSO. The OD value was measured at a wavelength of 540 nm using a BioTek spectrophotometer (USA).

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

$$\% I = [1- ((OD_{Sp} - OD_{Bl})/(OD_{DMSO} - OD_{Bl}))] \times 100\% \text{....(Eqn. 2)}$$

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

Data analysis

Experimental data was analyzed and recorded. All results were set up in triplicates, presented as the mean value ± standard deviation (S.D), and calculated using Microsoft Excel 2023 software. The IC_{50} value (µg/mL) (i.e., 50% inhibition concentration) was determined using TableCurve 2Dv4 software.

Results

Phytochemical evaluation

As a result in Table 1, fourteen volatile compounds from the *n*-hexane extract of *C. sahuynhensis* leaves were identified by gas chromatography-mass spectrometry (GC-MS). GC-MS analysis was able to identify less-polar/non-polar compounds in the leaf extract.

In total, 14 compounds from leaf extract with content (%) showed compounds such as 6,10,14-trimethyl-pentadecan-2-one (18.39%), phytol (16.97%), 1-ethylbutyl-hydroperoxide (12.92%), isoborneol (8.97%), 1-methyl-pentyl hydroperoxide (7.13%), neophytadiene (4.08%),

endo-borneol (2.98%), intermedeol (2.57%), caryophyllene oxide (2.46%), and γ-elemene (2.31%) are major volatile compounds. Meanwhile, ambrial (1.74%), caryophyllene (1.72%), exo-2-hydroxycineole acetate (1.21%), and alloaromadendrene (1.02%) were also detected with a lower content percentage of 2.0% (Table 1, Fig. 2 and 3).

Cytotoxic effect evaluation

As shown in Table 2, the cytotoxic activity of *C. sahuynhensis* leaf extract and the positive control drug (ellipticine) on the proliferation of cell lines was measured by the IC_{50} value (µg/mL). For the leaf extract acting on five human cancer cell lines (MCF-7, SK-LU-1, Hela, MKN-7, and HL-60), IC_{50} values (µg/mL) ranged from 221.70 ± 10.24 to 369.42 ± 10.60 µg/mL, corresponding to IC_{50} (µg/mL) for human breast carcinoma (221.70 ± 10.24 µg/mL), human lung

Table 1. Volatile constituents of the *n*-hexane extract from *C. sahuynhensis* leaves.

No.	RT (min)	Compound	MF	MW (g/mol)	RI (Exp.)	RI (Lit.)	Id. Method	Content (%)
1	3.664	1-Ethylbutyl hydroperoxide	$C_6H_{14}O_2$	118.17	-	-	MS	12.92
2	3.834	1-Methylpentyl hydroperoxide	$C_6H_{14}O_2$	118.17	-	-	MS	7.13
3	8.973	Isoborneol	$C_{10}H_{18}O$	154.25	1164	1157	MS, RI	8.97
4	9.177	endo-Borneol	$C_{10}H_{18}O$	154.25	1173	1167	MS, RI	2.98
5	12.714	exo-2-Hydroxycineole acetate	$C_{12}H_{20}O_3$	212.28	1345	1344	MS, RI	1.21
6	14.234	Caryophyllene	$C_{15}H_{24}$	204.35	1422	1419	MS, RI	1.72
7	14.418	γ-Elemene	$C_{15}H_{24}$	204.35	1430	1433	MS, RI	2.31
8	15.348	Alloaromadendrene	$C_{15}H_{24}$	204.35	1471	1461	MS, RI	1.02
9	16.868	Caryophyllene oxide	$C_{15}H_{24}O$	220.35	1592	1581	MS, RI	2.46
10	17.914	Intermedeol	$C_{15}H_{26}O$	222.37	1669	1667	MS, RI	2.57
11	19.944	Ambrial	$C_{16}H_{26}O$	234.38	1817	1809	MS, RI	1.74
12	20.249	Neophytadiene	$C_{20}H_{38}$	278.50	1842	1837	MS, RI	4.08
13	20.337	6,10,14-Trimethylpentadecan-2-one	$C_{18}H_{36}O$	268.50	1848	1844	MS, RI	18.39
14	23.576	Phytol	$C_{20}H_{40}O$	296.50	2116	2114	MS, RI	16.97
		Total (%)						**84.47**

Note: MW: Molecular Weight; MF: Molecular Formula; Id. Method: Identification method; RT: Retention time (min); RI (Exp.): Experimental retention indices; RI (Lit.): Retention Indices in literature.

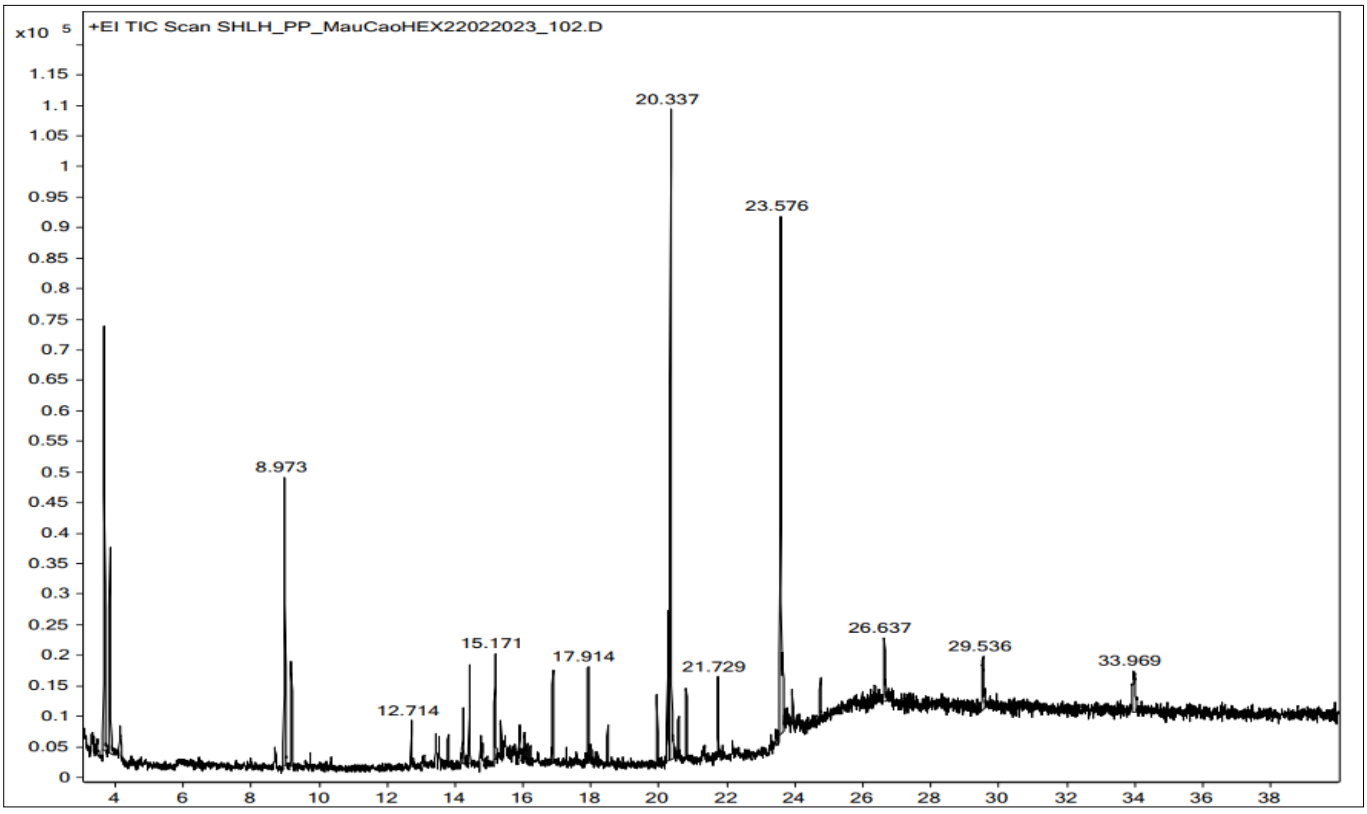

Fig. 2. GC-MS chromatogram of the *n*-hexane extract from *C. sahuynensis* leaves.

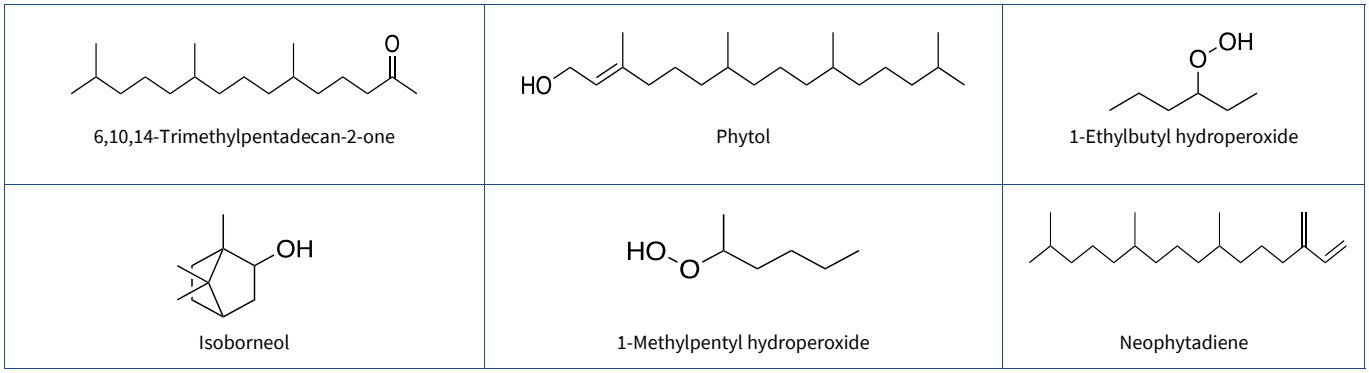

Fig. 3. Chemical structure of the major identified compounds in the *n*-hexane extract from *C. sahuynhensis* leaves.

carcinoma (369.42 ± 10.60 µg/mL), human cervical carcinoma (313.83 ± 9.55 µg/mL), human gastric carcinoma (341.55 ± 12.25 µg/mL), and human acute leukemia (266.43 ± 11.86 µg/mL) (Table 2). Thus, the leaf extract had cytotoxic effects on five human cancer cell lines. The experimental results showed that the IC_{50} value (µg/mL) of leaf extract was the highest for the human lung carcinoma line (SK-LU-1), which means the weakest cytotoxic activity (IC_{50} = 369.42±10.60 µg/mL). Meanwhile, this leaf extract had the strongest cytotoxic effect against the human breast carcinoma line (MCF-7, IC_{50} = 221.70±10.24 µg/mL). In this experiment, ellipticine, the positive control drug, was tested for cytotoxicity with IC_{50} values (µg/mL) ranging from 0.33±0.04 to 0.54±0.01 µg/mL on experimental cancer cell lines.

Table 2. Cytotoxic potential of *C. sahuynhensis* leaf extract and Ellipticine.

	% inhibition of cell growth of leaf extract				
Concentration (µg/mL)	**MCF-7**	**SK-LU-1**	**Hela**	**MKN-7**	**HL-60**
500	98.19±1.34	73.76±1.53	84.41±4.51	79.47±1.37	95.62±2.23
100	28.17±1.75	12.71±0.76	15.20±0.87	13.21±1.22	18.77±1.46
20.0	11.36±0.95	8.42±0.42	5.72±0.39	6.17±0.48	3.79±0.35
4.0	9.55±0.63	5.05±0.50	3.11±0.27	3.54±0.28	2.06±0.27
0.8	4.13±0.15	1.31±0.13	0.74±0.07	1.52±0.12	1.30±0.12
IC_{50}	221.70±10.24	369.42±10.60	313.83±9.55	341.55±12.25	266.43±11.86
Ellipticine (IC_{50})	0.48±0.02	0.54±0.01	0.42±0.04	0.43±0.01	0.33±0.04

Discussion

Natural compounds, particularly those derived from plants, are a secure, efficient, and non-toxic source that can be used as a substitute for chemical medications. Due to the growing demand for plant-based goods, scientists are more interested in finding these products in every part of the plant than just the primary section that is consumed. In the present study, the chemical profile and *in vitro* cytotoxic activity of the *n*-hexane extract of *Curcuma sahuynhensis* leaves were investigated. Using *C. sahuynhensis* for food in the traditional way prompted us to investigate biological effects such as cytotoxic activity, and subsequently, the chemical composition of *C. sahuynhensis* extract was investigated using GC-MS as a first step towards understanding the nature of cytotoxic effects *in vitro*. The volatile substances in *Curcuma* species play an important role. In a previous study, compounds such as cineole, camphor, caryophyllene, humulene, caryophyllene oxide, and humulene epoxide II were found in *C. sahuynhensis* rhizomes essential oil (18). This proves that volatile compounds and fatty acids were present in the *n*-hexane fraction. Additionally, *C. sahuynhensis* essential oil showed good activity against *Enterococcus faecalis* (ATCC 299212), *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 14579), and *Candida albicans* (ATCC 10231) with minimum inhibitory concentrations (MIC) of 64 µg/mL for each organism. However, this essential oil was inactive against *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Salmonella enterica* (ATCC 13076) as previously reported (18).

Chromatography-based untargeted metabolomics studies are quick-to-use and reliable approaches, and they have been used for quality studies on herbal medications recently (19, 20). Among the major phytochemicals detected (Fig. 3), 6,10,14-trimethylpentadecan-2-one (i.e., phytone) was a naturally occurring hydrocarbon. Interestingly, phytone has also been demonstrated to be volatile and is commonly found in some species such as *Hildegardia barteri* (Mast.) Kosterm. (21), *Veronica saturejoides* Vis. (22), *Curcuma longa* L. (23), *Reichardia tingitana* Roth (24), *Vicia ochroleuca* Ten. (25), etc. The literature indicates that phytone has a potent antibacterial effect and broad-spectrum suppression against a variety of fungi strains. It particularly has antioxidant, larvicidal, and anticancer activities (21, 22, 24, 25).

According to the previous studies, phytol shows immunostimulant activity and is safe and cost-effective for anti-schistosomal therapy (26). Based on its biological activities, interestingly, the anti-inflammatory, antioxi-

dant, anticancer, antimicrobial, antiallergic, anti-angio genic, and antiproliferative activities of this compound have been reported (27-31). The earlier report of Pejin *et al*. shows that phytol is cytotoxic against various cancer cell lines (MCF-7, HeLa, HT-29, A-549, Hs294 T, MDA-MB-231, and PC-3 cells) with IC_{50} values (μM) of 8.79±0.41, 15.51±0.76, 34.82±1.66, 56.98±2.68, 65.15±2.91, 69.67±2.99, and 77.85±1.93 μM, respectively (27). Also, available reports have shown the anticancer activities of phytol against Sarcoma (S-180), Human Leukemic (HL-60), and human gastric adenocarcinoma (AGS) cancer cells with IC_{50} values of 18.98±3.79, 1.17±0.34, and 147.67±5.63 μM, respectively (28, 31, 32).

It is known that isoborneol is a monoterpene present in a wide range of essential oils of aromatic plants (28). It has also been reported that at a concentration of 0.06%, isoborneol has strong activities against replication of herpes simplex virus-1 (HSV-1) (33-35).

Interestingly, neophytadiene is a diterpene commonly found in several species such as *Crateva nurvala* Buch.-Ham., *Blumea lacera* (Burm.f.) DC., *Turbinaria ornata* (Turner) J.Agardh, and *Aeschynomene elaphroxylon* (Guill. & Perr.) Taub., etc. This compound has various effects, especially anxiolytic-like activity, sedative properties, antidepressant-like action, anti-inflammatory and anti-cancer activities, etc. (36-38). Furthermore, other compounds detected from *C. sahuynhensis* leaves stand out for their multiple pharmacological properties and for their uses in the pharmaceutical, cosmetics, perfumery, and food industries. These potential candidates have been reported to have biological effects. For example, caryophyllene and caryophyllene oxide have anti-cancer, analgesic, anti-inflammatory, antioxidant (39, 40), and antimicrobial activities (40,41).

1-Ethylbutyl hydroperoxide and 1-methylpentyl hydroperoxide, are the compounds found in *C. sahuynhensis* leaf extract. These were detected at concentrations of 12.92% and 7.13%, respectively, by GC-MS analysis. In previous studies, 1-ethylbutyl hydroperoxide and 1-methylpentyl hydroperoxide compounds were also detected in the crude extract of *Moringa peregrina* Fiori leaves (42), the ethanol extract of *Ipomoea staphylina* leaves (43), and the methanol extracts from *Allium cepa* L. and *Allium sativum* L. wastes (44). To the best of our knowledge, there is no literature on the biological activity of 1-ethylbutyl hydroperoxide and 1-methylpentyl hydroperoxide.

The use of medicinal plants and extracts from them, which are abundant in polyphenolic compounds, may contribute to the explanation of the decline in cancer incidence. In particular, different herbal medicines have been found to have a variety of anticancer effects in numerous clinical trials (24, 45).

In our previous report, the extract of aerial parts of *C. sahuynhensis* contained phytochemicals such as polyphenols and flavonoids (12), which were reported to have potent anti-cancer activity through the regulation of pathways such as differential signaling of cancer cell growth and inhibition as well as the proliferation of oncogenes and tumorigenesis, regulation of enzyme activity, induction of apoptosis, antioxidants, metabolic regulation, immune system stimulation, and DNA repair (46, 47). In addition, polyphenols also have a significant protective role in the body in terms of inflammation, carcinogenesis, thrombosis, atherosclerosis, and antioxidant properties (47).

In this context, the potency of the cytotoxic activity of *C. sahuynhensis* against several cancer cell lines can be regarded as noticeable. The cytotoxic effect of *C. sahuynhensis* leaf extract showed concentration-dependent inhibition of the growth of experimental cancer cell lines. Our results showed that exposure to 100 μg/mL of *C. sahuyhensis* leaf extract inhibited the growth of human cancer cell lines MFC-7, SK-LU-1, Hela, MKN-7, and HL-60, with percentages of inhibition of cell growth being 28.17±1.75%, 12.71±0.76%, 15.20±0.87%, 13.21±1.22%, and 18.77±1.46%, respectively. The IC_{50} value varies according to the test cell line. Specifically, the cytotoxic effect of leaf extract per cell line was clearly expressed against MFC-7 (IC_{50} = 221.70±10.24 μg/mL) and HL-60 (IC_{50} = 266.43±11.86 μg/mL) cell lines (Table 2). This result demonstrates the difference in sensitivity of cancer cell lines to phytochemicals present in *C. sahuynhensis* leaves as well as the different molecular characteristics of these cells (48). Furthermore, the cytotoxicity of the leaf extract against different human cancer cell lines suggests that its use against different types of cancer may be fruitful. However, the IC_{50} value for the test cancer cell lines was found to be very high, which means that the extract did not show strong cytotoxic activity against the tested cancer cells. This may involve the complexity of the chemical composition of the leaf extract from *C. sahuynhensis*. For example, a study by Jambunathan *et al*. indicated that the methanol extract of *Curcuma amada* Roxb. leaves and rhizomes exhibited strong cytotoxicity towards breast cancer MCF-7 and MDA-MB-231 cell lines (7). In another study by Al-Amin *et al*., it was demonstrated that the *n*-hexane extract of *Curcuma caesia* Roxb. rhizomes exhibited MCF-7 cytotoxic effects against was stronger than the *n*-hexane extract of *C. sahuynhensis* leaves with IC_{50} values (μg/mL) of 59.1±0.40 and 221.70±10.24 μg/mL, respectively (49). Based on these reports, it can be hypothesized that different extraction methods and extraction solvents will influence the chemical compositions and cytotoxic effects.

Conclusion

In conclusion, the volatile compositions of the *n*-hexane extract from *Curcuma sahuynhensis* leaves were reported for the first time. The discovery of the anticancer activity of *C. sahuynhensis* leaves against five human cancer cell lines (MCF-7, SK-LU-1, Hela, MKN-7, and HL-60) is the novelty of this study. The presence of bioactive compounds, particularly 6,10,14-trimethylpentadecan-2-one, phytol, 1-ethylbutyl hydroperoxide, isoborneol, 1-methylpentyl hydroperoxide, and neophytadiene, etc., in the extract from *C. sahuynhensis* leaves, may be a source of therapeu-

tic interest for cancer.

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Authors contributions

The research idea was provided by TVC. GC-MS analysis and *in vitro* experiments were carried out by TVC, MNT, NHKL, and NTTN. Analysing, writing, and discussion were done by TVC, MNT, and TTTQ. Reading and revising the manuscript were done by TVC, NHKL, and TTTQ. All authors read and approved the final manuscript.

Compliance with ethical standards

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