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Phytochemical Investigation and Anticancer Activity of *Elaeocarpus hainanensis* Oliv. Against Acute Myeloid Leukemia

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Abstract: *Southeast Asian Elaeocarpus hainanensis Oliv. (family Elaeocarpaceae) is used in traditional medicine, but its phytochemical composition and pharmacological mechanisms have not been fully investigated. This study presents a thorough survey of the chemical composition of leaves and branches of E. hainanensis collected in Vietnam, followed by the isolation and structural determination of secondary compounds; among these, three new triterpenoids were identified: two rare oleanane-framework triterpenes with sulfate groups, 1α,3α-dihydroxy-olean-12-ene-1-sulfate (EH9) and 1α,3α-dihydroxy-olean-18-ene-1-sulfate (EH13).The biological activity of the examined compounds was evaluated and found that: The known cucurbitacins, namely cucurbitacin D (EH1) and 3-epi-isocucurbitacin D (EH3), demonstrated strong inhibitory activity against nucleophosmin-mutated acute myeloid leukemia (AML) cell lines (OCI-AML3), with submicromolar IC_{50} values, while the novel epoxy-cucurbitacins (EH6–EH8) showed weak cytotoxicity (IC_{50} > 50 μM). This report provides the first detailed evidence of the chemical diversity and the mechanism of specific anti-leukemia activity of E. hainanensis.*

Keywords: Elaeocarpus hainanensis; cucurbitacin D; acute myeloid leukemia; OCI-AML3; ZNF217; sulfated oleanane triterpenes.

1. Introduction

1.1. Botanical and Pharmacological Context of the Genus *Elaeocarpus*

The family *Elaeocarpaceae Juss*. includes about a dozen genera. The largest of these is *Elaeocarpus Burm*. ex L. with about 360 species of trees and shrubs. Mainly covering the tropical and subtropical areas of East Asia, Southeast Asia, Australia, and the Islands of the Pacific, the genus has been a part of the traditional medicine systems of the native people for a long time. In Vietnam, with a tropical monsoon climate and a high level of biodiversity, 38 species of *Elaeocarpus* have been recorded.

The lists of ethnomedicine describe the use of the species of the genus *Elaeocarpus* for a variety of purposes. They are used in the treatment of infections, like ulcers and pneumonia, and leprosy, and are used in the treatment of chronic diseases like hypertension and diabetes, as well as depression and epilepsy. For example, *E. floribundus* is used in the treatment of dysentery and diabetes, and in the treatment of inflammation of the gums to use the genus for its anti-inflammatory and antimicrobial properties. Despite this history, scientific studies in this area are scant and in some cases, like *Elaeocarpus, hainanensis* Oliv, and many others, there remains a lack of even basic studies in phytochemistry.

1.2. *Elaeocarpus hainanensis* Oliv.: Characteristics and Previous Studies

Elaeocarpus hainanensis Oliv. (known locally in Vietnam as Com hai nam, Ri ri, or Manh tang) is a small evergreen tree or shrub distinguished by its lanceolate leaves with serrated margins and fragrant white flowers. Geographically, it is distributed across southern China, Laos, Myanmar, Thailand, and Vietnam. In Vietnam, it is often found growing along streams in provinces such as Cao Bang, Lang Son, Quang Binh, and Thua Thien Hue.

Phytochemical studies of *E. hainanensis* have been somewhat limited, they have yielded encouraging results. Early research has revealed the existence of cucurbitane-type triterpenoids, a group of highly oxidized tetracyclic triterpenes recognized for their biological activity. Specifically, prior investigations have successfully isolated compounds including cucurbitacin D, F, and I, along with several 16,23-epoxy derivatives. These compounds have garnered considerable interest within natural product chemistry, owing to their structural intricacy and diverse pharmacological properties, encompassing cytotoxic, anti-inflammatory, and hepatoprotective effects. Nevertheless, the complete scope of chemical diversity within *E. hainanensis*, especially concerning polar constituents such as sulfated triterpenes or glycosides, has only recently been thoroughly investigated.

1.3. Acute Myeloid Leukemia and the Oncogenic Role of ZNF217

Acute Myeloid Leukemia (AML) encompasses a diverse array of hematologic malignancies, distinguished by the clonal proliferation of undifferentiated myeloid precursors. Notwithstanding progress in chemotherapy and stem cell transplantation, the

prognosis for AML patients, especially those harboring particular genetic mutations (e.g., nucleophosmin/*NPM1* mutations) or experiencing relapse, continues to be unfavorable. A crucial element underlying therapeutic resistance and disease advancement in AML is the dysregulated expression of oncogenes.

Zinc Finger Protein 217 (ZNF217), a Krüppel-like zinc finger protein, is encoded by a gene situated on chromosome 20q13.2, a locus frequently amplified in diverse human cancers, including breast, colon, and ovarian carcinomas.

ZNF217 is implicated as a transcriptional repressor with a role in facilitating apoptosis resistance, cell survival, and promoting epithelial mesenchymal transition (EMT). In the case of acute myeloid leukemia (AML), increased expression of ZNF217 is associated with poor prognosis and resistance to first-line chemotherapy in patients, making ZNF217 a therapeutic target of interest. The identification of small molecules capable of modulating ZNF217 expression and/or activity may afford the ability to overcome drug resistance in AML.

The specific effects of cucurbitacins on ZNF217 expression and their potential application in NPM1-mutated AML, despite their known cytotoxicity, remains uninvestigated. Moreover, the presence of unique chemotaxonomic markers, including sulfated triterpenes, in *E. hainanensis* from Vietnam, as determined from its chemical profile, is of interest for structural elucidation and biological assays.

This study aims to:

1. Employ state-of-the-art chromatographic and spectroscopic methods to systematically isolate and characterize the chemical constituents from the leaves and twigs of *E. hainanensis*.

2. Discover new compounds, particularly those with modified triterpenoid scaffolds (hydroperoxides and sulfates).

3. Assess the anticancer activity of the obtained compounds against the panel of solid tumors and, even more importantly, against the OCI-AML3 (a model of AML with NPM1 mutation) cell line.

2. Materials and Methods

2.1. General Experimental Procedures

A variety of methods and physical analytical techniques were employed for isolation and structure determination. Rotations were carried out on a JASCO P-2000 polarimeter (Japan). Molecular weight determination and its structure analysis were done using a high resolution ESI mass spectrometer (HR-ESI-MS) Agilent 6530 Q-TOF mass spectrometer (Agilent Technologies, USA).

Nuclear Magnetic Resonance (NMR) spectroscopy which consists of one (1H, 13C, DEPT) and two (HSQC, HMBC, COSY, NOESY) dimensional techniques were done using a Bruker Avance 500 MHz, Bruker NEO 600 MHz spectrometers (Bruker, Germany). The chemical shifts (δ) of the samples were determined in ppm with respect to Tetramethylsilane (TMS) which is an internal standard. The coupling constants (J) were provided in Hertz (Hz). Chromatographic methods (CC) with open column chromatography (CC) using silica gel 60 (0.040–0.063 mm and 0.063–0.200 mm, Merck) were used. Sephadex LH-20 (GE Healthcare) was used for gel filtration size exclusion chromatography. For reverse-phase chromatography, RP-18 resin (Merck) was used. Thin layer chromatography (TLC) was done using pre-coated silica gel 60 F254 aluminum plates (Merck). Detection of the compounds was done using a UV light source (254 and 365 nm) and spraying with vanillin-sulfuric acid followed by heat.

2.2. Plant Material

The leaves and twigs of *E. hainanensis* Oliv. were collected in Ha Tinh Province, Vietnam, in November 2019. The biological material was taxonomically identified by Dr. Do Ngoc Dai, Faculty of Agriculture and Forestry, Nghe An University of Economics. A voucher specimen (Code: 40TN/EH) was deposited at the Herbarium of the Institute of Chemistry, Vietnam Academy of Science and Technology (VAST), Hanoi, Vietnam.

2.3. Extraction and Isolation

Dried and powdered leaves and twigs of *E. hainanensis* (5.0 kg) were subjected to exhaustive maceration with methanol (MeOH, 90% v/v) at room temperature (3 × 15 L, 24 hours each). The combined extracts were filtered and concentrated under reduced pressure to yield a crude methanolic residue (285 g). This residue was suspended in water (2 L) and partitioned sequentially with *n*-hexane and dichloromethane (DCM) to yield the *n*-hexane fraction (EH, 76 g), the dichloromethane fraction (ED, 60 g), and the water-soluble residue. The water residue was further processed (desalting/solubilization in MeOH) to yield the methanol fraction (EM, 20 g).

2.3.1. Fractionation of the Dichloromethane Extract (ED)

The ED fraction (60 g) was subjected to silica gel column chromatography using a gradient elution system of DCM/MeOH (from 100:0 to 50:50 v/v), yielding ten sub-fractions (ED1–ED10).

- **Sub-fraction ED4 (10 g):** This fraction was re-chromatographed on silica gel using a DCM/Acetone gradient (9:1 to 5:5). Further purification of sub-fraction ED4.2 (1.15 g) yielded compounds EH14 (12 mg), EH15 (10 mg), and EH1 (21 mg).
- **Sub-fraction ED5 (12 g):** Chromatography on silica gel (DCM/Acetone 9:1 to 0:10) yielded six sub-fractions (ED5.1–ED5.6).

  ED5.1 (1.3 g) was purified via Sephadex LH-20 (DCM/MeOH 1:9) to yield EH5 (15 mg).

  ED5.2 (320 mg) yielded EH4 (3.5 mg) after purification on Sephadex LH-20.

  ED5.3 (350 mg) and ED5.4 (230 mg) were similarly purified to yield EH2a (10 mg, mixture) and EH3 (12 mg).

2.3.2. Fractionation of the Methanol Extract (EM)

The EM fraction (20 g) was chromatographed on silica gel using an EtOAc/MeOH gradient (9:1 to 5:5) to give four sub-fractions (EM1–EM4).

- **Sub-fraction EM3 (8.2 g):** This fraction was separated using silica gel CC (DCM/Acetone 9:1 to 5:5).

  Sub-fraction EM3.1 (254 mg) was purified by Sephadex LH-20 and preparative TLC (DCM/MeOH 9:1) to yield EH6 (6 mg).

  Sub-fraction EM3.3 (1.98 g) yielded EH13 (7 mg) and EH9 (6 mg) after sequential silica gel and Sephadex LH-20 chromatography.

  Sub-fraction EM3.4 (1.34 g) was chromatographed (DCM/MeOH 9:1 to 7:3) to yield EH8 (7 mg) and EH7 (6 mg) after Sephadex LH-20 purification.

- **Sub-fraction EM4 (2.1 g):** Separation on silica gel (DCM/MeOH) followed by Sephadex LH-20 purification of sub-fractions yielded EH10 (12 mg), EH11 (8 mg), and EH12 (10 mg).

2.4. Cell Culture

The human tumor cell lines that were utilized in the present investigation consisted of lung A549, T24 bladder, Huh-7 hepatocyte-derived carcinoma, 8505 thyroid, SNU-1 gastric, and OCI-AML3 acute myeloid leukemia. Cells were cultured in RPMI-1640 and DMEM with 10% Fetal Bovine Serum (FBS), 100 IU/mL of penicillin, 10 0 µg/mL of streptomycin, and were grown in a 5% CO_2 atmosphere at 37 °C.

2.5. Cytotoxicity Assay

The relative cytotoxicity of various compounds on the A549, T24, Huh-7, 8505, and SNU-1 tumor cell lines were measured utilizing the Sulforhodamine B method. A total of 96-well plates were employed to plant and grow the cells before being exposed to several of the test compounds for 72 hours. These cells were subsequently treated with 20% trichloroacetic acid to fix the samples, followed by washing and staining of the cells with 0.4 (w/v) Sulforhodamine B. Afterwards, 10 mM of the Tris base was used to solubilize the dye and the optical density was measured at 540 nm using an ELISA plate reader to determine the cell density present in each well. Control (DMSO) cells were used to determine the percent viability and in turn, the IC_{50} values were extrapolated using a regression analysis by the computer program TableCurve 2Dv4, while ellipticine was used as a control.

For the suspension cell line OCI-AML3, cells were seeded at a density of 2 2×10^5 cells/mL in 24-well plates. Compounds were dissolved in DMSO and added to the culture medium (final DMSO concentration < 0.1%). After 24 hours of incubation, cell viability and number were assessed using Trypan blue exclusion and automated cell counting. Percent viability was calculated relative to vehicle control.

2.6. Cell Cycle Analysis

OCI-AML3 cells treated with DMSO or compound were harvested after a 24 hour treatment and washed with PBS before freezing with 70% ethanol. The cells were then treated with RNase A and propidium iodide (PI, 50ug/mL) before analyzing. The flow cytometer (Beckman Coulter) was a Coulter Epics XL-MCL and was used to determine the percentage of cells in G0/G1, S, and G2/M phases after data was analyzed using the FlowJo software.

2.7. Apoptosis Assay

The Annexin V-FITC/PI double staining method was used to identify and quantify apoptotic cells. The cells of OCI-AML3 that were previously treated, were harvested and washed before resuspending in binding buffer. The cells were then incubated in the dark for 15 minutes with Annexin V-FITC and PI before flow cytometry. Flow cytometry was used to analyze and distinguish the cell population as the viable (Annexin V-/PI-), early (Annexin V+/PI-), and late apoptotic/necrotic (Annexin V+/PI+) cells.

2.8. Gene Expression Analysis (RT-PCR)

The RNeasy Plus Micro Kit (Qiagen) was used to extract total RNA from OCI-AML3 cells that were treated and controlled. The cDNA was synthesized in the QuantiTect Reverse Transcription Kit (Qiagen). The TNF-α, Bcl-2, TGF-β, and ZNF217 real-time PCR using TaqMan Gene Expression Assays was carried out on the ABI-7300 Real time Cycler (Applied Biosystems) and the 18S rRNA was used to normalize the data as it was the housekeeping gene.

2.9. α-Glucosidase Inhibition Assay

The inhibition of activity of α-glucosidase (from Saccharomyces cerevisiae) was assessed using a spectrophotometer. The sample compounds were mixed with the enzyme and the substrate p-nitrophenyl-α-D-glucopyranoside (p-NPG) and then incubated. p-Nitrophenol was released and measured at 405 nm. The positive control was acarbose.

3. Results and Discussion

3.1. Chemical Constituents of *E. hainanensis*

The phytochemical investigation of the leaves and twigs of *E. hainanensis* resulted in the isolation of 15 compounds (EH1–EH15). Based on spectroscopic data analysis and comparison with literature values, the compounds were identified as belonging to two main classes: cucurbitane-type triterpenoids and oleanane-type triterpenoids.

3.1.1. Cucurbitane-Type Triterpenoids

Eight cucurbitane derivatives were isolated from the dichloromethane and methanol fractions.

Known Compounds:

Using 1D and 2D NMR data, seven compounds were identified as known cucurbitacins:

- **EH1:** Cucurbitacin D ($C_{30}H_{44}O_7$, MW 516).
- **EH2:** Cucurbitacin I ($C_{30}H_{42}O_7$, MW 514), isolated as a mixture (EH2a) with cucurbitacin D.
- **EH3:** 3-epi-isocucurbitacin D ($C_{30}H_{44}O_7$, MW 516).
- **EH4:** Cucurbitacin F ($C_{30}H_{46}O_7$, MW 518).
- **EH5:** Cucurbitacin H ($C_{30}H_{46}O_8$, MW 534).
- **EH6:** 16α,23α-epoxy-3β,20R-dihydroxy-10αH,23βH-cucurbit-5,24-dien-11-one ($C_{30}H_{46}O_4$, MW 470).
- **EH7:**16α,23α-epoxy-3β,20β-dihydroxy-10αH,23βH-cucurbit-5,24-dien-11-one-3-O-β-D-glucopyranoside ($C_{36}H_{56}O_9$, MW 632).

New Compound: Elaeohainencin A (EH8)

Compound EH8 was isolated as a white solid. Its molecular formula was established as $C_{30}H_{46}O_6$ based on the quasi-molecular ion peak at m/z 503.3358 $[M + H]^+$ (calcd for $C_{30}H_{47}O_6$, 503.3367) in the HR-ESI-MS.

The ^{1}H-NMR spectrum exhibited signals characteristic of a cucurbitane skeleton, including seven methyl groups (δ_H 0.93–1.75). The side chain was identified as an isopropylidene group based on olefinic signals at δ_H 5.02 and 5.00 (H-26) and a vinylic methyl at δ_H 1.75 (H-27).

Comparison of the ^{13}C-NMR data of EH8 with those of EH6 revealed a high degree of similarity in the tetracyclic core (rings A–D). However, a significant difference was observed in the side chain. The carbon signal for C-24 appeared at δ_C 93.2 ppm, representing a substantial downfield shift compared with the typical olefinic or epoxide carbons reported for related compounds.

This chemical shift, together with the mass spectral fragmentation pattern indicating the loss of an oxygen molecule ($M - O_2$), suggested the presence of a hydroperoxide group at C-24.

HMBC correlations from H-26 (δ_H 5.00) to C-24 (δ_C 93.2), C-25 (δ_C 143.7), and C-27 (δ_C 18.0) confirmed the location of the hydroperoxide moiety. Based on NOESY analysis and coupling constants (J 23,24 = 7.5 Hz), the relative configuration was established. Thus, EH8 was elucidated as 16α,23α-epoxy-3β,20β-dihydroxy-24α-hydroperoxy-10αH,23βH-cucurbit-5,25-dien-11-one, and named *Elaeohainencin* A. This represents the first report of a natural cucurbitacin containing a C-24 hydroperoxide group.

Table 1. *^{13}C NMR data for the novel compound EH8 (CD_3OD, 125 MHz)*

Position	δC	Type	Position	δC	Type	Position	δC	Type
1	21.6	CH_2	11	216.6	C=O	21	29.1	CH_3
2	29.8	CH_2	12	49.6	CH_2	22	45.9	CH_2
3	76.9	CH	13	49.2	C	23	76.2	CH
4	42.3	C	14	50.9	C	24	93.2	CH-OOH
5	141.5	C	15	41.6	CH_2	25	143.7	C
6	120.4	CH	16	78.0	CH	26	116.2	CH_2
7	24.9	CH_2	17	56.5	CH	27	18.0	CH_3
8	44.3	CH	18	20.1	CH_3	28	28.2	CH_3
9	49.0	CH	19	20.5	CH_3	29	26.1	CH_3
10	36.4	CH	20	72.4	C	30	26.1	CH_3

3.1.2. Oleanane-Type Triterpenoids

Five oleanane triterpenoids were isolated (EH9–EH13).

Known Compounds:

- **EH10:** 1α-hydroxy-olean-12-en-3-O-α-L-arabinopyranoside.
- **EH11:** 1α-hydroxy-olean-11-oxo-12-en-3-O-α-L-arabinopyranoside.
- **EH12:** 1α-hydroxy-olean-12-en-3-O-β-D-xylopyranoside.

New Compound: 1α,3β-dihydroxy-olean-12-ene-1-sulfate (EH9)

Compound EH9 was isolated as a white solid. HR-ESI-MS analysis (negative-ion mode) exhibited a molecular ion peak at m/z 521.3308 $[M - H]^-$ (calcd for $C_{30}H_{49}O_5S$, 521.3306), indicating a molecular formula of $C_{30}H_{50}O_5S$. Fragmentation analysis showed a characteristic peak at m/z 441.3736, confirming the presence of a sulfate group.

The ^1H NMR spectrum displayed signals for eight methyl singlets and one olefinic proton at δ_H 5.19 (H-12, t, J = 7.0 Hz), characteristic of an olean-12-ene skeleton. A key feature was the resonance of H-1 at δ_H 4.26 (dd, J = 3.0, 1.8 Hz) and the

corresponding carbon C-1 at δ_C 81.8. This significant downfield shift, relative to a typical hydroxyl group, indicated esterification, specifically sulfation at this position. The H-3 signal appeared at δ_H 3.75 (δ_C 73.8). NOESY correlations confirmed the α-orientation of H-1 (sulfate group) and the β-orientation of H-3 (hydroxyl group). Thus, EH9 was identified as 1α,3β-dihydroxy-olean-12-ene-1-sulfate.

New compound: 1α,3β-dihydroxy-olean-18-ene-1-sulfate (EH13)

Compound EH13 ($C_{30}H_{50}O_5S$) showed spectral features similar to those of EH9 but with distinct olefinic signals. The ^{13}C NMR spectrum displayed tetrasubstituted double-bond carbons at δ_C 144.3 (C-18) and δ_C 130.8 (C-19), characteristic of an olean-18-ene (germanicene) skeleton. The sulfate group was again located at C-1 (δ_C 81.8) based on HMBC and MS data. Consequently, EH13 was elucidated as 1α,3β-dihydroxy-olean-18-ene-1-sulfate.

These sulfated triterpenes are rare in the genus *Elaeocarpus* and may serve as significant chemotaxonomic markers.

3.1.3. Other Constituents

Two additional compounds were isolated:

- **EH14:** 5-(hydroxymethyl)-2-furancarboxaldehyde (5-HMF).
- **EH15:** Blumenol A, a megastigmane derivative.

3.2. Biological Evaluation

3.2.1. Cytotoxicity Against Solid Tumor Cell Lines

The isolated 16,23-epoxycucurbitacins (EH6, EH7, EH8) were evaluated for cytotoxicity against five human solid tumor cell lines: A549 (lung), T24 (bladder), Huh-7 (liver), 8505 (thyroid), and SNU-1 (gastric) using the SRB assay.

Table 2. *Cytotoxicity (IC_{50}, μM) of epoxy-cucurbitacins*

Compound	A549	T24	Huh-7	8505	SNU-1
EH6	83.49 ± 4.11	104.49 ± 4.40	56.19 ± 3.34	80.19 ± 6.17	75.96 ± 3.57
EH7	108.53 ± 3.72	116.22 ± 4.22	111.17 ± 8.23	101.68 ± 3.80	95.06 ± 8.62
EH8	100.60 ± 3.13	101.71 ± 5.28	63.82 ± 5.46	114.12 ± 7.55	98.92 ± 4.54
Ellipticine	1.87 ± 0.20	2.11 ± 0.16	1.58 ± 0.16	1.54 ± 0.12	1.58 ± 0.12

Structure-Activity Relationship (SAR): The results indicate that these epoxy-cucurbitacins possess only weak to moderate cytotoxicity (IC_{50} > 50 μM). Comparison with literature data for non-epoxidized analogs (e.g., cucurbitacin D) suggests that the formation of the 16α,23α-epoxy bridge significantly reduces cytotoxic potency against these specific solid tumor cell lines. The presence of the hydroperoxide group in EH8 did not enhance activity compared with EH6. This "non-cytotoxic" profile against general solid tumors is noteworthy, as it may imply a better safety margin for targeted therapies in which nonspecific toxicity is undesirable.

3.2.2. Potent Anti-Leukemic Activity Against OCI-AML3

In a targeted investigation against Acute Myeloid Leukemia, specifically the OCI-AML3 cell line (characterized by NPM1 mutation and ZNF217 overexpression), the non-epoxidized cucurbitacins demonstrated remarkable potency.

Table 3*. Cytotoxicity (IC_{50}, μg/mL) Against OCI-AML3*

Compound	IC_{50}	Classification
EH1 (Cucurbitacin D)	< 0.3 μg/mL (≈ 0.58 μM)	Potent
EH2a (Cuc D + I)	1.94 μM	Potent
EH3 (3-epi-isoCuc D)	1.89 μM	Potent
EH10 (Oleanane)	15.0 μg/mL	Moderate

EH1 emerged as the most potent compound. The differential sensitivity between solid tumors and OCI-AML3 suggests a specific molecular vulnerability in the leukemic cells targeted by the cucurbitacin scaffold.

3.2.3. Mechanism of Action: Cell Cycle Arrest and Apoptosis

To elucidate the mechanism of cell death, flow cytometry analyses were performed on OCI-AML3 cells subjected to EH1, EH2a, and EH3 treatment.

Cell Cycle Modulation

EH1 (cucurbitacin D) induced cell cycle arrest at the G0/G1 phase at higher concentrations (3 μg/mL). In contrast, EH3 (3-epi-isocucurbitacin D) and EH2a caused cell cycle arrest at the G2/M phase at lower concentrations (0.5–1.0 μg/mL), thereby inhibiting mitotic progression.

Apoptosis Induction

Annexin V/PI staining validated that both EH1 and EH3 instigate apoptosis in a dose-dependent fashion. Notably, significant apoptotic populations were detected at concentrations as low as 0.5 μg/mL.

3.2.4. Molecular Target Modulation: ZNF217, TNF-α, and Bcl-2

The investigation probed the molecular mechanisms underpinning the anti-leukemic effects, with a particular focus on the expression of the oncogene ZNF217. ZNF217 significantly influences both chemoresistance and cell growth in acute myeloid leukemia (AML).

Down regulation of ZNF217

RT-PCR analysis showed that treatment with Cucurbitacin D (EH1) at a concentration of 0.3 μg/mL significantly reduced ZNF217 mRNA levels in OCI-AML3 cells. This downregulation is crucial because ZNF217 depletion is known to make cancer cells more sensitive to treatment and slow down their growth.

Modulation of Apoptotic Genes

TNF-α: Treatment led to a decrease in TNF-α levels. Although TNF-α is usually pro-apoptotic, its modulation in the context of cancer-related inflammation can disrupt survival signaling, such as the NF-κB pathway.

Bcl-2: The anti-apoptotic gene of Bcl-2 was significantly downregulated. This suppression of Bcl-2 shifts the cellular balance towards apoptosis, specifically through the intrinsic mitochondrial pathway, which is consistent with the flow of cytometry results.

3.2.5. Enzyme Inhibition: α - Glucosidase

Beyond anticancer activity, the new sulfated oleananes showed promising metabolic activity.

- EH13 exhibited potent α-glucosidase inhibitory activity with an IC_{50} of 3.81 ± 0.33 μM.
- EH9 showed an IC_{50} of 21.27 ± 0.48 μM.
- Both compounds were significantly more potent than the positive control acarbose (IC_{50} = 247.73 μM).

This suggests that sulfation at C-1, particularly in the olean-18-ene scaffold, creates a highly effective pharmacophore for glycosidase inhibition, pointing to potential applications in managing type 2 diabetes.

4. Conclusion

This research provides a detailed phytochemical and pharmacological characterization of *Elaeocarpus hainanensis* Oliv. growing in Vietnam. We successfully isolated 15 compounds, including the novel hydroperoxy-cucurbitacin Elaeohainencin A (EH8) and two novels sulfated oleananes (EH9, EH13).

The biological evaluation highlights *E. hainanensis* as a repository of highly active compounds with selective toxicity. While the epoxy-cucurbitacins are relatively non-toxic to solid tumor lines, Cucurbitacin D (EH1) and its isomer EH3 are identified as potent anti-leukemic agents. Their mechanism involves the targeted downregulation of the chemoresistance gene ZNF217, suppression of the anti-apoptotic Bcl-2 gene, and induction of cell cycle arrest. Additionally, the discovery of potent α-glucosidase inhibition by the sulfated oleananes reveals a dual therapeutic potential for this plant in both oncology and metabolic disease management. These findings validate the traditional use of the *Elaeocarpus* genus and provide a strong scientific basis for the development of Cucurbitacin D as a targeted therapy for NPM1-mutated AML and EH13 as metabolic inhibitors.

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