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CHEMICAL CONSTITUENTS AND *IN VITRO* ANTI-INFLAMMATORY ACTIVITY OF *Aglaia odorata* AND *Aphanamixis polystachya* (Meliaceae)

SUMMARY OF DISSERTATION ON SCIENCE OF MATTER

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INTRODUCTION

The urgency of the thesis

In recent decades, the demand for biologically active compounds derived from natural sources has steadily increased, particularly in the context of complex diseases such as cancer, chronic inflammation, and antibiotic resistance, which pose major challenges to modern medicine. According to the World Health Organization (WHO), up to 80% of the population in developing countries still relies on natural products for primary healthcare. Moreover, more than 60% of currently used anticancer drugs are either directly derived from natural sources or developed based on natural structural scaffolds.

Vietnam ranks among the 16 most biodiverse countries in the world, hosting approximately 12,000 species of vascular plants, many of which are medicinal and possess significant yet underexplored potential. The family Meliaceae comprises about 50 genera and 600 species globally, with more than 40 species recorded in Vietnam, primarily distributed in tropical forests. Members of this family are notable for their richness in secondary metabolites, particularly triterpenoids, limonoids, and flavonoids, which exhibit potent biological activities, including anticancer, anti-inflammatory, antibacterial, and antioxidant effects.

The genera *Aglaia* and *Aphanamixis*, both belonging to the family Meliaceae, have long been used in traditional medicine in Vietnam and neighboring countries such as China and Indonesia. The genus *Aglaia* comprises more than 120 species, mainly distributed throughout Southeast Asia, and is a rich source of flavaglines—compounds with distinctive chemical structures and diverse biological activities. Although *Aphanamixis* includes fewer species, it contains rare limonoids and triterpenoids with considerable biological potential.

To date, research on the chemical constituents and biological activities of these two genera in Vietnam remains fragmented and unsystematic. Therefore, in-depth studies of *Aglaia* and *Aphanamixis* species are essential not only to expand the repository of natural compounds with promising biological activities but also to support the development of novel pharmaceuticals.

In this context, the present study, entitled "Chemical constituents and *in vitro* anti-inflammatory activity of *Aglaia odorata* and *Aphanamixis polystachya* (Meliaceae)", is proposed to enhance scientific understanding of the chemical composition and biological activities of these two promising plant species, thereby contributing to research on natural product–based drug development in Vietnam.

Objectives of the dissertation:

– To elucidate the major chemical constituents and determine the absolute configurations of compounds isolated from two species, *Aglaia odorata* and *Aphanamixis polystachya*, collected in Vietnam.

– To evaluate the *in vitro* nitric oxide (NO) inhibitory activity of the isolated compounds in RAW264.7 cells and to assess their anti-inflammatory effects by examining their ability to inhibit the production of inflammatory mediators, including iNOS, IL-6, and TNF-α, in selected promising compounds.

Contents of the dissertation:

– Isolation of the compounds from *Aglaia odorata* and *Aphanamixis polystachya* collected in Vietnam.

– Determination of the chemical structures of the isolated compounds using modern physical methods.

– Evaluation of the *in vitro* inhibitory effects of the isolated compounds on nitric oxide (NO) production and on the production of inflammatory mediators, including IL-6, TNF-α, and iNOS, in relevant cellular models.

CHAPTER 1. OVERVIEW

This chapter provides an overview of the chemical components and biological activity of species belonging to the genera *Aglaia* and *Aphanamixis*.

1.1. Overview of the genus *Aglaia*

1.1.1. General introduction to the genus Aglaia

The genus *Aglaia* is one of the largest genera within the family Meliaceae (mahogany family), order Sapindales, class Magnoliopsida, comprising more than 120 described species. Members of this genus are predominantly distributed in tropical regions, particularly across Southeast Asia, South Asia, the Pacific Islands, and Australia. *Aglaia* exhibits especially high species diversity in Malaysia, Indonesia, Thailand, Vietnam, and India, with eight species also recorded in southern China. Of the more than 120 known species, approximately 65 are considered endemic to Indonesia.

1.1.2. Chemical constituents of the genus Aglaia

Extensive and diverse phytochemical investigations of the genus *Aglaia* have revealed several classes of secondary metabolites, including flavaglines, triterpenoids, sesquiterpenoids, steroids, alkaloids, and limonoids. Notably, flavaglines−structurally characterized as derivatives formed through the fusion of flavonoid units with cinnamic acid moieties−represent a unique class of compounds found exclusively in *Aglaia* species. Owing to their chemical distinctiveness and taxonomic specificity, flavaglines are regarded as important chemotaxonomic markers for species identification within the genus.

1.1.3. Biological activities of the genus Aglaia

Many species of *Aglaia* have long been utilized in traditional medicine. Extracts and isolated compounds from *Aglaia* have demonstrated a wide

range of biological activities, including cytotoxic, anti-inflammatory, insecticidal, antifungal, antitubercular, and antiviral effects.

1.2. Overview of the genus *Aphanamixis*

1.2.1. General introduction to the genus Aphanamixis

The genus *Aphanamixis*, belonging to the family Meliaceae, order Sapindales, and class Magnoliopsida, comprises approximately 25 species, which are predominantly distributed in tropical forests. Species of this genus are mainly found in India, Thailand, Malaysia, Indonesia, China, and Vietnam. Among them, *Aphanamixis polystachya* and *Aphanamixis grandifolia* are the most widely recognized and extensively studied.

1.2.2. Chemical constituents of the genus Aphanamixis

Compounds isolated from the genus *Aphanamixis* include several major classes, such as limonoids (triterpenoids), sesquiterpenoids, diterpenoids, sterols, alkaloids, and other minor groups. Limonoids represent the most characteristic and abundant class within the genus. These compounds contain 26 carbon atoms and belong to the group of modified triterpenoids derived from the tirucallane (C_{30}) skeleton, which is either incorporated into or rearranged from the 4,4,8-trimethyl-17-furanyl steroidal core. Limonoids found in *Aphanamixis* species exhibit remarkable structural diversity and complexity, resulting from extensive oxidation and rearrangements of their original carbon frameworks.

1.2.3. Biological activities of the genus Aphanamixis

Both isolated compounds and crude extracts from *Aphanamixis* species have demonstrated a wide range of biological activities, notably cytotoxic, anti-inflammatory, insecticidal, antibacterial, and antifungal properties.

CHAPTER 2. EXPERIMENT AND RESULTS

2.1. Materials

The species *Aglaia odorata* Lour. was collected from the primary forest of Ru Linh, Quang Tri Province, in September 2022. The scientific name of the specimen was authenticated by Dr. Le Tuan Anh, Vietnam National Museum of Nature, Vietnam Academy of Science and Technology (VAST). A voucher specimen (NCCT-P145) has been deposited at the Institute of Chemistry, VAST.

The species *Aphanamixis polystachya* (Wall.) R. Parker was collected from Tam Dao National Park, Phu Tho Province, in September 2022. The scientific name of the specimen was identified by Dr. Nguyen The Cuong, Institute of Biology, Vietnam Academy of Science and Technology (VAST). A voucher specimen (NCCT-P125) has been preserved at the Institute of Chemistry, VAST.

2.2. Methods

Methods for isolation of secondary metabolites

Combining several chromatographic methods including thin-layer chromatography (TLC), column chromatography (CC), silica gel, RP-18, Sephadex LH-20 and high-performance liquid chromatography (HPLC).

Methods for determination of chemical structure of compounds

The general method used to determine the chemical structure of compounds is the combination between physical parameters and modern spectroscopic including high-resolution electrospray ionization mass spectrometry (HR-ESI-MS), one/two-dimension nuclear magnetic resonance (NMR) spectroscopy, electronic circular dichroism (ECD) and optical rotation.

Theoretical ECD calculation method

Conformational searches were performed using Spartan 18. Conformers with Boltzmann populations greater than 1% were further optimized and subjected to time-dependent density functional theory (TD-DFT) calculations to obtain their ECD spectra using Gaussian 16 at the B3LYP/6-31G(d,p) level. The calculated ECD spectra of all conformers were then Boltzmann-averaged using SpecDis v1.71 to generate the theoretical ECD spectrum for each compound.

Theoretical ^{13}C NMR calculation method

Conformational searches were conducted using Spartan 18. All obtained conformers were optimized, and their NMR chemical shifts were calculated using the GIAO ^{13}C NMR method implemented in Gaussian 16. The calculated NMR data of individual conformers were Boltzmann-averaged to obtain the theoretical NMR values for each compound. Finally, the calculated NMR data were compared with the experimental data using the STS (Sorted Training Sets) protocol.

Methods for evaluating inhibitory activity on NO Production and the expression of related cytokines (IL-6, TNF-α, and iNOS)

2.3. Isolation of compounds

2.3.1. Isolation of compounds from A. odorata

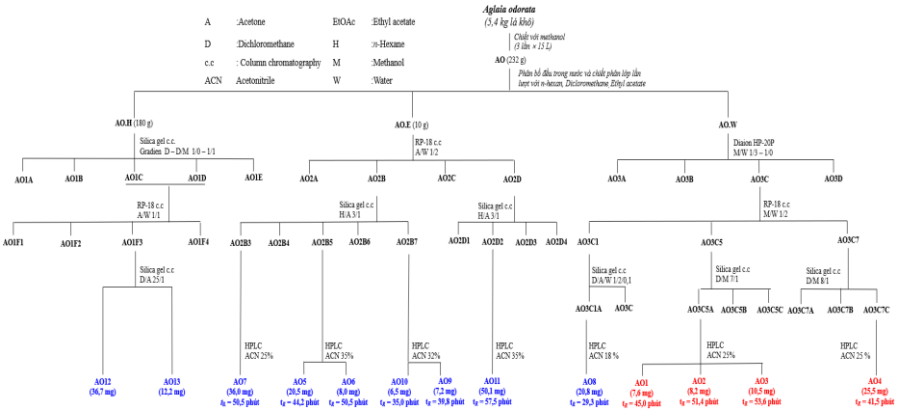

Figure 2.1. *Isolation of compounds from A. odorata*

2.3.2. Isolation of compounds from A. polystachya

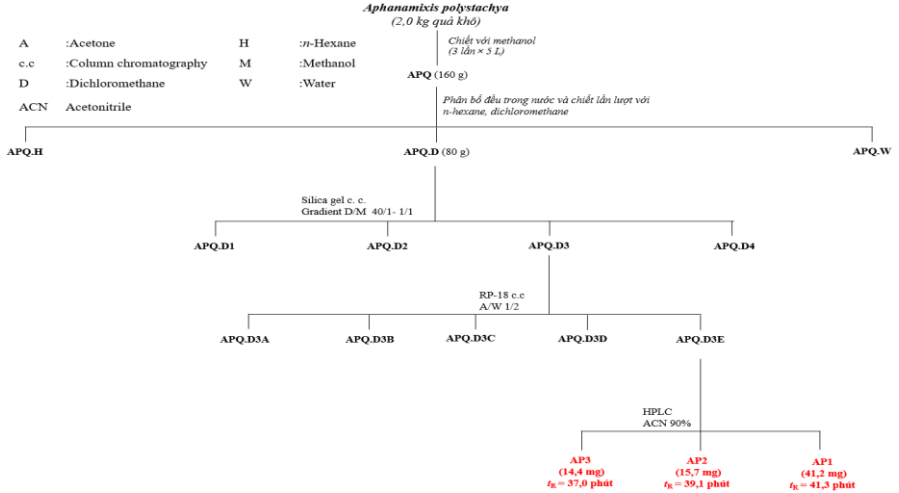

Figure 2.2. *Isolation of compounds from A polystachya*

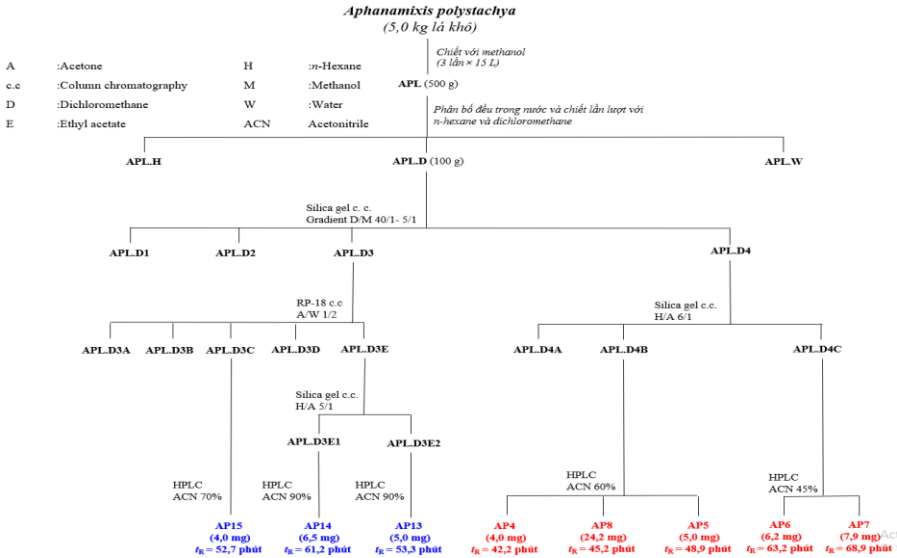

Figure 2.3. *Isolation of compounds from A polystachya*

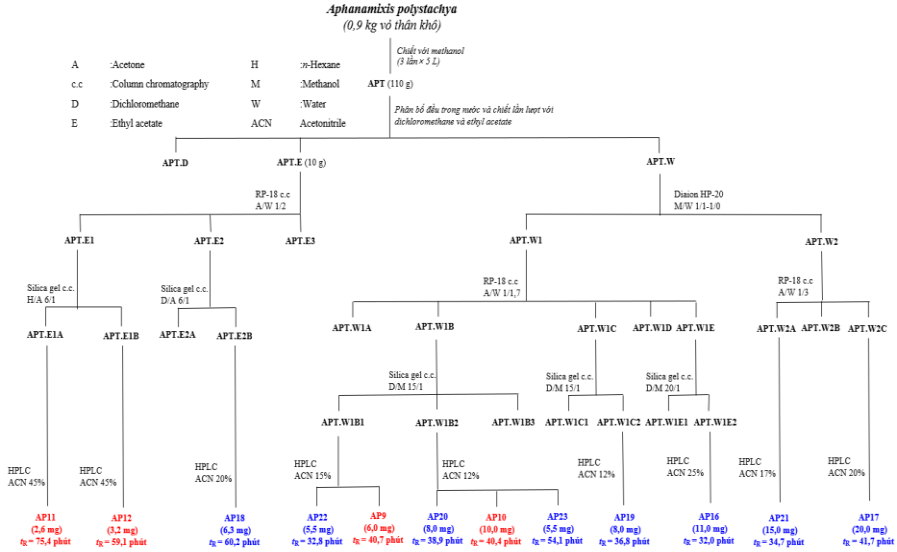

Figure 2.4. *Isolation of compounds from A polystachya*

2.4. Physical and spectroscopic data of compounds

2.5. Results on activity of compounds

2.5.1. Results of the NO production inhibition assay

Table 2.1. *NO production inhibition assay results for* ***AO1–AO13***

Compounds	IC_{50} (μM)	Compounds	IC_{50} (μM)	Compounds	IC_{50} (μM)
AO1	24,3 ± 1,2	**AO6**	66,5 ± 2,2	**AO11**	43,2 ± 1,8
AO2	22,7 ± 1,4	**AO7**	> 100	**AO12**	16,2 ± 2,4
AO3	21,4 ± 1,2	**AO8**	> 100	**AO13**	17,3 ± 2,6
AO4	33,1 ± 2,1	**AO9**	> 100	**L-NMMA[a]**	30,2 ± 1,7
AO5	75,1 ± 2,5	**AO10**	82,4 ± 1,9		

* L-NMMA: N^G-monomethyl-L-arginine acetate used as a positive control.

Table 2.2. *NO production inhibition assay results for* ***AP1-AP23***

Compounds	IC_{50} (μM)	Compounds	IC_{50} (μM)	Compounds	IC_{50} (μM)
AP1	1,7 ± 0,2	**AP9**	42,0 ± 1,2	**AP17**	>100
AP2	3,0 ± 1,5	**AP10**	>100	**AP18**	>100
AP3	5,3 ± 0,3	**AP11**	67,9 ± 4,1	**AP19**	>100
AP4	25,3 ± 1,5	**AP12**	20,5 ± 2,8	**AP20**	78,6 ± 2,6
AP5	37,7 ± 2,1	**AP13**	31,1 ± 2,7	**AP21**	>100
AP6	16,8 ± 1,0	**AP14**	36,6 ± 1,8	**AP22**	92,4 ± 3,1
AP7	18,8 ± 1,4	**AP15**	10,2 ± 0,2	**AP23**	>100
AP8	20,2 ± 2,4	**AP16**	>100	**L-NMMA[a]**	31,5 ± 2,6

* L-NMMA: N^G-monomethyl-L-arginine acetate used as a positive control.

2.5.2. Inhibitory effects of the compounds on the pro-inflammatory mediators (IL-6, TNF-α, and iNOS).

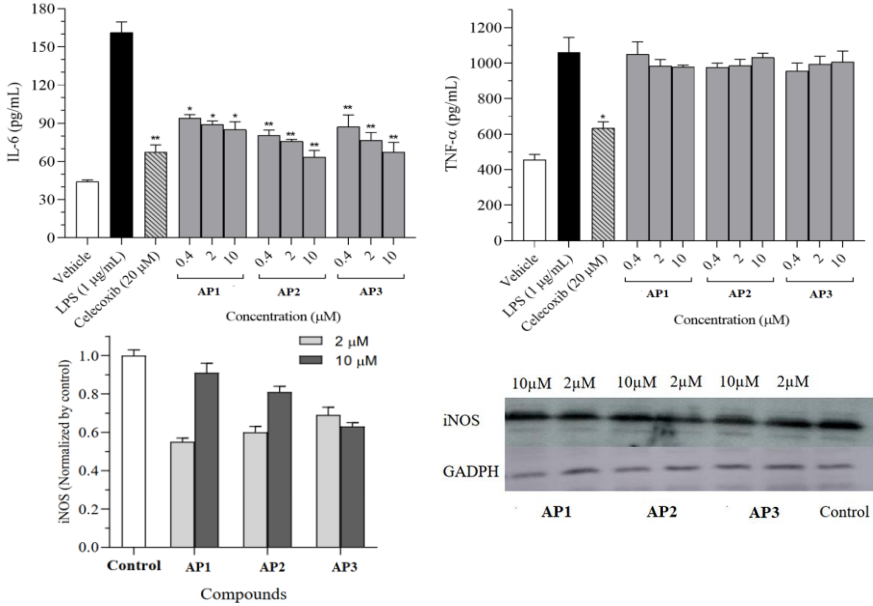

Figure 2.5. *Effects of compounds AP1–AP3 on the expression of iNOS, IL-6, and TNF-α in LPS-stimulated RAW264.7 cells*

CHAPTER 3. DISCUSSIONS

3.1. Chemical constituents of *A. odorata* and *A. polystachya*

3.1.1. Chemical constituents of A. odorata

A total of thirteen compounds (**AO1**–**AO13**) were isolated and characterized from *A. odorata*. Among them, four were identified as new structures – aglaodorata A–C (**AO1**–**AO3**) and aglaodoratin J (**AO4**) – while the remaining nine were known compounds, namely 4-methoxysalicifoliol (**AO5**), 7*β*-caruilignan C (**AO6**), loliolide (**AO7**), icariside B1 (**AO8**), grasshopper ketone (**AO9**), (+)-*ent*-ficusol (**AO10**), (−)-odorinol (**AO11**), odorine (**AO12**), and *epi*-odorine (**AO13**).

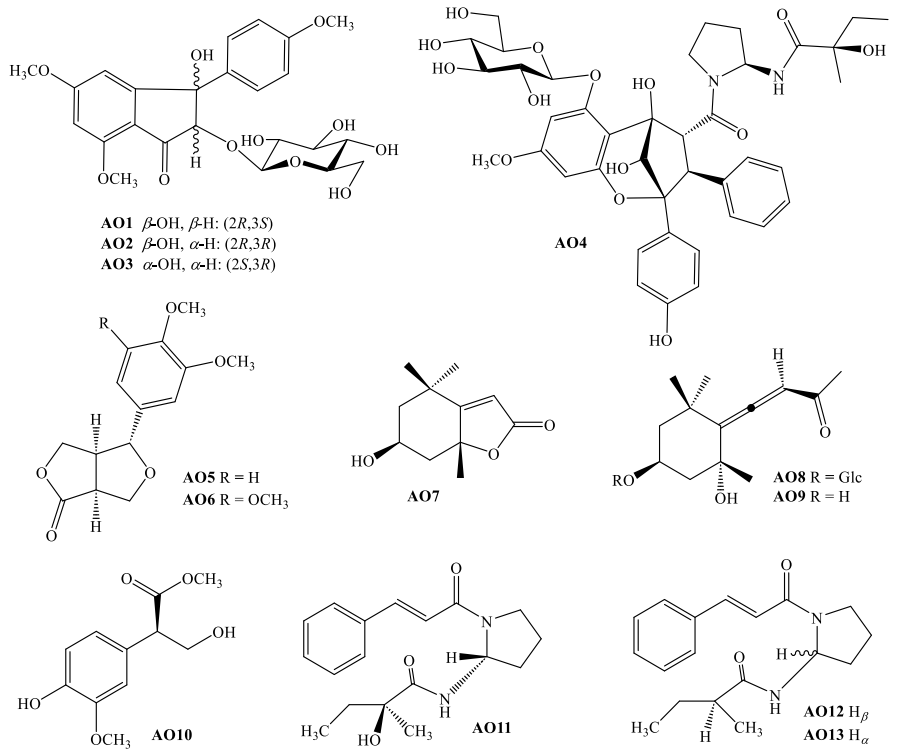

Figure 3.1. *Chemical structures of the compounds isolated from A. odorata*

The newly identified compounds **AO1**–**AO3** possess a previously unreported "2,9-deoxyflavonoid" scaffold, characterized by the absence of an oxygen atom at C-1 and the formation of an intramolecular C–C bond between C-2 and C-9. Consequently, **AO1**–**AO3** represent the first examples of this unique structural framework, distinct from all flavonoids reported to date. Moreover, **AO1**, **AO2** and **AO3** are stereoisomers that differ only in the absolute configurations at C-2 and C-3, as demonstrated by NOESY correlations and ECD spectral analysis.

Compound **AO4** is a new member of the flavonol-diamide [3+2] structural class, also known as the flavaglines—a characteristic group of secondary metabolites in the genus *Aglaia*. A notable structural feature of **AO4** is the presence of a glucose moiety attached at C-6. Compounds **AO5**–

AO13 are known metabolites: **AO5** and **AO6** are lignan-type compounds; **AO7** is a terpenoid; **AO8** and **AO9** belong to the megastigmane class; **AO11**, **AO12**, and **AO13** are biamide derivatives; and **AO10** is a phenolic compound.

*Structural elucidation of compound **AO1**: aglaodorata A (new compound)*

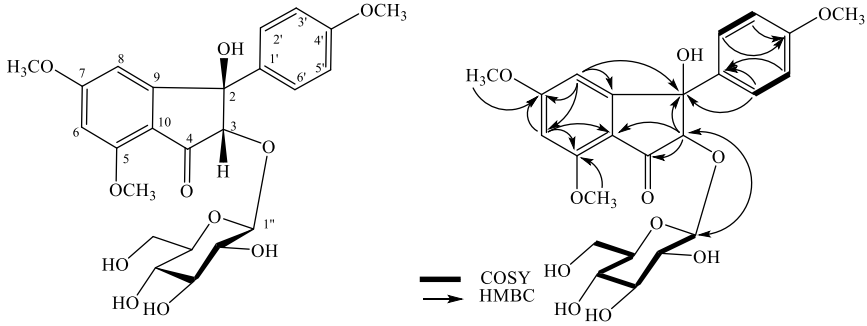

Figure 3.2.** Chemical structures of compound **AO1

Compound **AO1** (Figure 3.2) was obtained as a pale-yellow powder. Its molecular formula was determined to be $C_{24}H_{28}O_{11}$ on the basis of HR-ESI-MS data (*m/z* 493,1705 $[M+H]^+$, 515,1533 $[M + Na]^+$, and *m/z* 491,1538 $[M–H]^–$), indicating 11 degrees of unsaturation. The IR spectrum of **AO1** displayed absorption bands at 3405, 1705, 1606, and 1079 cm^{-1} indicating the presence of OH, C=O, C=C, and C-O-C functional groups, respectively. The ^1H NMR spectrum of **AO1** showed two *meta*-coupled olefinic protons [δ_H 6,51 and 6,64 (each 1H, d, J = 2,4 Hz)], attributed to H-6 and H-8 of a flavone. Four other olefinic proton signals [δ_H 7,11 and 6.97 (each 2H, d, J = 8,0 Hz)] indicative of an AA′BB′ coupled system was assigned to a *para*-disubstituted benzene ring. Three methoxy groups were recognized based on their proton signals at δ_H 3,74, 3,86, and 3,94 (each 3H). In addition, signals of an anomeric proton [δ_H 4,46 (d, J = 7,8 Hz)] and two oxymethylene protons [δ_H 3,47 (dd, J = 12,0, 8,4 Hz) and 2,98 (dd, J = 12,0, 2,4 Hz)] were attributed to H-1″ and H_2-6″ of the sugar moiety, respectively. The ^{13}C NMR spectrum of **AO1** included 24 carbon signals, six of which were assigned to a

glucopyranosyl moiety, three of which were attributed to three methoxy groups, and the remaining 15 of which were attributed to a flavonoid. Six of the carbon signals (δ_C 105,8, 75,0, 77,6, 72,2, 77,8, and 63,3) well matched a glucopyranosyl moiety, confirmed by HSQC, HMBC, and COSY spectra (Figure 3.2).

Table 3.1. *NMR spectral data of compound* ***AO1***

C	δ_C[a,b]	δ_H[a,c] (mult., *J*, Hz)	C	δ_C[a,b]	δ_H[a,c] (mult., *J*, Hz)
2	81,5	-	4'	159,9	-
3	93,9	4,74 (s)	5-OCH_3	56,4	3,94 (s)
4	197,5	-	7-OCH_3	56,6	3,86 (s)
5	160,4	-	4'-OCH_3	55,6	3,74 (s)
6	100,0	6,51 (d, 2,4)	3-*O*-Glc		
7	170,4	-	1"	105,8	4,46 (d, 7,8)
8	101,6	6,64 (d, 2,4)	2"	75,0	3,08 (dd, 9,0, 7,8)
9	160,9	-	3"	77,6	3,36 (dd, 9,0, 9,0)
10	115,4	-	4"	72,2	3,03 (dd, 9,0, 9,0)
1'	135,8	-	5"	77,8	3,38 (ddd, 9,0, 5,4, 2,4)
2', 6'	128,5	7,11 (d, 8,0)	6"	63,3	3,47 (dd, 12,0, 5,4)
3', 5'	114,1	6,97 (d, 8,0)			3,98 (dd, 12,0, 2,4)

[a]measured in CD_3OD,[b]150MHz, [c]600MHz

In the aglycone moiety, four carbon signals [δ_C 135,8 (C), 128,5 (CH), 114,1 (CH), 159,9 (C)] were attributed to a *para*-disubstituted benzene ring and thus were assigned to the B ring. Other six olefinic carbon signals [δ_C 160,4 (C), 100,0 (CH), 170,4 (C), 101,6 (CH), 160,9 (C), and 115,4 (C)] corresponded to the A ring. Three remaining carbon signals [δ_C 81,5 (C), 93,9 (CH), and 197,5 (C=O)] were assigned to C-2, C-3, and C-4 of the flavonoid. The downfield-shifted signals of C-2 and C-3 indicated that these were oxygen-bearing carbons. Intriguingly, the HMBC spectrum clearly showed a correlation between H-8 (δ_H 6,64) and C-2 (δ_C 81,5), consistent with a direct C-C linkage between C-9 and C-2 rather than an ether bridge (C-O-C) between C-9 and C-2, as in a normal flavonoid skeleton. To the best of our

knowledge, the formation of a cyclopentane ring by the C-C cyclization of C-2/C-9 (2,9-deoxyflavonoid) is unprecedented with respect to the carbon framework of the flavonoid. In the HMBC spectrum, the methoxy protons [δ_H 3,94, 3,86, and 3,74] correlated with C-5 (δ_C 160,4), C-7 (δ_C 170,4), and C-4′ (δ_C 159,9), respectively, evidenced by three methoxy groups at C-5, C-7, and C-4′. On the other hand, the correlation of Glc H-1″ (δ_H 4,46) with C-3 (δ_C 93,9) indicated an *O*-glycosidic linkage at C-3, and the large $J_{H\text{-}1''/H\text{-}2''}$ (7,8 Hz) of the anomeric proton indicated a *β*-glucoside linkage. The D-glucose moiety was also identified by acid hydrolysis of **AO1**, treatment with cysteine methyl ester and *O*-tolyl isothiocyanate, followed by HPLC analysis and comparison with the t_R values of authentic D/L-glucose.

Despite determination of the *O*-glycosidic linkage at C-3 and the absolute configuration of the sugar moiety (D-glucose), the absolute configurations at C-2 and C-3 could not be elucidated from the experimental NMR data due to the unprecedented 2,9-deoxyflavonoid skeleton of compounds **AO1**. However, they could be established by experimental ECD, TD-DFT computational ECD, and GIAO computational NMR. Then four possible diastereoisomers, **AO1a**-(2*S*,3*R*), **AO1b**-(2*S*,3*S*), **AO1c**-(2*R*,3*R*), **AO1d**-(2*R*,3*S*), were generated for computations. In recent years, several strategies based on GIAO NMR calculation have been successfully developed to assign single diastereoisomers. Of these, the accuracy and reliability of the structural assignments based on GIAO ^{13}C NMR calculation using sorted training sets (STS) strategy has been demonstrated. Therefore, GIAO ^{13}C NMR calculations were performed for diastereoisomers **AO1a**–**AO1d** using an STS strategy. Comparisons between the experimental ^{13}C NMR data for **AO1** and computational ^{13}C NMR results for **AO1a**– **AO1d** indicated that the absolute configurations of **AO1** was **1d**-(2*R*,3*S*) (relative probability P_{rel} = 99.96%). Additionally, experimental ECD of **AO1** and the TD-DFT calculation ECD for the four isomers **AO1a**– **AO1d** were performed to

confirm the absolute configurations of **AO1**. The good agreements between the experimental ECD of this compound and the corresponding calculated ECD for isomers **AO1a**– **AO1d** allowed the complete assignment of the absolute configurations of **AO1**-(2*R*,3*S*) (Figure 3.3). The NOESY spectrum of **AO1** showed that correlations of H-3/H-2′ were not observed. The NOESY correlations did indicate that H-3 and the *p*-methoxyphenyl group were located on the opposite side for **AO1**. Following the successful determination of the chemical structures of the 2,9-deoxyflavonoids, it was named aglaodorata A.

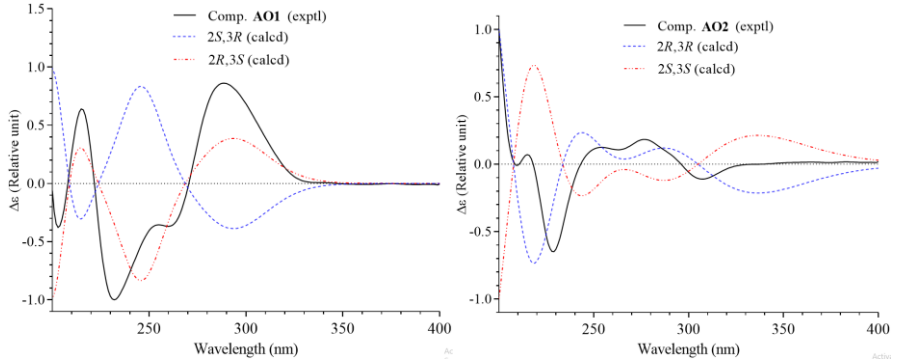

Figure 3.3. *The experimental ECD of* ***AO1*** *and the TD-DFT calculation ECD for the four isomers*

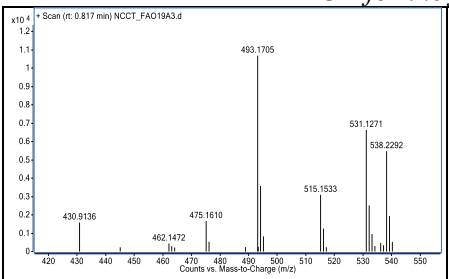

Figure 3.4. *(+)-HR-ESI-MS of compound* ***AO1***

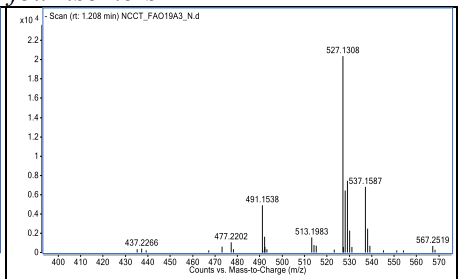

Figure 3.5. *(–)-HR-ESI-MS of compound* ***AO1***

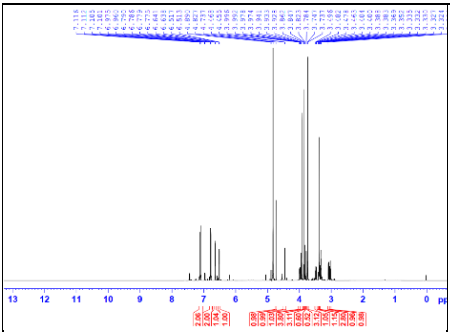

Figure 3.6. *^{1}H-NMR spectrum of compound **AO1** in CD_3OD*

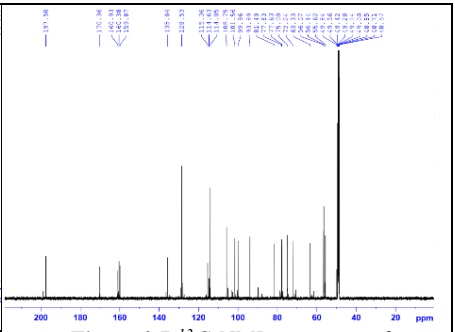

Figure 3.7. *^{13}C-NMR spectrum of compound **AO1** in CD_3OD*

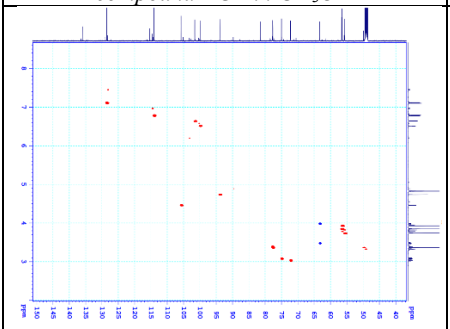

Figure 3.8. *HSQC spectrum of compound **AO1***

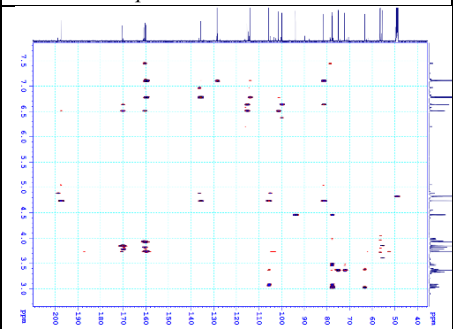

Figure 3.9. *HMBC spectrum of compound **AO1***

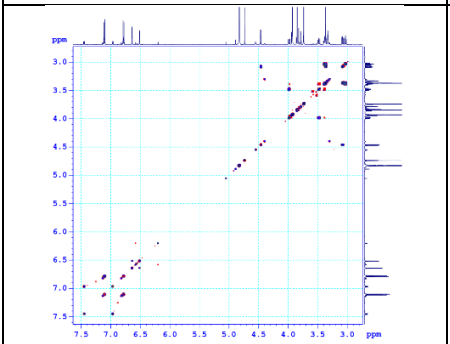

Figure 3.10. *COSY spectrum of compound **AO1***

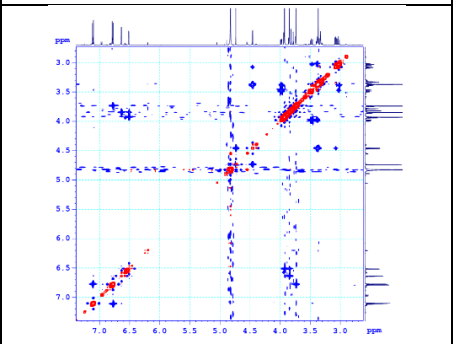

Figure 3.11. *NOESY spectrum of compound **AO1***

3.1.2. Chemical constituents of A. polystachya

From *Aphanamixis polystachya*, a total of 23 compounds (**AP1**–**AP23**) were isolated and structurally characterized, including **12 new metabolites**: aphanapolystachone A-C (**AP1**-**AP3**), aphanamixionolide A-E (**AP4**-**AP8**), 11-methoxysawaranospiroride C (**AP9**), 6*α*,9*ξ*,10,13-tetrahydroxymegastigmane-3-one (**AP10**), 11-hydroxyaphanamixin B (**AP11**), (2*Z*,6*E*,13*E*)-2,6,13-triene-11,15-dihydroxyphytanic acid (**AP12**). The remaining 11 compounds were identified as known metabolites: piscidinol A (**AP13**), hispidone (**AP14**), bourjotinolone A (**AP15**), cinnacasside D (**AP16**), cinnacasside E (**AP17**), vilsonol F (**AP18**), (3*S*,5*R*,6*S*,7*E*,9*R*)-3,5,6,9-tetrahydroxy-7-en-megastigmane (**AP19**), (3*S*,5*R*,6*R*,7*E*,9*R*)-3,6,9,10-tetrahydroxy-7-en-megastigmane (**AP20**), citroside A (**AP21**), *threo*-1-(3,4,5-trimethoxyphenyl)-1,2,3-propanetriol (**AP22**) and 3,4,5-trimethoxyphenyl-1-*O*-*β*-D-glucopyranoside (**AP23**).

The new compounds **AP1**–**AP3** are sesquiterpene–diterpene dimers produced by the fusion of a guaia-1,4,5-triene sesquiterpene unit with a nemoralisim-type diterpene unit. A plausible biosynthetic pathway for these metabolites involves an enzyme-mediated [4+2] Diels–Alder cycloaddition between the two precursors. Notably, this sesquiterpene–diterpene dimeric scaffold, as observed in **AP1**–**AP3**, has not been previously reported in any vascular plant.

AP4–**AP8** constitute a group of open-chain diterpene lactone derivatives composed of two structural fragments: a γ-/*δ*-lactone moiety and a furan-3-one unit. This structural class is considered rare in nature. The four additional new compounds **AP9**–**AP12** include a phenolic γ-butyrolactone derivative (**AP9**), a megastigmane-type compound (**AP10**), and two open-chain diterpene derivatives (**AP11**–**AP12**). The absolute configurations of these compounds were established by combining NOESY correlations, experimental ECD spectra, and comparison with TD-DFT-calculated ECD data.

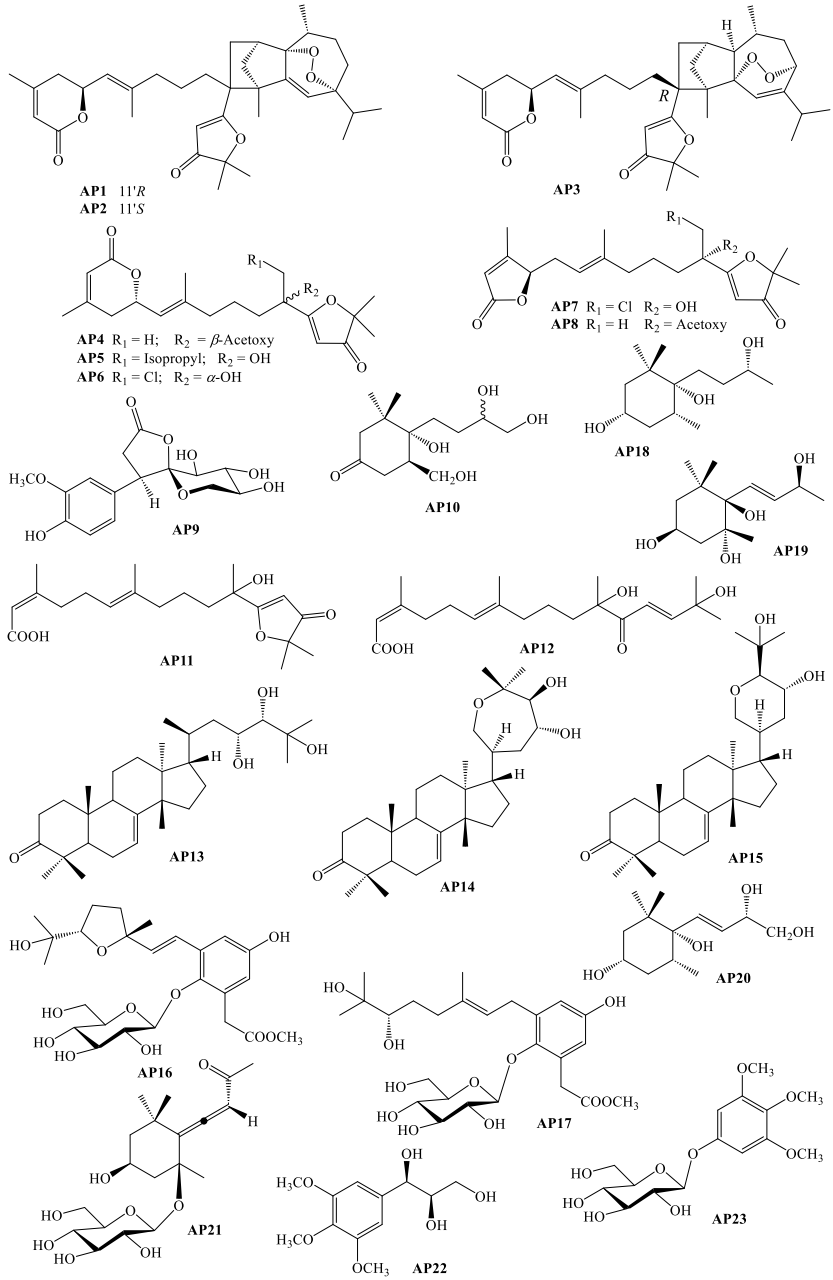

Figure 3.12. *Chemical structures of the compounds isolated from A. polystachya*

3.2. Biological activities of the compounds isolated from *A. odorata* and *A. polystachya*

3.2.1. NO-inhibitory activity of the compounds isolated from A. odorata

The compounds **AO1**–**AO13** isolated from *A. odorata* were evaluated for their anti-inflammatory potential by assessing their ability to inhibit NO production in LPS-stimulated RAW264.7 macrophages, following the previously described procedure. At 100 μM, none of the tested compounds exhibited noticeable cytotoxicity in the MTT assay. Among them, **AO12** and **AO13** showed the most pronounced inhibition, with IC_{50} values of 16,2 and 17,3 μM, respectively – stronger than the positive control L-NMMA (IC_{50} = 30,2 μM). Compounds **AO1–AO4** and **AO11** displayed moderate activity, with IC_{50} values ranging from 24,3 to 43,2 μM. By contrast, **AO5**, **AO6**, and **AO10** exhibited only weak inhibition (IC_{50} = 66,5–82,4 μM) and the remaining compounds were considered inactive (IC_{50} > 100 μM).

From these results, it is evident that **AO1**–**AO3** display notable NO-inhibitory activity, suggesting that the "2,9-deoxyflavonoid" scaffold – characterized by the absence of an oxygen atom at C-1 and the C–C ring closure between C-2 and C-9 – confers stronger activity compared with more common flavonoid skeletons. AO4 is a glycosylated derivative of aglaxiflorin D, a flavagline previously reported to strongly inhibit NO production as well as PGE_2 release. However, **AO4** showed only moderate activity, implying that the presence of sugar moiety may reduce bioactivity. This observation is consistent with earlier findings on flavagline glycosides such as aglapervirisin J–M.

Compounds **AO11**–**AO13** belong to the bisamide class, all of which displayed NO-inhibitory activity consistent with previous reports. Among them, **AO12** and **AO13** exhibited markedly stronger activity than **AO11**, suggesting that the presence of a hydroxy group reduces the potency of these bisamide-type compounds.

3.2.2. Inhibitory effects on pro-inflammatory mediators of compounds from A. polystachya

The compounds **AP1**–**AP23** isolated from *A. polystachya* were evaluated for their anti-inflammatory potential based on their ability to inhibit NO production in LPS-stimulated RAW264.7 macrophages, following the established protocol. At 100 μM, all tested compounds except **AP12** showed no appreciable cytotoxicity in the MTT assay. For **AP12**, cytotoxicity was eliminated when the concentration was reduced to 50 μM.

The NO inhibitory assay revealed that **AP1–AP4, AP6–AP8, AP12, and AP15** exhibited strong activity, with IC_{50} values ranging from 1,7 to 20,5 μM, outperforming the positive control L-NMMA (IC_{50} = 31,5 μM). Compounds **AP5, AP9, AP11, AP13, AP14, AP20**, and **AP22** showed moderate inhibition (IC_{50} = 31,1–92,4 μM), while the remaining compounds were considered inactive (IC_{50} > 100 μM). Notably, the three newly discovered compounds **AP1–AP3**, isolated from the fruits of *A. polystachya*, displayed the most potent activity, with IC_{50} values of 1,7, 3,0 and 5,3 μM, respectively – far stronger than L-NMMA.

Given their promising NO-inhibitory profiles, **AP1**–**AP3** were further investigated for their effects on key inflammatory mediators, including iNOS, IL-6, and TNF-α. Compounds **AP1** and **AP2** markedly suppressed iNOS expression at both tested concentrations (2 and 10 μM). **AP3** also reduced iNOS expression, though in a concentration-independent manner; interestingly, it exerted a stronger effect at 2 μM than at 10 μM.

Assessment of cytokine production showed that all three compounds significantly inhibited the secretion of IL-6 but did not affect the secretion of TNF-α cytokines at tested concentrations 0.4, 2, and 10 μM. Taken together, these results suggest that AP1–AP3 selectively modulate inflammatory pathways by inhibiting NO production through downregulation of iNOS and

suppressing IL-6 production, while having no significant effect on TNF-α secretion.

The anti-inflammatory properties of these compounds may be attributable to the peroxy-bridged cage structure present in their molecular framework. Peroxide-containing natural products are well known for their pharmacological potential. A classic example is artemisinin, discovered in 1972 as a potent antimalarial agent. Since then, numerous peroxide metabolites from both marine organisms and terrestrial plants have been reported to possess antiviral, anticancer, and anti-inflammatory activities.

CONCLUSIONS

This study reveals previously unreported chemical constituents and noteworthy anti-inflammatory activities from *Aglaia odorata* and *Aphanamixis polystachya* collected in Vietnam. Using a combination of chromatographic separations and modern spectroscopic techniques, a total of 36 compounds were isolated and structurally elucidated from the two species, including 16 new natural products.

1. Chemical Constituents

- From *A. odorata*, thirteen compounds (AO1–AO13) were isolated, including **four new structures** aglaodorata A-C (**AO1-AO3**), and aglaodoratin J (**AO4**), together with nine known compounds**,** namely 4-methoxysalicifoliol (**AO5**), 7*β*-caruilignan C (**AO6**), loliolide (**AO7**), icariside B1 (**AO8**), grasshoper ketone (**AO9**), (+)-*ent*-ficusol (**AO10**), (–)-odorinol (**AO11**), odorine (**AO12**) and *epi*-odorine (**AO13**).

Three new compounds **AO1**–**AO3** possess a carbon skeleton that has not been previously reported, representing an unusual structural motif within the flavonoid family. **AO4** is a new flavonol-diamide [3+2] (flavagline) derivative; notably, whereas known flavaglines typically bear a 6-*O*-methoxy group, AO4 uniquely carries a 6-*O*-glucopyranosyl substituent, a feature not previously observed in this class of compounds.

- From *A. polystachya*, twenty-three compounds (AP1–AP23) were isolated, including **twelve new compounds**: aphanapolystachone A-C (**AP1-AP3**), aphanamixionolide A-E (**AP4-AP8**), 11-methoxysawaranospiroride C (**AP9**), 6*α*,9ξ,10,13-tetrahydroxymegastigmane-3-one (**AP10**), 11-hydroxyaphanamixin B (**AP11**) and (2*Z*,6*E*,13*E*)-2,6,13-triene-11,15-dihydroxyphytanic acid (**AP12**). Eleven additional known compounds were also identified, including piscidinol A (**AP13**), hispidone (**AP14**), bourjotinolone A (**AP15**), cinnacasside D (**AP16**), cinnacasside E (**AP17**), vilsonol F (**AP18**), (3*S*,5*R*,6*S*,7*E*,9*R*)-3,5,6,9-tetrahydroxy-7-en-megastigmane (**AP19**), (3*S*,5*R*,6*R*,7*E*,9*R*)-3,6,9,10-tetrahydroxy-7-en-megastigmane (**AP20**), citroside A (**AP21**), *threo*-1-(3,4,5-trimethoxyphenyl)-1,2,3-propanetriol (**AP22**) and 3,4,5-trimethoxyphenyl-1-*O*-*β*-D-glucopyranoside (**AP23**).

Compounds **AP1**–**AP3** represent a novel class of sesquiterpene–diterpene dimers, formed through the coupling of a guaia-1,4,5-triene sesquiterpene unit with a nemoralisim-type diterpene. This type of sesquiterpene–diterpene dimer has never been reported in vascular plants. **AP4**–**AP8** are open-chain diterpene lactone derivatives, constructed from *γ*-/*δ*-lactone fragments and a furan-3-one unit, with some members featuring chlorine atoms, giving rise to an uncommon structural scaffold in nature. The remaining new compounds include a phenolic derivative (**AP9**), a megastigmane (**AP10**), and two acyclic diterpenes (**AP11**–**AP12**). The absolute configurations of the new compounds were established by comparing experimental ECD spectra with TD-DFT-calculated ECD data.

2. Biological Activity

The inhibitory activity on NO production was evaluated for thirteen compounds (**AO1**–**AO13**) isolated from *A. odorata* and twenty-three compounds (**AP1**–**AP23**) isolated from *A. polystachya*. The results showed that:

- From *A. odorata*, compounds **AO12** and **AO13** exhibited potent inhibitory effects on NO production, with IC_{50} values of 16,2 and 17,3 μM, respectively. Compounds **AO1**–**AO4** and **AO11** also demonstrated noticeable inhibitory activity, with IC_{50} values comparable to that of the positive control. The remaining compounds showed very weak or no activity, as their IC_{50} values exceeded 100 μM.

- From *A. polystachya*, compounds **AP1**–**AP4**, **AP6**–**AP8**, **AP12**, and **AP15** strongly inhibited NO production. Compounds **AP5**, **AP9**, **AP11**, **AP13**, **AP14**, **AP20**, and **AP22** exhibited moderate to weak inhibitory effects, whereas the remaining compounds were inactive due to IC_{50} values greater than 100 μM. Notably, compounds **AP1**–**AP3** displayed remarkably potent NO-inhibitory activity, with IC_{50} values of 1,7, 3,0, and 5,3 μM, respectively—significantly lower than that of the positive control L-NMMA (IC_{50} = 31,5 μM).

- Compounds **AP1**–**AP3** were further investigated for their effects on inflammation-related mediators, including iNOS, IL-6, and TNF-α. The results indicated that all three compounds effectively suppressed iNOS expression at the tested concentrations. In addition, **AP1**–**AP3** inhibited IL-6 production at all evaluated concentrations but did not affect TNF-α levels. These findings suggest that **AP1**–**AP3** modulate inflammatory responses in a selective manner toward specific pro-inflammatory mediators.

RECOMMENDATIONS

The chemical composition and biological activity studies on *Aglaia odorata* and *Aphanamixis polystachya* indicate that compounds **AO12** and **AO13** from *A. odorata*, together with **AP6**, **AP7**, and **AP15** from *A. polystachya*, exhibit notable inhibitory effects on NO production in RAW264.7 macrophages. Notably, compounds **AP1**–**AP3** show exceptionally strong NO-inhibitory activity in RAW264.7 cells and display selective modulation of inflammatory mediators, including suppression of

NO biosynthesis through down-regulation of iNOS expression, as well as inhibition of IL-6 production. Taken together, these results indicate that the compounds warrant further in-depth studies to elucidate their mechanisms of action.

NEW CONTRIBUTIONS OF THE DISSERTATION

1. **Four new compounds** were isolated and structurally elucidated from *Aglaia odorata*, namely aglaodorata A–C **(AO1–AO3)** and aglaodoratin J **(AO4).** Among them, **AO1**–**AO3** possess previously unreported carbon skeletons, while **AO4** belongs to the characteristic flavagline class. The absolute configurations of **AO1**–**AO3** were established using modern spectroscopic techniques, including IR, 1D and 2D NMR, HR-ESI-MS, and ECD, in combination with theoretical calculations (^{13}C GIAO and TD-DFT ECD). All four new compounds (**AO1**–**AO4**) exhibited inhibitory effects on LPS-induced NO production in RAW264.7 macrophages, with IC_{50} values of 24,3, 22,7, 21,4 and 33,1 μM, respectively.

2. **Twelve new compounds** were isolated and characterized from *Aphanamixis polystachya*: aphanapolystachone A-C (**AP1-AP3**), aphanamixionolide A-E (**AP4**-**AP8**), 11-methoxysawaranospiroride C (**AP9**), 6α,9ξ,10,13-tetrahydroxymegastigmane-3-one (**AP10**), 11-hydroxyaphanamixin B (**AP11**), (2*Z*,6*E*,13*E*)-2,6,13-triene-11,15-dihydroxyphytanic acid (**AP12**). **AP1**–**AP3** represent unprecedented dimeric terpenoids formed from two terpenoid units—a structural motif not previously reported in vascular plants. **AP4**–**AP8** are new open-chain diterpene derivatives featuring γ-/δ-lactone units, a furan-3-one moiety, and the presence of chlorine atoms, constituting a rare structural framework in nature. The remaining new compounds belong to diverse structural classes. The isolated compounds exhibited inhibitory activity against LPS-induced NO production in RAW264.7 macrophages, with IC_{50} values ranging from 1,7 to 92,4 μM. Notably, **AP1**–**AP3** showed strong inhibition of iNOS expression at both tested concentrations (2 μM and 10 μM), and all three compounds effectively suppressed IL-6 production at 0,4, 2,0, and 10 μM.

LIST OF THE PUBLICATIONS RELATED TO THE DISSERTATION

1. **Ngo Anh Bang**, Duong Thi Hai Yen, Dan Thi Thuy Hang, Pham Hai Yen, Nguyen Huy Hoang, Do Thi Trang, Duong Thi Dung, Nguyen Thi Cuc, Nguyen The Cuong, Nguyen Xuan Nhiem, Bui Huu Tai and Phan Van Kiem. New diterpene lactone derivatives from *Aphanamixis polystachya* leaves inhibit nitric oxide production in RAW 264.7 cells. *RSC Advances*, **2024**, 14(29), e20536.
2. **Ngo Anh Bang**, Dan Thi Thuy Hang, Duong Thi Hai Yen, Nguyen Huy Hoang, Duong Thi Dung, Nguyen The Cuong, Pham Hai Yen, Nguyen Xuan Nhiem, Bui Huu Tai and Phan Van Kiem. Four Unidentified Compounds Isolated from the Stem Barks of *Aphanamixis polystachya* and Their NO Production Inhibition in LPS Activated RAW 264.7 Cells. *Chemistry & Biodiversity*, **2024**, 21(8), e202401118.
3. **Ngo Anh Bang**, Bui Huu Tai, Pham Hai Yen, Phan Van Kiem. The genus *Aglaia*: Diversity in chemical structure and biological activity. *Vietnam Jounal of Chemistry*, **2024**, 62(6), 737-757.
4. Bui Huu Tai, **Ngo Anh Bang**, Pham Hai Yen, Duong Thi Hai Yen, Nguyen Thi Cuc, Duong Thi Dung, Phan Thi Thanh Huong, Do Thi Trang, Nguyen Xuan Nhiem, Nguyen The Cuong, Phan Van Kiem. Undescribed sesquiterpene-diterpene heterodimers from the fruits of *Aphanamixis polystachya* selectively modulate inflammatory markers in RAW 264.7 cells. *Phytochemistry,* **2024**, 220, e113997.
5. Pham Hai Yen, **Ngo Anh Bang**, Do Thi Trang, Duong Thi Hai Yen, Duong Thi Dung, Phan Thi Thanh Huong, Nguyen Huy Hoang, Bui Huu Tai, Le Tuan Anh, Phan Van Kiem. Undescribed 2,9-deoxyflavonoids and flavonol-diamide [3+2] adduct from the leaves of *Aglaia odorata* Lour. Inhibit nitric oxide production. *Phytochemistry,* **2023**, 214, e113792.
6. Phan Van Kiem, Bui Huu Tai, **Ngo Anh Bang**, Pham Hai Yen, Duong Thi Hai Yen, Nguyen Thi Cuc, Duong Thi Dung, Phan Thi Thanh Huong, Do Thi Trang, Nguyen Xuan Nhiem. The anti-inflammatory activity of sesquiterpene–diterpene heterodimers and the isolation method of these compounds from *Aphanamixis polystachya.* Vietnam Patent. No 1-2024-02063.