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BUI THI NHA TRANG

RESEARCH ON CHEMICAL COMPOSITION AND INHIBITION OF NO PRODUCTION, CYTOTOXIC ACTIVITY OF S*UAEDA MARITIMA* (L.) DUMORT AND *DERRIS TRIFOLATA LOUR.*

SUMMARY OF DOCTORAL DISSERTATION IN MATERIALS SCIENCE

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INTRODUCTION

1. Rationale of the dissertation

Vietnam is geographically located in the tropical monsoon climate zone, with a predominantly mountainous terrain that results in distinct differentiation in climate and natural characteristics. These conditions have given rise to diverse ecosystems and rich vegetation, comprising approximately 12,000 species, of which more than one-third are used as medicines in traditional and folk practices.

With the advancement of science and technology, particularly in spectroscopic methods such as nuclear magnetic resonance (NMR), mass spectrometry (MS), and infrared spectroscopy (IR), the past decades have witnessed a growing trend in conducting in-depth research on medicinal plants and animals. The primary aim is to discover natural compounds with significant biological activity for the development of high-value pharmaceuticals and functional foods to improve human health and quality of life.

Suaeda maritima, commonly known as sea-blite, is a small halophytic shrub widely distributed around the world. It naturally grows in moist habitats, along brackish waters, estuarine mudflats, and coastal lagoons. According to the Dictionary of Vietnamese Medicinal Plants, *Suaeda maritima* possesses multiple medicinal uses, including the treatment of hepatitis and allergies, and exhibits laxative and diuretic properties.

Derris trifoliata Lour., locally known as “cóc kèn nước,” is a heliophilous and hygrophilous species that often grows within shrub clusters along brackish or saline canals and streams. Studies on its biological activities have demonstrated that extracts and isolated compounds from *Derris trifoliata* exhibit noteworthy properties such as

cytotoxicity against cancer cells, antibacterial activity, and antioxidant effects. However, in Vietnam, research on the chemical composition and biological activities of *Suaeda maritima* and *Derris trifoliata* remains considerably limited to date.

In order to gain deeper insights into the chemical constituents and nitric oxide (NO) production inhibitory effects of *Suaeda maritima* and *Derris trifoliata* in Vietnam, thereby providing a scientific basis for the sustainable use and development of natural resources, as well as laying the groundwork for subsequent studies in developing formulations for community healthcare and disease prevention, I have selected the research topic: "**Research on chemical composition and inhibition of NO production, cytotoxic activity of S*uaeda maritima* (L.) Dumort and *Derris trifolata* Lour**."

Objectives of the dissertation:

- To identify the major chemical constituents of *Suaeda maritima* (L.) Dumort and *Derris trifoliata* Lour. in Vietnam.

- To evaluate the inhibitory effects on nitric oxide production in RAW264.7 cells and the cytotoxic activity against two human cancer cell lines—lung cancer (SK-LU-1) and liver cancer (HepG2)—of the compounds isolated from these two species.

Scope of the dissertation includes:

1. Isolation of the main compounds from *Suaeda maritima* (L.) Dumort and *Derris trifoliata* Lour. in Vietnam.

2. Structural elucidation of the isolated compounds using modern spectroscopic methods.

3. Evaluation of the inhibitory effects on nitric oxide production in RAW264.7 cells and the cytotoxic activity against two human cancer cell lines—lung cancer (SK-LU-1) and liver cancer (HepG2)—of the

compounds isolated from *Suaeda maritima* (L.) Dumort and *Derris trifoliata* Lour.

CHAPTER 1. LITERATURE REVIEW

This chapter provides an overview of national and international studies on the chemical constituents and biological activities of the genus *Suaeda* and the genus *Derris* in general, with a particular focus on the two species *Suaeda maritima* and *Derris trifoliata*.

1.1. Overview of the genus Suaeda and the species Suaeda maritima

1.1.1. General introduction

The genus *Suaeda* belongs to the order Caryophyllales and the family Amaranthaceae. It comprises more than 100 species distributed across all continents. Research on this genus has mainly focused on species such as *S. fruticosa, S. moquinii, S. salsa, S. vera,* and *S. vermiculata*.

1.1.2. Studies on the chemical constituents of the genus Suaeda

Chemical investigations of this genus have led to the isolation of more than 120 compounds, with the major groups being phenolics, terpenoids, and alkaloids.

+ Phenolic compounds: Phenolics represent the largest group of compounds found in the genus *Suaeda*, with 91 identified constituents belonging to subclasses such as simple phenols and phenolic acids, phenylpropanoids, and flavonoids. The flavonoids are further divided into flavones, flavonols, flavanones, flavanonols, flavanols (catechins), anthocyanins, and chalcones.

+ Alkaloids: To date, 19 alkaloids—most of which are heterocyclic alkaloids—have been isolated from species of the genus *Suaeda*.

+ Other constituents: In addition to the main groups mentioned above, the genus also contains other classes of compounds, such as quinines, steroids, polysaccharides, and fatty acids.

1.1.3. Studies on the Biological Activities of the Genus Suaeda

Research has demonstrated that extracts and phenolic fractions isolated from *Suaeda* exhibit antioxidant activity. Less polar fractions and alkaloid constituents have been associated with antibacterial activity. In addition to anti-inflammatory, hepatoprotective, and cytotoxic properties, several studies have also reported other biological activities of the genus, including hypoglycemic, antiparasitic, analgesic, and thrombolytic effects.

1.1.4. Botanical characteristics of Suaeda maritima

Suaeda maritima (L.) Dumort, also locally known as "phì diệp biển" or "rau nhót biển," is a small halophytic shrub with a wide distribution in mangrove areas across the world, particularly in tropical, equatorial, and subtropical coastal regions. In Vietnam, preliminary surveys have identified the presence of *Suaeda maritima* in provinces such as Thái Bình, Nam Định, Nghệ An, and Hà Tĩnh, where it grows scattered across salt fields, coastal marshes, and shrimp ponds.

Since 1999, eleven studies worldwide have investigated the chemical composition, biological activities, and pharmacological properties of *Suaeda maritima*. Its chemical constituents have been shown to consist mainly of phenolic compounds (phenylpropanoids and flavonoids), along with smaller quantities of other groups such as essential oils and sterols. International studies have primarily focused on isolating additional phenolic compounds and examining their biological activities. However, comprehensive investigations into the chemical constituents and biological activities of the genus *Suaeda*, and *Suaeda maritima* in particular, remain limited compared with its biological potential

1.2. Overview of the genus Derris and the species Derris trifoliata

1.2.1. General introduction

The genus *Derris* comprises approximately 160 climbing species belonging to the family Fabaceae, distributed across tropical and

subtropical regions. In Vietnam, 21 species of *Derris* have been identified, among which *Derris elliptica* (known locally as "dây mật" or "thuốc cá") and *Derris trifoliata* (known as "cóc kèn nước") are the most commonly used.

1.2.2. Studies on the chemical constituents of the genus Derris

Research on the genus *Derris* has revealed around 240 compounds, with the major chemical groups including:

+ Rotenoids: Thirty rotenoids have been identified. Their structural frameworks can be categorized into: (i) those with a double bond between rings B and C, (ii) those with geometric stereoisomers at C-6a and C-12a (cis/trans), and (iii) those with an opened C-ring structure.

+ Flavonoids: This is another major class of compounds in the genus. A total of 56 flavonoids and derivatives, 75 isoflavonoids, 12 chalcones, and 23 pterocarpans have been reported from species such as *D. elliptica, D. eriocarpa, Derris trifoliata, D. scandens,* and *D. laxiflora*.

+ Other constituents: In addition, the genus also contains triterpenoids, coumarins, neolignans, megastigmanes, and phenolic derivatives.

1.2.3. Studies on the biological activities of the genus Derris

The genus *Derris* exhibits a wide spectrum of biological activities, including insecticidal, antioxidant, antibacterial, antifungal, hypoglycemic, and cytotoxic properties. Among these, the most prominent is its cytotoxic activity against various human cancer cell types, such as cholangiocarcinoma, hepatocellular carcinoma, nasopharyngeal carcinoma (KB), breast adenocarcinoma (MCF-7), and lung carcinoma.

1.2.3. Botanical characteristics of Derris trifoliata

Derris trifoliata Lour., locally referred to as "cóc kèn nước," is a heliophilous and hygrophilous plant that typically grows interspersed

among shrubs along brackish or saline canals and streams. Domestic research on the chemical constituents and biological activities of the genus *Derris* in general, and *Derris trifoliata* in particular, remains very limited. Existing studies on *Derris trifoliata* mainly focus on two aspects: its distribution and rotenone extraction.

To date, only 29 compounds from *Derris trifoliata* have been reported in 15 international publications. These include 16 rotenoids, 11 flavonoids and derivatives, and 2 neolignans. Thus, the principal chemical groups of this species are rotenoids and flavonoids, which together account for over 93% of the reported compounds as of 2025.

The notable biological activities of *Derris trifoliata* include cytotoxic activity, potent larvicidal effects, antioxidant capacity, as well as antibacterial and anti-inflammatory properties. These findings open up promising research directions for applications of the genus *Derris* in general, and *Derris trifoliata* in particular, in modern pharmaceutical and medical sciences.

CHAPTER 2. EXPERIMENTAL METHODS AND RESULTS

2.1. Research materials

Samples of *Suaeda maritima* were collected at Xuan Thuy National Park, Nam Dinh Province, in May 2023. The species identification was conducted in May 2024 by Dr. Nguyen The Cuong, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology (VAST). A voucher specimen of *Suaeda maritima* (NCCT-P103) has been deposited at the Institute of Chemistry, VAST.

Samples of *Derris trifoliata* were collected at Xuan Thuy National Park, Nam Dinh Province, in May 2024, and the species was identified by Dr. Nguyen The Cuong, Institute of Ecology and Biological Resources,

VAST. A voucher specimen of *Derris trifoliata* (NCCT-P127) has also been deposited at the Institute of Chemistry, VAST.

2.2. Research Methods

Isolation of compounds: A combination of chromatographic techniques was employed, including thin-layer chromatography (TLC), column chromatography (CC), and high-performance liquid chromatography (HPLC).

Structural elucidation: The general approach involved integrating physicochemical data with modern spectroscopic methods, including high-resolution electrospray ionization mass spectrometry (HR-ESI-MS), nuclear magnetic resonance spectroscopy (NMR), circular dichroism (CD), and optical rotation [α]D[α]D[α]D.

Biological activity evaluation:

+ *Cytotoxic activity:* Cytotoxicity assays against cancer cell lines were performed at the Biological Testing Laboratory, Institute of Biotechnology, VAST. In vitro cytotoxicity was evaluated following the method of Skehan (1990). The IC_{50} value (concentration required to inhibit 50% of cell growth) was determined using TableCurve 2D v4 software.

+ *Inhibition of NO production:* The in vitro inhibitory effect on nitric oxide production was assessed by examining the cytotoxicity of the tested samples on LPS-activated RAW264.7 cells using the MTT method, as described by Tim Mosmann (1983). IC_{50} values were determined by nonlinear regression analysis using a sigmoidal model in GraphPad Prism 8.0, based on percentage inhibition at different concentrations.

Determination of absolute configuration of monosaccharides:

+ The compound was dissolved in 1.0 M HCl prepared in a dioxane/water mixture (1/1, v/v) and heated in a water bath at 80 °C for 3

hours. The resulting acidic solution was neutralized with silver carbonate, and the precipitate was removed by filtration.

+ The solution was evaporated under a nitrogen stream. The residue was dissolved in 1.0 mL of water and extracted with chloroform (three times, 1 mL each). The aqueous layer was collected and further evaporated under nitrogen. The monosaccharide units in the residue were analyzed and compared with standard sugars by thin-layer chromatography, followed by purification using preparative TLC on pre-coated silica gel plates.

+ The optical rotations of the monosaccharides in water were measured and compared with reference data.

2.3. Isolation of Compounds

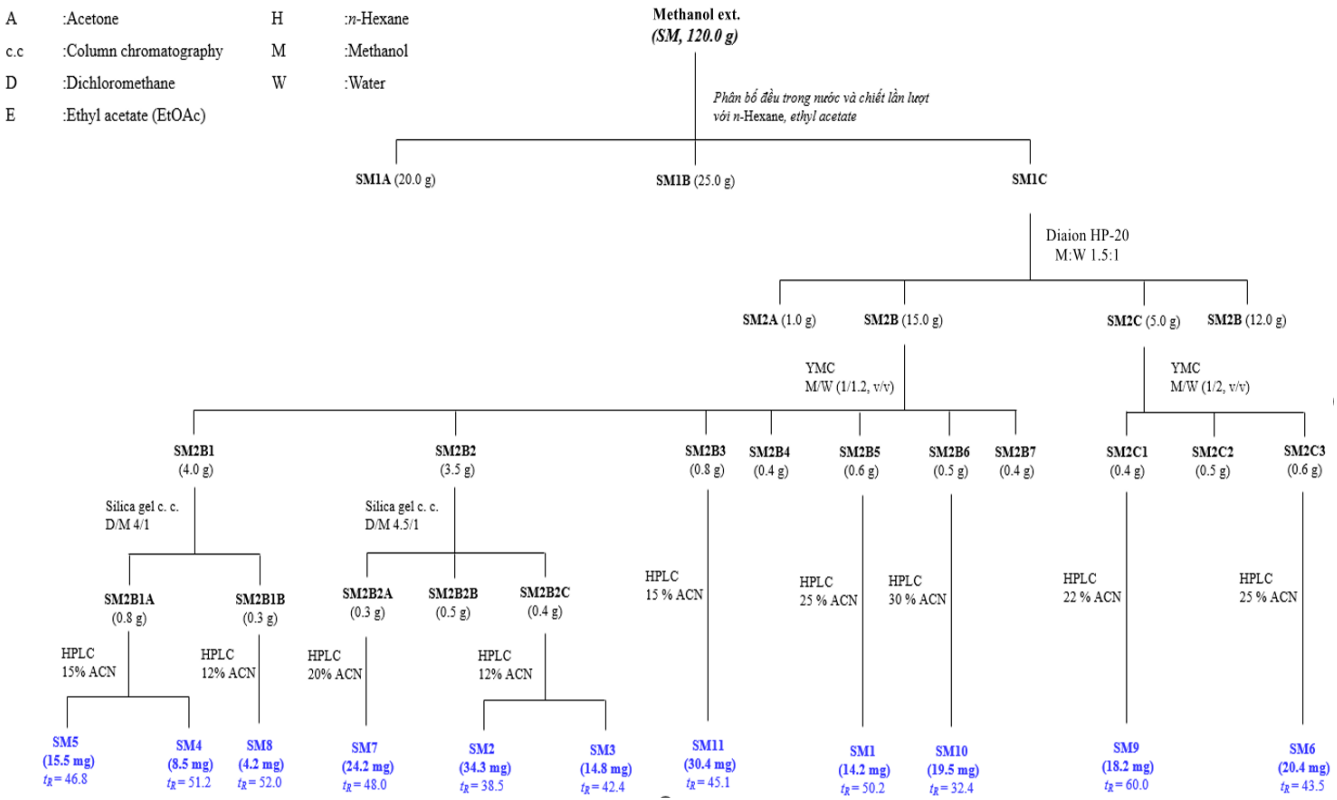

Figure 2.1. Isolation scheme of compounds from Suaeda maritima

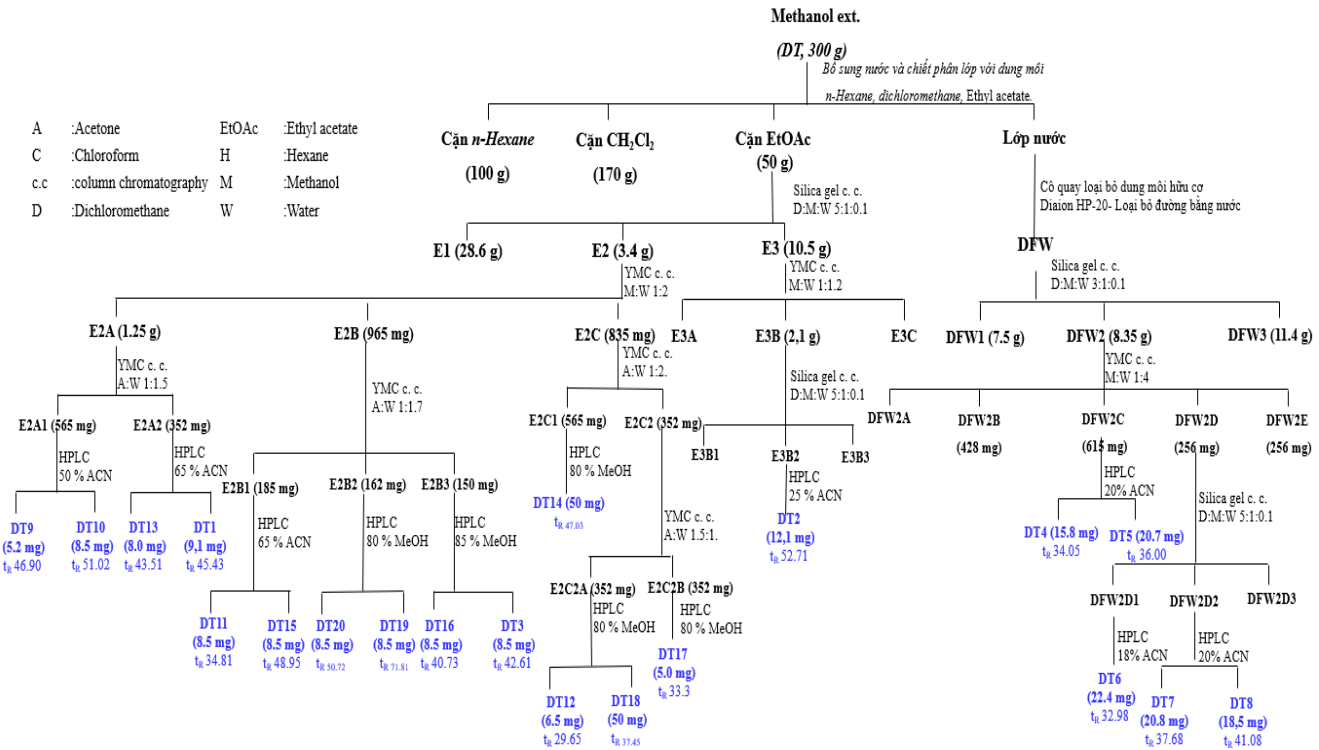

Figure 2.2. Isolation scheme of compounds from Derris trifoliata

2.4. Biological cctivity results of compounds isolated from *Suaeda maritima* and *Derris trifoliata*

2.4.1. Inhibitory effects on no production of compounds isolated from Suaeda maritima

Table 2.1. Inhibitory effects on NO production of compounds isolated from Suaeda maritima

compound	IC_{50} (μM)	compound	IC_{50} (μM)
SM1	66,5±3,1	**SM7**	>100
SM2	29,3±2,7	**SM8**	>100
SM3	67,3±2,8	**SM9**	>100
SM4	85,5±8,2	**SM10**	>100
SM5	65,7±4,0	**SM11**	>100
SM6	63,7±3,3	**Dex**	13,4±1,5

Dex: Dexamethasone was used as a positive control

2.4.2. Inhibitory effects on no production of compounds isolated from Derris trifoliata

Table 2.2. Inhibitory effects on NO production of compounds isolated from Derris trifoliata

compound	IC_{50} (μM)	compound	IC_{50} (μM)
DT1	12,35±0,57	**DT12**	NĐ
DT2	22,06±1,15	**DT13**	11,87±1,02
DT3	>100	**DT14**	26,35±1,71
DT4	>100	**DT15**	25,91±0,96
DT5	>100	**DT16**	>100
DT6	NĐ	**DT17**	11,40±1,03
DT7	>100	**DT18**	18,39±0,95
DT8	>100	**DT19**	NĐ
DT9	NĐ	**DT20**	NĐ
DT10	NĐ	**Dex**	13,4±1,5
DT11	NĐ		

Dex: Dexamethasone was used as a positive control

2.4.3. Cytotoxic activity results of compounds isolated from Suaeda maritima

Table 2.3. Cytotoxic activity of compounds isolated from Suaeda maritima

compound	IC_{50} (μM)	
	SK-LU-1	HepG2
SM1	>100	>100
SM2	>100	>100
SM3	54,29 ± 2,35	58,91 ± 3,23
SM4	68,97 ± 1,37	78,76 ± 2,70
SM5	63,15 ± 3,21	82,93 ± 2,55
Ellipticine	1,87 ± 0,20	1,67 ± 0,20

Ellipticine was used as a positive control

2.4.4. Cytotoxic activity results of compounds isolated from Derris trifoliata

Table 2.4. Cytotoxic activity of compounds isolated from Derris trifoliata

Comp.	IC_{50} (µM)		Comp.	IC_{50} (µM)	
	SK-LU-1	HepG2		SK-LU-1	HepG2
DT1	77,28 ± 6,57	70,88 ± 2,38	DT12	>100	>100
DT2	>100	>100	DT13	28,89 ± 1,70	23,59 ± 1,07
DT3	>100	>100	DT14	>100	>100
DT4	>100	>100	DT15	>100	>100
DT5	>100	>100	DT16	>100	>100
DT6	>100	>100	DT17	>100	>100
DT7	>100	>100	DT18	>100	>100
DT8	>100	>100	DT19	13,71 ± 0,95	10,04 ± 0,52
DT9	20,19 ± 1,98	34,85 ± 2,89	DT20	8,52 ± 0,29	7,11 ± 0,23
DT10	60,24 ± 5,25	40,84 ± 3,01	Ell	1,38 ± 0,12	1,26 ± 0,08
DT11	89,46 ± 7,64	95,90 ± 4,12			

Ell: Ellipticine was used as a positive control

CHAPTER 3. DISCUSSION OF RESULTS

3.1. Compounds isolated from Suaeda maritima

3.1.1 Compounds isolated from Suaeda maritima

From *Suaeda maritima*, a total of 11 compounds (**SM1**-**SM11**) were isolated and structurally elucidated. Among these, four new compounds were designated as *Suaeda*ma A–D (**SM1**-**SM4**). The remaining seven known compounds included: (1,3*S*,8*S*)-trihydroxydec-9-en-4,6-diyne 1-*O*-*β*-D-glucopyranoside (**SM5**), acantrifoside E (**SM6**), methyl chlorogenate (**SM7**), methyl 3,4-di-*O*-caffeoylquinate (**SM8**), methyl 3,5-di-*O*-caffeoylquinate (**SM9**), methyl 4,5-di-*O*-caffeoylquinate (**SM10**) and (7*S*,8*S*)-*threo*-4,9,9′-trihydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan-7-*O*-*β*-D-glucopyranoside (**SM11**).

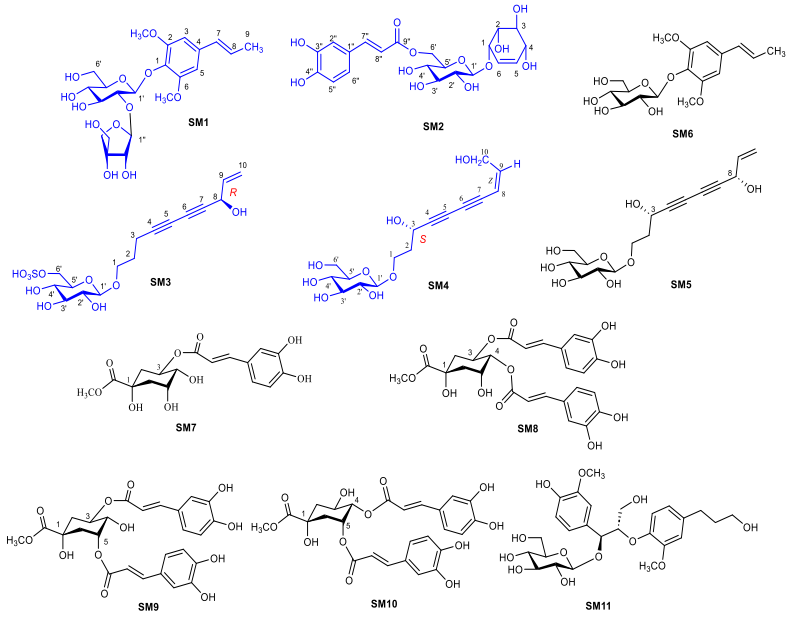

Figure 3.1. Chemical structures of compounds isolated from Suaeda maritima

- Compounds **SM1**, **SM2**, and **SM6–SM11** belong to the phenolic group, including: phenylpropanoid glycosides (**SM1** and **SM6**), a caffeoyl polyhydroxycyclohexen glycoside (**SM2**), methyl caffeoyl quinates (**SM7–SM10**), and a neolignan (**SM11**).

- Compounds **SM3–SM5** are deca-4,6-diyne glucoside derivatives, among which **SM3** and **SM4** are novel compounds, formed through double-bond migration or hydration of a saturated carbon atom on the deca-4,6-diyne backbone. Compound **SM5** is a previously known compound isolated from Artemisia capillaris and reported here for the first time from *Suaeda maritima*.

3.1.2. Compound SM1: Suaedama A (new compound)

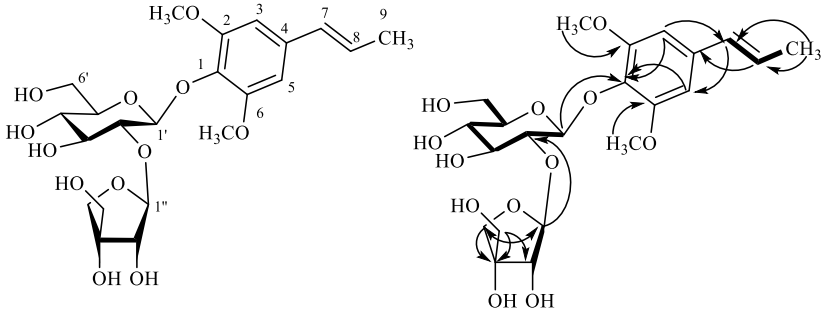

*Figure 3.2. Structure and interactions of compound **SM1***

Compound **SM1** was obtained as a white amorphous powder. The FT-IR spectrum of **SM1** exhibited characteristic absorption bands corresponding to hydroxyl groups, olefinic double bonds, and C–O–C functional groups at 3404, 1586, 1462, and 1070 cm^{-1}, respectively. High-resolution electrospray ionization mass spectrometry (HR-ESI-MS) analysis showed a quasi-molecular ion peak at m/z 511.1779 $[M+Na]^+$ (calculated for $[C_{22}H_{32}O_{12}Na]^+$: 511.1786, Δ = –1.4 ppm), establishing the molecular formula of **SM1** as $C_{22}H_{32}O_{12}$, corresponding to seven degrees of unsaturation.

The ^{1}H-NMR, ^{13}C-NMR, HSQC, and ^{1}H–^{1}H COSY spectra of **SM1** revealed structural fragments, including: a tetrasubstituted symmetrical benzene ring indicated by signals at δC/δH: 134.9 (C-1), 154.5 (C-2, C-6), 105.0 (C-3, C-5)/6.67 (2H, s), and 135.9 (C-4); a trans-configured double bond with a large coupling constant (^{3}J = 16.2 Hz) at δC/δH: 132.1 (C-7)/6.36 (1H, d, J = 16.2 Hz) and 126.2 (C-8)/6.23 (1H, dq, J = 16.2, 7.8 Hz); a secondary methyl group at δC/δH: 18.5 (C-9)/1.88 (3H, d, J = 7.8 Hz); and two methoxy groups at δC/δH: 57.0/3.86 (6H, s). These spectral features indicated that the aglycone structure of **SM1** was 4-[(1*E*)-1-propen-1-yl]-2,6-dimethoxyphenol, analogous to the aglycone of acantrifoside E.

Comparison of the NMR spectra of **SM1** with those of acantrifoside E revealed additional signals attributable to a pentose unit at δC/δH: 110.4 (CH)/5.49, 78.1 (CH)/4.03, 80.9 (C), 75.6 (CH_2)/4.06 and 3.71, and 66.4 (CH_2)/3.73 and 3.62. Notably, the presence of a tertiary carbinol carbon at δC 80.9 and an anomeric carbon at δC 110.4 indicated that this sugar was an apiofuranosyl unit. Moreover, the broad singlet anomeric proton at δH 5.49 (1H, br s) confirmed the β-apiofuranoside configuration.

The HMBC spectrum of **SM1** showed correlations including: H_3-9 to C-7/C-8; H-7 to C-3/C-4/C-5; H-3/H-5 to C-1/C-4/C-7; methoxy protons to C-2/C-3; anomeric proton of glucose H-1′ (δH 5.10) to C-1 (δC 134.9); and anomeric proton of apiose H-1″ (δH 5.49) to C-2′ (δC 78.7). These correlations established that the apiose unit was linked to the acantrifoside E core at C-2′. The downfield shift of C-2′ and the COSY correlation between H-1′ and H-2′ further confirmed this linkage.

Thus, the structure of **SM1** was elucidated as 4-[(1E)-1-propen-1-yl]-2,6-dimethoxyphenol glycosylated with a glucose unit at C-1 (forming the acantrifoside E moiety), and an apiofuranosyl unit linked to glucose at C-2′. The coupling constants of the anomeric protons, J = 7.5 Hz (for glucose, δH 5.10) and $J \approx 0$ Hz (br s, for apiose, δH 5.49), confirmed the β-glycosidic linkages, consistent with previously reported structures such as 4-[(1E)-1-propen-1-yl]phenyl 1-O-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside.

Table 3.1. NMR spectroscopic data of compound SM1

C	[a,b]δ_C	[a,c]δ_H (*J* = Hz)	**C**	[a,b]δ_C	[a,c]δ_H (*J* = Hz)
1	134,9	-	4′	71,4	3,48 (t, 9,0)
2	154,5	-	5′	78,0	3,19 (m)
3	105,0	6,67 (s)	6′	62,6	3,74 (m)
4	135,9	-			3,65 (dd, 12,0, 5,0)
5	105,0	6,67 (s)	2′-*O*-Api		
6	154,5	-	1″	10,4	5,49 (br s)
7	132,1	6,36 (br d, 16,0)	2″	78,1	4,03 (br s)
8	126,2	6,23 (dq, 16,0, 8.0)	3″	80,9	-
9	18,5	1,88 (d, 8.0)	4″	75,6	4,06 (d, 10,0)
1-*O*-Glc					3,71 (d, 10,0)
1′	102,8	5,10 (d, 7,5)	5″	66,4	3,73 (m)
2′	78,7	3,71 (dd, 9,0.7,5)			3,62 (d, 11,0)
3′	78,7	3,56 (t, 9,0)	OCH_3	57,0	3,86 (s)

([a]recorded in CD_3OD; [b]125 MHz; [c]500 MHz)

Compound **SM1** was hydrolyzed under acidic conditions following the procedure described in Section 2.2. The hydrolysis products were identified as:

+ D-glucose (0.9 mg), with $[\alpha]_D^{25}$ = +48.0 (c 0.08, H_2O), compared with the reported value $[\alpha]_D^{25}$ = +44.0.

+ D-apiose (0.6 mg), with $[\alpha]_D^{25}$ = +8.8 (c 0.05, H_2O), compared with the reported value $[\alpha]_D^{25}$ = +6.4.

These results confirm that the sugar units in **SM1** are D-glucose and D-apiose, which is consistent with the NMR spectral data.

Based on the combined evidence from NMR analyses (^1H, ^{13}C, HSQC, COSY, HMBC) and acid hydrolysis, the structure of compound **SM1** was established as 4-[(1*E*)-1-propen-1-yl]-2,6-dimethoxyphenyl 1-*O*-*β*-D-apiofuranosyl-(1→2)-*β*-D-glucopyranoside. A literature survey indicated that this compound has not been previously reported, and it was therefore designated as *Suaeda*ma A.

3.2. Compounds isolated from Derris trifoliata

3.2.1 Compounds isolated from Derris trifoliata

From *Derris trifoliata*, twenty compounds were isolated and their structures elucidated, including eight new compounds: (2*S*)-6-(2-hydroxyethyl)-8-(3-methylbut-2-en-1-yl)-5,7,4'-trihydroxy flavanone(**DT1**), kaempferol 3-*O*-[6-*O*-(*E*)-feruloyl-*β*-D-glucopyranosyl]-(1→2)-*O*-[*α*-L-rhamnopyranosyl (1→6) -*β*-D-glucopyranoside (**DT2**), (+)-13-homo-13-oxa-6a,12a-dehydrorotenone (**DT3**), Derristrifoside A-E (**DT4**-**DT8**); along with twelve known compounds: 6a*α*,12a*α*-12a-hydroxyelliptone (**DT9**), Derrisfolin B (**DT10**), rotenonic acid (**DT11**), *cis*-12a-hydroxyrot-2-enonic acid (**DT12**), (2S)-lonchocarpol D (**DT13**), derriflavanone C (**DT14**), lupinenol (**DT15**), euchrenone B_{10} (**DT16**), 6,8-

diprenylaromadendrin (**DT17**), (-)-8-prenylnaringenin (**DT18**), lonchocarpol C_1 (**DT19**) and lonchocarpol C_2 (**DT20**).

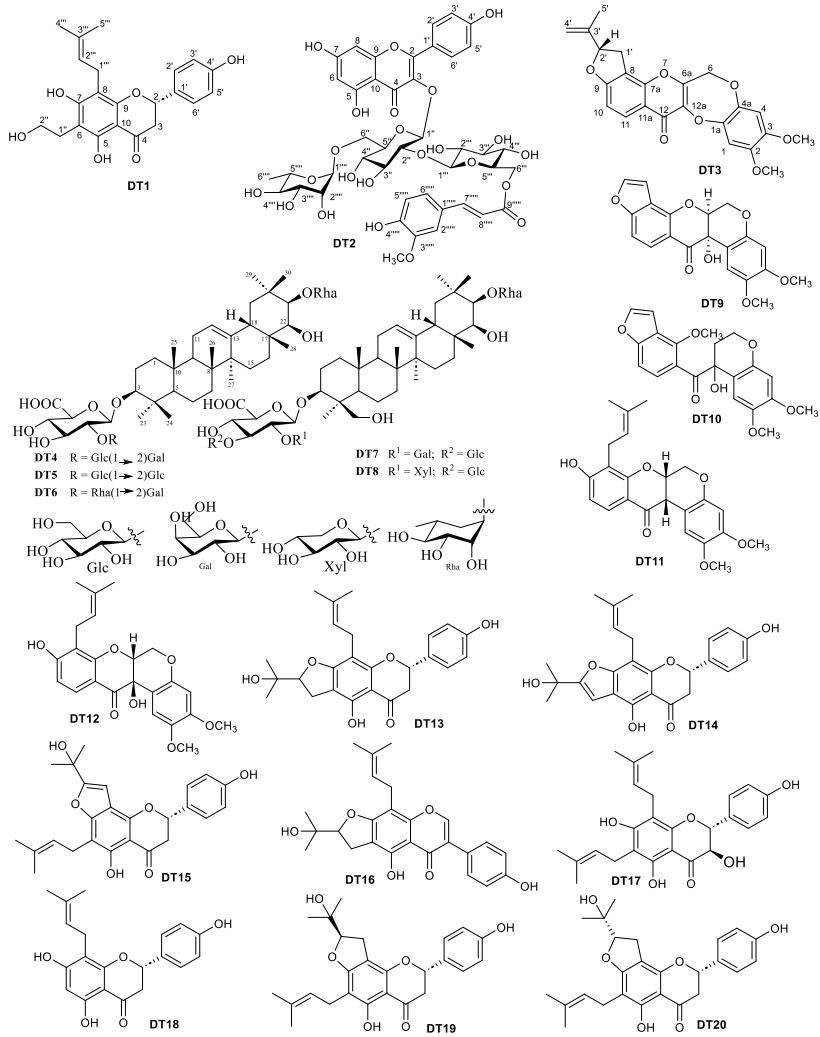

Figure 3.1. Chemical structures of compounds isolated from Derris trifoliata

With respect to their chemical structures, compounds **DT4–DT8** belong to the oleane-type triterpene saponins; compound **DT3** and **DT9–DT12** are rotenoids; while compounds **DT1**, **DT2**, and **DT13–DT20** are flavonoids. These compound classes are characteristic constituents of the genus *Derris*..

3.2.2. Compound DT1: (new compound)

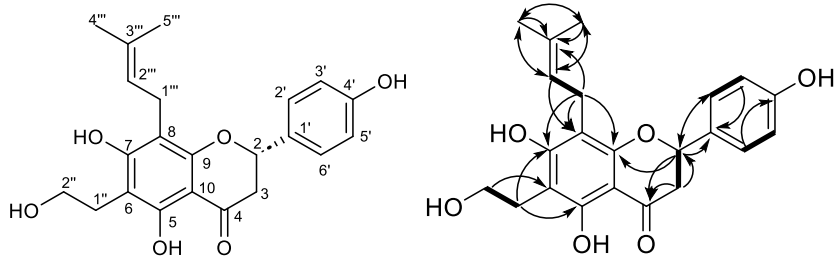

*Figure 3.4. Chemical structure and key HMBC (H→C) and COSY (H–H) correlations of compound **DT1***

Compound **DT1** was obtained as a pale yellow amorphous powder. The FT-IR spectrum of **DT1** exhibited characteristic absorption bands corresponding to hydroxyl, ketone, olefinic, and C–O–C functional groups at 3227, 1635, 1467, and 1047 cm^{-1}, respectively. High-resolution mass spectrometry (HR-ESI-MS) of **DT1** revealed a pseudomolecular ion peak at m/z 385.1633 $[M+H]^+$ (calculated for $[C_{22}H_{25}O_6]^+$, 385.1646; Δ = –3.4 ppm), which established its molecular formula as $C_{22}H_{24}O_6$, corresponding to 11 degrees of unsaturation.

The ^{1}H-NMR spectrum of **DT1** indicated the presence of a para-substituted aromatic ring [δH 7.31 (2H, d, J = 8.4 Hz) and 6.83 (2H, d, J = 8.4 Hz)], an isoprenyl group [δH 3.23 (2H, d, J = 6.6 Hz, H-1‴″), 5.15 (1H, t, J = 6.6 Hz, H-2‴″), 1.61 and 1.59 (each 3H, s)], and a hydroxyethyl group [δH 2.86 (2H, t, J = 6.6 Hz, H-1‴) and

3.74 (2H, t, J = 6.6 Hz, H-2‴)]. In addition, the signals of an sp^3 methylene group [δH 2.70 (dd, J = 16.8, 12.6 Hz, H-3ax) and 3.05 (dd, J = 16.8, 3.0 Hz, H-3eq)] and an sp^3 carbinol methine proton at δH 5.27 (dd, J = 12.6, 3.0 Hz, H-2) suggested a flavanone skeleton.

The ^{13}C-NMR and HSQC spectra of **DT1** further confirmed the structural fragments, including a flavanone core (15C) bearing an isoprenyl group [δC 22.9 (CH_2, C-1⁗), 124.1 (CH, C-2⁗), 131.6 (C, C-3⁗), 25.9 (CH_3, C-4⁗), 17.9 (CH_3, C-5⁗)], and a hydroxyethyl group [δC 26.4 (CH_2, C-1‴) and 63.4 (CH_2, C-2‴)]. This structure represents a prenylated flavanone framework bearing a hydroxyethyl substituent (laxiflorin type), which has previously been reported from *Derris* laxiflora

Table 3.2. NMR spectroscopic data of compound DT1

C	[a,b]δ_C	[a,c]δ_H (J = **Hz**)	**C**	[a,b]δ_C	[a,c]δ_H (J = **Hz**)
2	80,1	5,27 (dd, 12,6, 3,0)	3′	116,3	6,83 (d, 8,4)
3	44,0	2,70 (dd, 16,8,12,6)	4′	158,8	-
		3,05 (dd, 16,8, 3,0)	5′	116,3	6,83 (d, 8,4)
4	198,2	-	6′	128,9	7,31 (d, 8,4)
5	160,6	-	1″	26,4	2,86 (t, 6,0)
6	107,7	-	2″	63,4	3,74 (t, 6,0)
7	165,3	-	1‴	22,9	3,23 (d, 6,6)
8	109,5	-	2‴	124,1	5,15 (t, 6,6)
9	159,9	-	3‴	131,6	-
10	103,2	-	4‴	25,9	1,62 (s)
1′	131,5	-	5‴	17,9	1,59 (s)
2′	128,9	7,31 (d, 8,4)			

([a]recorded in CD_3OD; [b]125 MHz; [c]500 MHz)

The NMR signals of **DT1**, in combination with ^{1}H–^{1}H COSY and HMBC spectra, revealed that the data of **DT1** were comparable to those of lonchocarpol A, except for the additional signals of a hydroxyethyl group at C-6. This assignment was further confirmed by HMBC correlations from H-2″ (δH 3.74) to C-1″ (δC 26.4)/C-6 (δC 107.7) and from H-1″ (δH 2.86) to C-5 (δC 160.6)/C-6 (δC 107.7)/C-7 (δC 165.3). The isoprenyl group attached to C-8 was established by HMBC correlations from H-1‴ (δH 3.23) to C-7 (δC 165.3)/C-8 (δC 109.5)/C-9 (δC 159.9). The absolute configuration of **DT1** was determined by ECD spectroscopy, which exhibited a negative Cotton effect at 293 nm, thereby confirming the 2S configuration. Consequently, **DT1** was identified as a new compound, (2*S*)-6-(2-hydroxyethyl)-8-(3-methylbut-2-en-1-yl)-5,7,4′-trihydroxyflavanone, with the trivial name demethoxy laxiflorin.

3.3. Biological activities of the compounds

3.3.1 Biological activities of compounds isolated from Suaeda maritima

The evaluation of NO inhibitory activity of 11 compounds (**SM1**–**SM11**) isolated from *S. maritima* showed that, at 100 μM, these compounds did not exhibit statistically significant cytotoxicity (cell viability ranging from 79.22% to 94.44%). Therefore, the observed inhibitory effects on NO production were not attributed to compound-induced cell death. At the same concentration (100 μM), **SM1**–**SM11** inhibited NO production with inhibition rates ranging from 37.25% to 77.02%. Among them, compounds **SM1**–**SM6** displayed the strongest NO inhibitory effects, with inhibition rates above 50% (52.79–

77.02%). These compounds were thus selected for further dose-dependent assays to determine IC_{50} values.

The results demonstrated that **SM1**–**SM6** inhibited NO production with IC_{50} values of 66.5 μM, 29.3 μM, 85.5 μM, 65.7 μM, 63.7 μM, and 67.3 μM, respectively, compared with the positive control dexamethasone (IC_{50} = 13.4 μM). These findings indicate that phenylpropanoid glycosides (**SM1** and **SM6**), a polyhydroxycyclohexene glycoside derivative (**SM2**), and acetylenic glucosides (**SM3–SM5**) are responsible for the NO inhibitory activity observed in *S. maritima*.

Compounds **SM1**–**SM5** were further evaluated for cytotoxic activity against two cancer cell lines, SK-LU-1 and HepG2. Compounds **SM1** and **SM2** did not exhibit cytotoxic effects (IC_{50} > 100 μM), whereas **SM3**–**SM5** showed cytotoxicity against both tested cell lines with IC_{50} values ranging from 54.29 to 82.93 μM

3.3.2. Biological activities of compounds isolated from Derris trifoliata

The evaluation of NO inhibitory activity of 20 compounds isolated from *Derris trifoliata* revealed that **DT2–DT5**, **DT7**, **DT8**, and **DT14–DT18** (100 μM) did not induce statistically significant cytotoxicity (cell viability ranging from 86.37% to 99.75%). Therefore, the assessment of NO inhibition at this concentration or lower concentrations (<100 μM) was not affected by compound-induced cell death. In contrast, **DT1**, **DT6**, **DT9–DT13**, **DT19**, and **DT20** exhibited cytotoxicity against RAW264.7 cells at 100 μM. These compounds were further evaluated for their effects on RAW264.7 cell viability at lower concentrations (20, 4, 0.8, 0.16, and 0.032 μM) to determine their potential NO inhibitory activity under

non-cytotoxic conditions. However, except for **DT1** and **DT13**, the compounds **DT6**, **DT9–DT12**, **DT19**, and **DT20** did not show significant NO inhibition (>50%) when tested at concentrations that were non-cytotoxic to RAW264.7 cells.

Compounds **DT1–DT5**, **DT7**, **DT8**, and **DT13–DT18**, which were non-cytotoxic at the highest tested concentrations (100 μM or 20 μM), exhibited NO inhibitory activity with inhibition rates ranging from 23.02% to 98.99%. Among these, **DT1**, **DT2**, **DT13–DT15**, **DT17**, and **DT18** showed inhibition rates above 50% and were therefore subjected to further dose-dependent assays to determine their IC_{50} values. The results demonstrated that **DT1**, **DT2**, **DT13–DT15**, **DT17**, and **DT18** inhibited NO production with IC_{50} values ranging from 11.40 to 26.35 μM. These findings indicate that prenylated flavonoids represent the main contributors to the NO inhibitory activity of *Derris trifoliata*. Notably, **DT1**, **DT13**, and **DT17** exhibited stronger activity than the positive control dexamethasone.

All 20 compounds (**DT1–DT20**) were screened for cytotoxic effects against SK-LU-1 and HepG2 cancer cell lines. The results showed that **DT9**, **DT13**, **DT19**, and **DT20** exhibited cytotoxicity against both cell lines with IC_{50} values ranging from 7.11 to 34.85 μM. Compounds **DT1**, **DT10**, and **DT11** demonstrated weak cytotoxicity, with IC_{50} values between 60.24 and 95.90 μM.

CONCLUSIONS

By combining chromatographic techniques with modern spectroscopic methods, a total of 31 compounds were isolated and structurally elucidated: 11 compounds from *Suaeda maritima* and 20 compounds from *Derris trifoliata*, of which 12 were new.

1. Findings on chemical constituents

From *Suaeda maritima*, 11 compounds (**SM1**–**SM11**) were isolated and identified, including 4 new compounds, sueadama A–D (**SM1**–**SM4**), and 7 known compounds.

From *Derris trifoliata*, 20 compounds (**DT1–DT20**) were isolated and identified, including 8 new compounds: (2*S*)-6-(2-hydroxyethyl)-8-(3-methylbut-2-en-1-yl)-5,7,4'-trihydroxyflavanone (**DT1**), kaempferol 3-*O*-[6-*O*-(*E*)-feruloyl-*β*-D-glucopyranosyl]-(1→2)-*O*-[*α*-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside(**DT2**), (+)-13-homo-13-oxa-6a,12a-dehydro- rotenone (**DT3**), derristrifoside A-E (**DT4**-**DT8**), together with 12 known compounds.

2. Findings on biological activities

Compounds **SM1**–**SM6** inhibited NO production with IC_{50} values ranging from 29.3 to 85.5 μM.

Compounds **SM3–SM5** exhibited cytotoxicity against both SK-LU-1 and HepG2 cell lines with IC_{50} values ranging from 54.29 to 82.93 μM, whereas the remaining compounds were inactive.

Compounds **DT1**, **DT2**, **DT13–DT15**, **DT17**, and **DT18** inhibited NO production with IC_{50} values of 11.40–26.35 μM. Among these, **DT1**, **DT13**, and **DT17** displayed stronger activity than the positive control dexamethasone (IC_{50} = 13.4 μM).

Compounds **DT9**, **DT13**, **DT19**, and **DT20** exhibited cytotoxicity against both SK-LU-1 and HepG2 cell lines, with IC_{50} values ranging from 7.11 to 34.85 μM. Compounds **DT1**, **DT10**, and **DT11** showed weak cytotoxicity (IC_{50} = 60.24–95.90 μM), while the others were inactive (IC_{50} > 100 μM).

RECOMMENDATIONS

Further studies are needed to investigate the anti-inflammatory and anticancer effects of the bioactive compounds isolated from *S. maritima* and *Derris trifoliata*, including evaluations on other inflammatory mediators (TNF-α, interleukins, etc.), as well as studies on their anti-inflammatory mechanisms and cytotoxic mechanisms.

NOVEL CONTRIBUTIONS OF THE DISSERTATION

Four new compounds were isolated and identified from S. maritima: sueadama A–D (**SM1**–**SM4**). In addition, compounds **SM7–SM11** were reported for the first time from this species.

Eight new compounds were isolated and identified from *Derris trifoliata*: (2*S*)-6-(2-hydroxyethyl)-8-(3-methylbut-2-en-1-yl)-5,7,4'-trihydroxyflavanone (**DT1**), kaempferol 3-*O*-[6-*O*-(*E*)-feruloyl-*β*-D-glucopyranosyl]-(1→2)-*O*-[*α*-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside(**DT2**), (+)-13-homo-13-oxa-6a,12a-dehydro- rotenone (**DT3**), derristrifoside A-E (**DT4-DT8**).

The biological activities of 12 new compounds (**SM1**–**SM4**, **DT1–DT8**) were reported for the first time, including their NO inhibitory effects (**SM1**–**SM4**, **DT1–DT8**) and cytotoxic activities against lung cancer (SK-LU-1) and liver cancer (HepG2) cell lines (**SM3–SM5**, **DT1–DT8**).

LIST OF PUBLISHED PAPERS RELATED TO THE DISSERTATION

1. **Bui Thi Nha Trang**, Bui Thi Mai Anh, Nguyen Thi Mai, Nguyen Thi Cuc, Le Thi Huyen, Nhu Thi Hang Nga, Phan Van Kiem, and Bui Huu Tai. "*Suaedamas A-D: Four new compounds from the aerial parts of Suaeda maritima with inhibition of NO production activity*". Chemistry and Biodiversity, 2024, 21, e202401787.
2. **Bui Thi Nha Trang**, Bui Thi Mai Anh, Nguyen Thi Mai, Tran Thuy Nga, Dan Thi Thuy Hang, Bui Huu Tai, Phan Van Kiem. "*Discovery of undescribed flavonoid and rotenoid derivatives from the leaves of Derris trifoliata with their cytotoxic activity*". Chemistry and Biodiversity, 2025, e202500006.
3. **Bui Thi Nha Trang**, Nguyen Thi Mai, Bui Thi Mai Anh, Duong Thi Dung, Bui Huu Tai, Phan Van Kiem. "*The genus Derris Lour., a potential source of valuable biologically active ingredients*". Vietnam Jounal of Chemistry, 2024, vjch.202400124.