MINISTRY OF DUCATION AND TRAINING

VIETNAM ACADEMY OF SCIENCE AND TECHNOLOGY

**GRADUATE UNIVERSITY OF SCIENCE AND TECHNOLOGY**


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# STUDY ON THE CHEMICAL CONSTITUENTS OF *Syzygium cerasiforme* (Blume) Merr. & L.M.Perry, *Syzygium bullockii* (Hance) Merr. & L.M.Perry, *Syzygium attopeuense* (Gagnep.) Merr. & L.M.Perry IN VIET NAM AND THEIR NITRIC OXIDE INHIBITORY ACTIVITY

**SUMMARY OF DISSERTATION ON ORGANIC CHEMISTRY**
**Code: 9.44.01.14**

**Ha Noi - 2023**

This dissertation is completed at: Graduate university of Science and Technology, Vietnam Academy of Science and Technology

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The dissertation will be examined by Examination Board of Graduate University of Science and Technology, Vietnam Academy of Science and Technology at…hour…..date…. month…. 2023.

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# INTRODUCTION

## The urgency of the thesis

Now a day, although modern medicine is developing strongly, traditional medicine still plays an important role as well as tobe a treatment method that attracted many people. Numerous of researchs aimed to find biologically active substances that has been attented by many scientists all over the world. In recent years, many projects on medicinal plants of the Vietnamese flora have been carried out and have made important contributions to the health care of human.

The genus *Syzygium* belongs to the Myrtaceae family and is known as a genus of flowering plants. This genus includes thousands of species, and its habitat ranges from Africa, Madagascar, Southeast Asia, and the Pacific. This genus has high biodiversity, with some species that have not yet been described in terms of botanical classification. Most species are evergreen trees and shrubs.

Species of the genus *Syzygium* have been focused on researching by many scientists around the world. In particular, scientists mainly focus on the species *Syzygium* aromaticum. Studies on the chemical composition of this species have shown the presence of phenolics, terpenoids, flavonoids, sterols, etc. These compounds exhibit antioxidant, antifungal, antibacterial and immune-enhancing activities. strong translation. However, there have been no studies on the chemical composition and biological activities of this genus in Vietnam.

From that point, the topic "Study on the chemical constituents of *Syzygium cerasiforme* (Blume) Merr. & L.M.Perry, *Syzygium bullockii* (Hance) Merr. & L.M.Perry and *Syzygium attopeuense* (Gagnep.) Merr. & L.M.Perry in Vietnam and their nitric oxide inhibitory activity "

was chosen to get orientation of research, isolation and structure determination of compounds which were isolated from the genus *Syzygium* in Vietnam towards anti-inflammation.

**The objectives of the thesis**:

Study on chemical constituents of *Syzygium cerasiforme* (Blume) Merr. & L.M.Perry, *Syzygium bullockii* (Hance) Merr. & L.M.Perry and *Syzygium attopeuense* (Gagnep.) Merr. & L.M.Perry collected in Viet Nam. Evaluation of the NO inhibitory activity of isolated compounds on RAW264.7 cell line stimulated by LPS in an *in vitro* model

**The main contents of the thesis:**

1. Isolation of compounds from three species: *Syzygium cerasiforme*, *Syzygium bullockii* and *Syzygium attopeuense*.
2. Determination of chemical structures of the isolated compounds
3. Evaluation of the NO inhibitory activity of isolated compounds on RAW264.7 cell line stimulated by LPS in an *in vitro* model.

## CHAPTER 1: OVERVIEW

### 1.1. Introduction to the genus *Syzygium*

#### *1.1.1. Botany of the genus Syzygium*

The genus *Syzygium* belongs to the family Myrtaceae, order Myrtales, class Magnoliopsida, phylum Magnoliophyta. Most *Syzygium* species are evergreen trees and shrubs. In the world, according to the world flora online page, the *Syzygium* genus has about 1,217 species. In Vietnam, according to Nguyen Tien Ban and colleagues, Vietnam has up to 70 species of *Syzygium*.

#### *1.1.2. The status of study on chemical composition of Syzygium genus*

The result of studies showed that species of the genus *Syzygium* are rich in secondary metabolites including flavonoids, terpenoids, lignans, alkyl phloroglucinol, tannins, and chromene derivatives. The flavonoid compounds and their glycoside derivatives are also most commonly isolated in species of the genus *Syzygium*. Following flavonoids, the terpenoid compounds also accounts for a large number. Beside of them, there are chromone glycoside compounds, steroid, tannin and phenolic compounds.

### *1.1.3. The status of study on biological activity of the genus Syzygium*

The studies have shown that the extracts and compounds isolated from some species of the genus *Syzygium* have expressed notable antioxidant, antibacterial, cytotoxic with cancer cells, antidiabetic and anti-inflammatory activities.

### *1.1.4. The status of study on the genus Syzygium in Viet Nam*

Currently, there is a little bit study about the genus syzygium in Vietnam and even no data was provided to determine the chemical structure and biological activity of compounds from the three species *S. cerasiforme*, *S. bullockii* and *S. attopeuense* in the world as well as in Vietnam.

## 1.2. Introduction to S. cerasiforme, S. bullockii and S. attopeuense

The botany characteristics of three species *S. cerasiforme*, *S. bullockii* and *S. attopeuense* are describled and they distribute in the Northern, Central and Southern provinces.

## 1.3. Introduction to anti-inflammatory activity

The anti-inflammatory activity is researched based on understanding the inflammatory process, changes in inflammation, the effects of the inflammatory response on the body and especially the role of factors IL6, TNF-α, NO and COX-2 in inflammation.

# CHAPTER 2. RESEARCH SUBJECTS AND METHODS

## 2.1. Subjects

The subjects was chosen include *S. cerasiforme* leaves collected in Vinh Phuc in April 2022, the leaves and branches of *S. bullockii* collected in Vinh Linh, Quang Tri in July 2022 and the leaves and branches of *S. attopeuense* harvested in Vinh Linh, Quang Tri in September 2022. These samples were all assessed by scientists and reputable organizations in Vietnam. The samples have been keeping at the Institute of Marine Biochemistry, Vietnam Academy of Science and Technology.

## 2.2. Methods

### *2.2.1. Methods for isolation*

The thesis topic uses research methods including:

- Compound isolation method: Combination of chromatography methods including thin layer chromatography (TLC), column chromatography (CC), high performance liquid chromatography (HPLC),

- Structure determination method: Using modern spectroscopic methods including high resolution mass spectrometry (HR-ESI-MS), 1-dimensional and 2-dimensional nuclear magnetic resonance (NMR) spectroscopy, circular dichroism spectroscopy (CD), infrared spectrum (IR), polar rotation ($[\alpha]_D$), melting point measurement and sugar determination method.

- Method to evaluate the NO production inhibitory activity: Use the method to evaluate the NO production inhibitory activity on the RAW264.7 cell line stimulated by LPS after testing cytotoxicity by colorimetric method (MTT).

# CHAPTER 3: EXPERIMENT AND RESULTS

## 3.1. Isolation of compounds

### *3.1.1. Isolation of compounds from S. cerasiforme*

From *S. cersiforme* species, 20 compounds were isolated using chromatographic methods according to the following diagram:

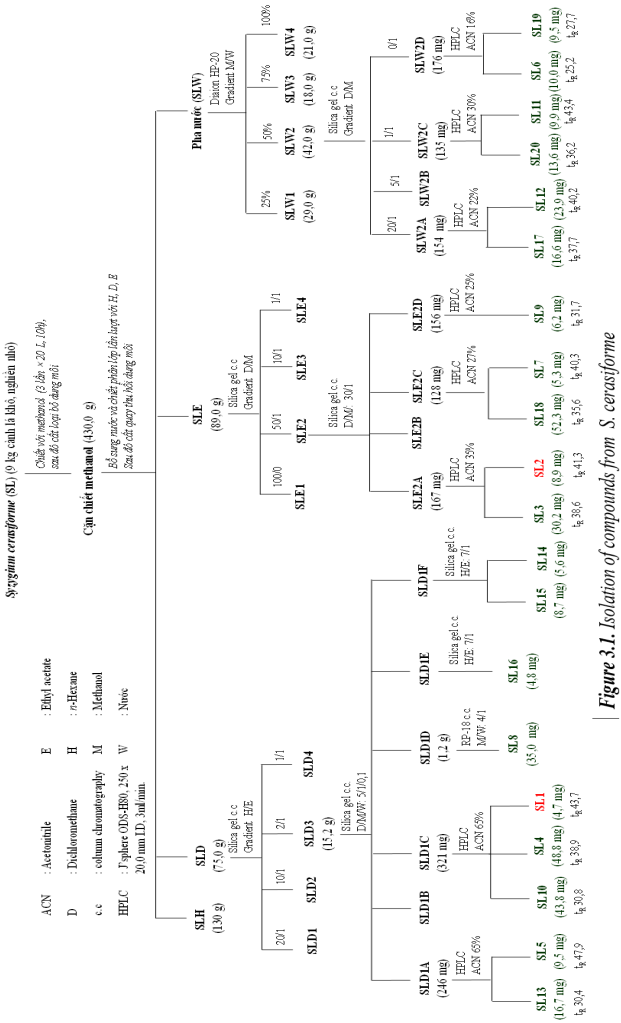



*Figure 3.1. Isolation of compounds from S. cerasiforme*

### *3.1.2. Isolation of compounds from S. bullockii*

From *S. bullockii* species, 17 compounds were isolated using chromatographic methods according to the following diagram:

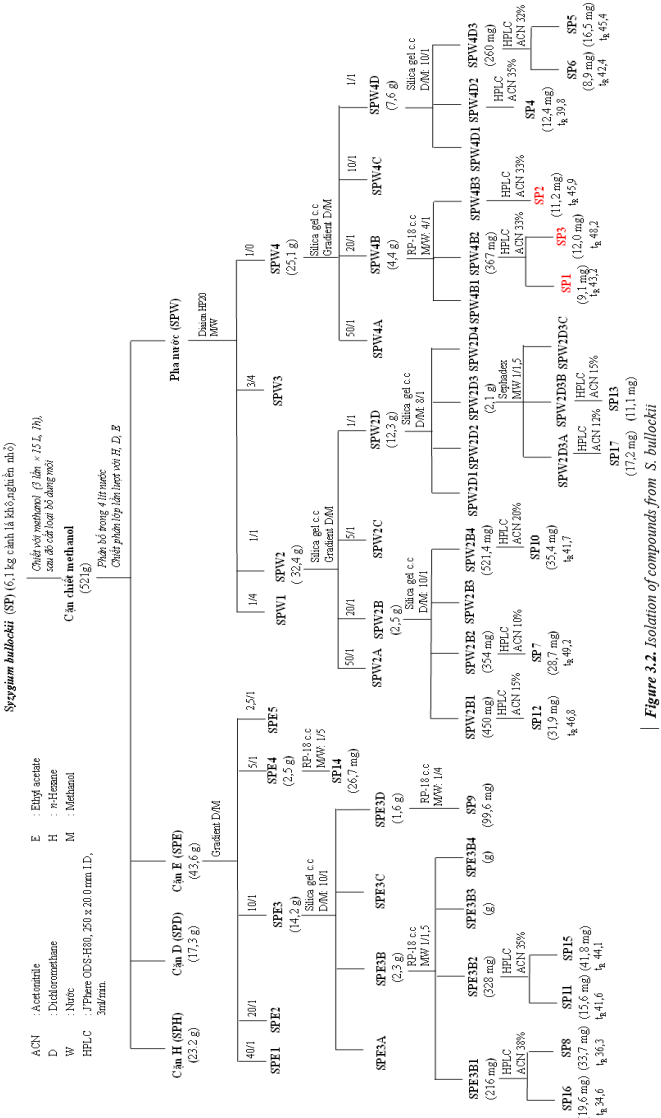



***Figure 3.2.** Isolation of compounds from S. bullockii*

### *3.1.3. Isolation of compounds from S. attopeuense*

From *S. attopeuense* species, 17 compounds were isolated using chromatographic methods according to the following diagram:

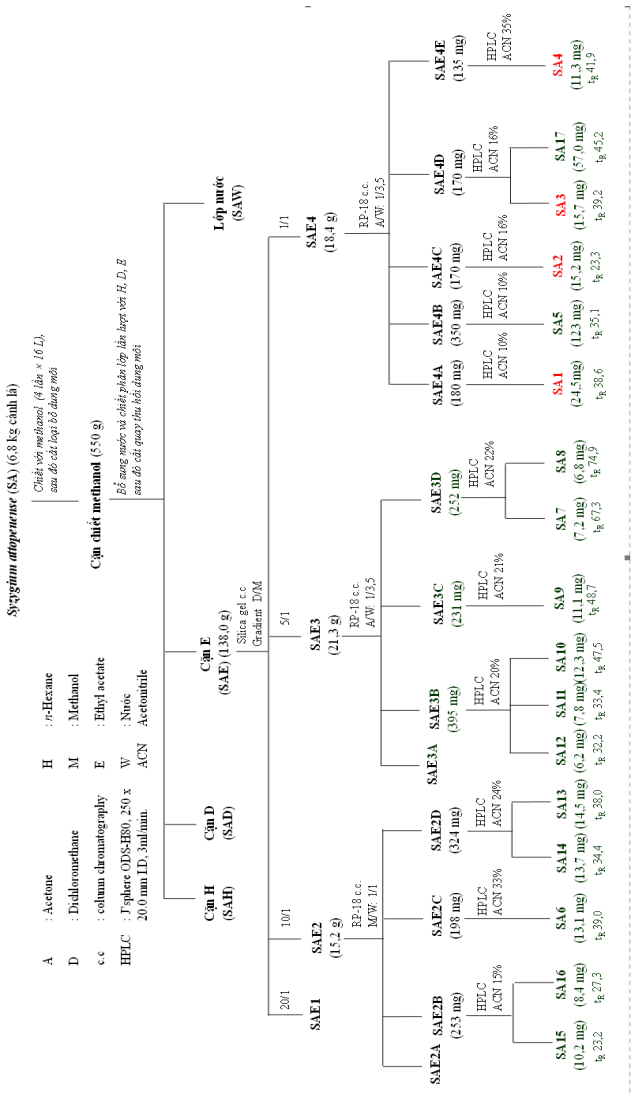



*Figure 3.3. Isolation of compounds from S. attopeuense*

### 3.2. Physical and spectroscopic data of compounds

54 compounds isolated from three species S. *cerasiforme*, *S. bullockii* and *S. attopeuense* are presented with physical parameters such as existing forms, color, melting temperature, specific rotation and infrared spectroscopy (IR) data, high resolution mass spectrometry (HR-ESI-MS), molecular formula, molecular mass (M),

### 3.3. Results of testing the NO production inhibitory activity of the isolated compounds

The results of testing the NO production inhibitory activity of compounds isolated from species are summarized in the following tables.

- From *S. cerasiforme* species with 20 compounds (**SL1-SL20**)

*Table 0.1. NO production inhibitory activity of **SL1-SL20***

| Comp. | % survival cell[#] | $IC_{50}$ (µM) | Comp. | % survival cell[#] | $IC_{50}$ (µM) |
|---|---|---|---|---|---|
| **SL1** | 100.00 | 12.28 ± 1.15 | **SL11** | 98.27 | 51.72 ± 2.85 |
| **SL2** | 99.25 | 8.52 ± 1.62 | **SL12** | 98.67 | 45.28 ± 2.18 |
| **SL3** | 97.09 | 7.68 ± 0.87 | **SL13** | 99.12 | 33.38 ± 0.78 |
| **SL4** | 99.56 | 42.27 ± 1.29 | **SL14** | 98.13 | 51.09 ± 2.13 |
| **SL5** | 99.11 | 45.13 ± 2.16 | **SL15** | 97.66 | 54.17 ± 2.34 |
| **SL6** | 99.25 | 9.67 ± 0.57 | **SL16** | 96.03 | 39.54 ± 1.32 |
| **SL7** | 99.24 | 86.52 ± 2.98 | **SL17** | 95.77 | 6.98 ± 0.57 |
| **SL8** | 99.12 | 53.71 ± 1.23 | **SL18** | 95.11 | 33.17 ± 0.78 |
| **SL9** | 98.26 | 45.12 ± 0.98 | **SL19** | 95.23 | 25.51 ± 1.02 |
| **SL10** | 98.67 | 6.69 ± 0.34 | **SL20** | 98.12 | 58.12 ± 2.34 |
| L-NMMA[a] | 90.83 | 32.5 ± 1.00 | | | |

*[a)] Positive control, [#] at concentration of 100 µM*

- From *S. bullockii* species with 17 compounds (**SP1-SP17**)

*Table 3.2. NO production inhibitory activity of **SP1-SP17***

| Comp. | % survival cell[#] | $IC_{50}$ (µM) | Comp. | % survival cell[#] | $IC_{50}$ (µM) |
|---|---|---|---|---|---|
| **SP1** | 94.32 | 11.58 ± 0.71 | **SP10** | 95.78 | 1.42 ± 0.19 |
| **SP2** | 94.85 | 13.61 ± 2.55 | **SP11** | 91.25 | 58.08 ± 2.38 |
| **SP3** | 96.04 | 6.93 ± 0.41 | **SP12** | 94.85 | 13.70 ± 1.25 |
| **SP4** | 97.85 | 7.09 ± 0.62 | **SP13** | 97.58 | 8.59 ± 0.68 |
| **SP5** | 98.86 | 7.20 ± 0.51 | **SP14** | 94.46 | 5.71 ± 0.61 |
| **SP6** | 97.41 | 7.91 ± 0.95 | **SP15** | 98.98 | 6.47 ± 0.69 |
| **SP7** | 81.57 | 1.89 ± 0.29 | **SP16** | 94.15 | 4.23 ± 0.66 |
| **SP8** | 92.39 | 9.06 ± 0.55 | **SP17** | 94.32 | 11.30 ± 0.14 |
| **SP9** | 97.43 | 11.95 ± 0.82 | **L-NMMA[a]** | 86.76 | 33.8 ± 2.6 |

*[a)] Positive control, [#] at concentration of 100 µM*

- From *S. attopeuense* species with 17 compounds (**SA1-SA17***)*

*Table 3.3. NO production inhibitory activity of **SA1-SA17***

| Comp. | % survival cell[#] | $IC_{50}$ (µM) | Comp. | % survival cell[#] | $IC_{50}$ (µM) |
|---|---|---|---|---|---|
| **SA1** | 94.21 | 18.37 ± 1.38 | **SA10** | 97.01 | 95.14 ± 3.67 |
| **SA2** | 89.68 | 31.23 ± 2.18 | **SA11** | 99.12 | > 100 |
| **SA3** | 88.38 | 35.12 ± 2.53 | **SA12** | 97.22 | > 100 |
| **SA4** | 93.67 | >100 | **SA13** | 95.71 | 98.46 ± 3.51 |
| **SA5** | 87.17 | 34.89 ± 2.13 | **SA14** | 99.26 | > 100 |
| **SA6** | 77.22 | 28.24 ± 1.79 | **SA15** | 97.72 | 78.35 ± 1.66 |
| **SA7** | 94.59 | 88.55 ± 1.78 | **SA16** | 97.72 | 76.39 ± 2.41 |
| **SA8** | 98.21 | 89.85 ± 2.08 | **SA17** | 98.32 | > 100 |
| **SA9** | 99.72 | > 100 | **D*** | 97.88 | 15.37 ± 1.42 |

*D* dexamethasone is positive control, [#] at concentration of 100 µM*

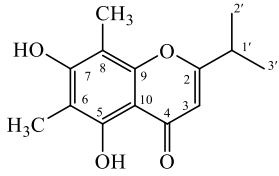
# CHAPTER 4: DISCUSSIONS

## 4.1. Chemical composition and inhibitory activity on NO production of *S. cerasiforme*

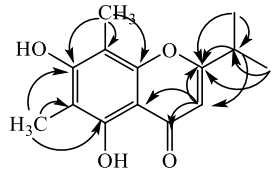
### *4.1.1. Determination of chemical structures of compounds isolated from S. cerasiforme*

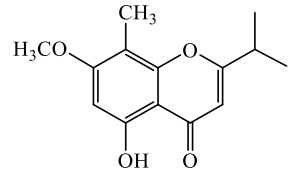
*4.1.1.1. Compound **SL1**: 5,7-Dihydroxy-2-isopropyl-6,8-dimethyl-4*H*-chromen-4-one (new compound)*

Compound **SL1** was obtained as pale-yellow needle-shaped crystals.




**SL1**




5-hydroxy-7-methoxy-2-isopropyl -8-methyl-chromone

*Figure 0.1 Chemical structure and important HMBC correlations of compound* **SL1**

*Table 0.4. The NMR data of* **SL1**

| C | $\delta_C$[a,b] | $\delta_H$[a,c] (độ bội, *J* = Hz) | C | $\delta_C$[a,b] | $\delta_H$[a,c] (độ bội, *J* = Hz) |
|---|---|---|---|---|---|
| 2 | 176,4 | - | 9 | 154,9 | - |
| 3 | 105,8 | 6,07 (s) | 10 | 105,0 | - |
| 4 | 184,8 | - | 1′ | 34,6 | 2,96 (sept, 6,6) |
| 5 | 157,7 | - | 2′ | 20,5 | 1,36 (d, 6,6) |
| 6 | 108,5 | - | 3′ | 20,5 | 1,36 (d, 6,6) |
| 7 | 161,6 | - | 6-$CH_3$ | 7,8 | 2,11 (s) |
| 8 | 103,2 | - | 8-$CH_3$ | 8,0 | 2,24 (s) |

*[a]Measured in $CD_3OD$, [b]150MHz, [c]600MHz.*

On the HR-ESI-MS high-resolution mass spectrum of compound **SL1**, a pseudo molecular ion peak appears at *m/z* 249.1128 $[M+H]^+$ (positive ion spectrum) and *m/z* 247.0975 $[M-H]^-$ (negative ion spectrum), combined with $^{13}C$ NMR and HSQC spectrum data,

allows determining the molecular formula $C_{14}H_{16}O_4$. $^{13}C$ NMR and HSQC spectra show a resonance signal of 14 carbon atoms including 4 methyl groups, 2 methine groups and 8 quaternary carbons. Two methine protons at $\delta_H$ 6.07 (H-3) and $\delta_H$ 2.96 (H-1′) has HSQC interactions with carbon of $\delta_C$ 105.8 and 34.6, respectively. The two methyl groups appear as doublet signals at $\delta_H$ 1.36 (6H, d, $J$ = 6.6 Hz) along with the methine group signal ($\delta_H$ 2.96, sept.) indicating the presence of an isopropyl group [15]. NMR spectrum data of compound **SL1** (Table 4.1) shows similarities with data of compound 5-hydroxy-7-methoxy-2-isopropyl-8-methylchromone. Therefore, it can be predicted that compound **SL1** has a chromen-4-one framework [15]. The isopropyl branch was determined to be bound to C-2 through analysis of HMBC spectral data, with the appearance of interactions between protons H-2′ and H-3′ ($\delta_H$ 1.36) with C-2 ($\delta_C$ 176.4)/C-1′ ($\delta_C$ 34.6), between H-1′ ($\delta_H$ 2.96) and C-2 and C-3 ($\delta_C$ 105.8), and between H-3 ($\delta_H$ 6 .07) with C-4 ($\delta_C$ 184.8)/C-2/C-1′.

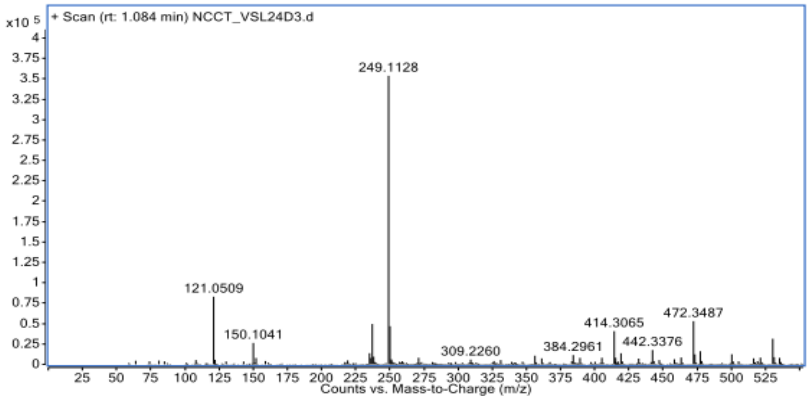



*Figure 0.2. (+)-HR-ESI-MS của hợp chất **SL1***

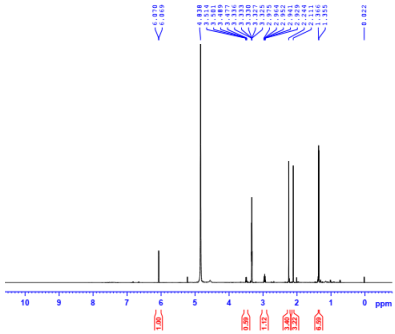


*Figure 0.3. $^{1}H$ NMR spectrum of **SL1***

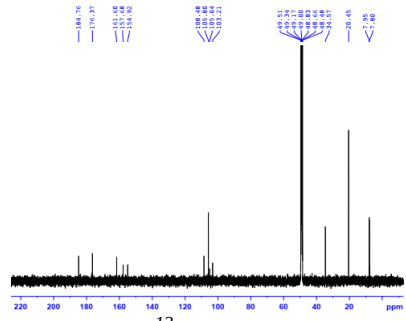


*Figure 0.4. $^{13}C$ NMR spectrum of **SL1***

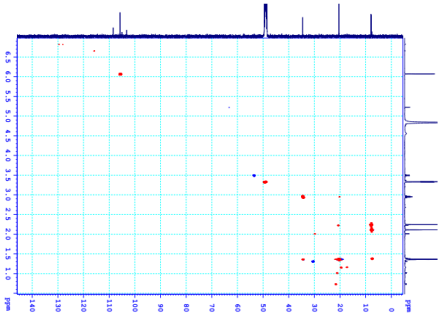


*Figure 0.5. HSQC spectrum of **SL1***

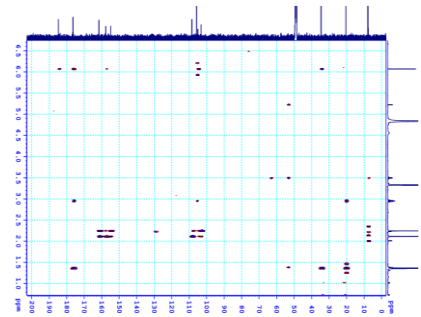


*Figure 0.6. HMBC spectrum of **SL1***

Two methyl groups were identified attached to carbons C-6, C-8 and two hydroxyl groups attached to C-5 and C-7 by HMBC interaction signals between 6-CH3 protons ($\delta_H$ 2.11) with C -5 ($\delta_C$ 157.7)/C-6 ($\delta_C$ 108.5)/C-7 ($\delta_C$ 161.6) and between the proton of 8-CH3 ($\delta_H$ 2.24) and C-7/C-8 ( $\delta_C$ 103.2)/C-9 ($\delta_C$ 154.9), From the above spectral data analysis, compound **SL1** was identified as 5,7-dihydroxy-2-isopropyl-6,8-dimethyl-4H chromen-4-one. This is a new compound.

By combining analysis of HR-ESI-MS spectrum data, 1-dimensional and 2-dimensional NMR spectra, and similar CD spectra, the structures of 20 compounds isolated from *S. cerasiforme*

were determined, including 2 new compounds **SL1** and **SL2** (Figre 4.34)

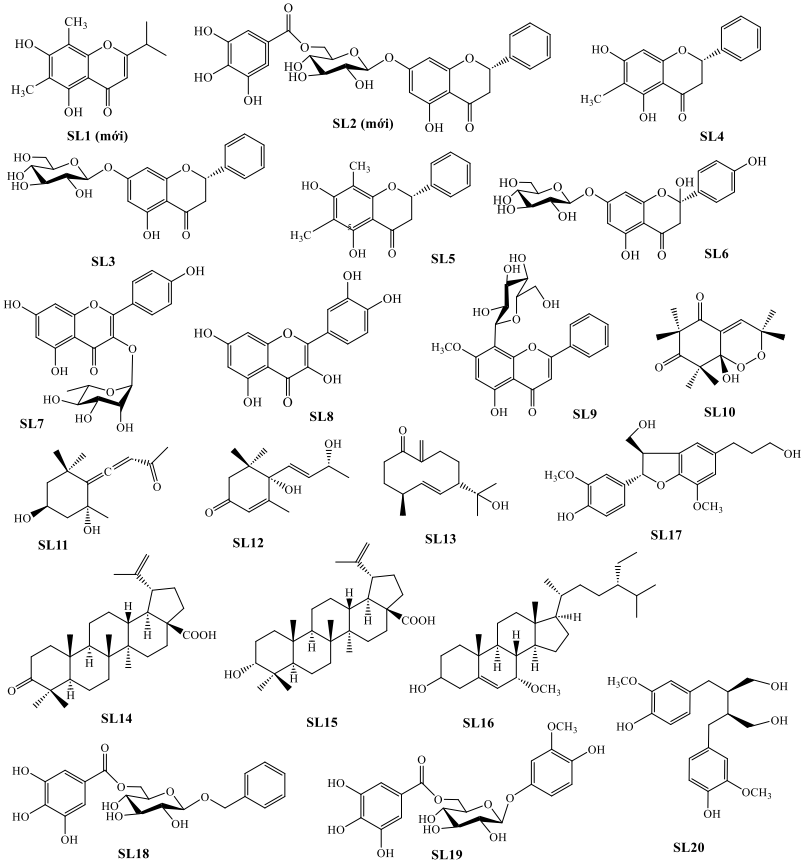



*Figure 4.34. Chemical structures of compounds **SL1-SL20***

The new compound **SL1** (5,7-dihydroxy-2-isopropyl-6,8-dimethyl-4H chromen-4-one) has a chromone skeleton structure and the compound **SL2** (5,7-dihydroxyflavanone 7-*O*-*β*-D -(6″-*O*-galloyl glucopyranoside) is a galloyl glycoside derivative of flavanone with the novelty of a galloyl branch added to the sugar moiety. The absolute configuration of the C-2 stereogenic center was determined

to be 2S through through CD circular dichroism spectrum data. Structures **SL1, SL2** were found for the first time from *S. cerasiforme*. The known compounds **SL3-SL9** are compounds belonging to the flavonoid group in which **SL3-SL6** are compounds flavanone and glycoside derivatives of flavanone, compounds **SL7, SL8** are compounds with flavonol frame, **SL9** has flavonone frame. Three compounds **SL14-SL16** have terpenoid frame. The remaining compounds belong to different frame groups.

### *4.1.2. The NO production inhibitory activity of compounds isolated from S. cerasiforme*

Results in Table 3.1 shows that all compounds did not show significant toxicity in the cytotoxicity assay that using the MTT colorimetric method. Compounds **SL1, SL2, SL5, SL6, SL10, SL17** have the ability to effectively inhibit NO production on LPS-activated cell lines with $IC_{50}$ inhibitory concentrations in the range of 6.69 - 12.28. µM and **SL19** inhibited an average $IC_{50}$ of 25.51 µM compared to the positive control L-NMMA with an $IC_{50}$ value of 32.50 µM. The remaining compounds showed weaker inhibitory activity with $IC_{50}$ values ranging from 33.17-86.51 µM. Considering the relationship between the structure and anti-inflammatory effects of the isolated compounds, it can be concluded that flavanone compounds have more significant NO production inhibitory activity than flavone compounds.

## 4.2. Chemical composition and inhibitory activity on NO production of *S. bullockii*.

### *4.2.1. Determination of chemical structures of compounds isolated from S. bullockii*

*4.2.1.2. Compound **SP1:** Syzybulloside A (2α,3α,6α-trihydroxyurs-12,20(30)-dien-28-oic acid 28-O-α-D-glucopyranosyl ester)*

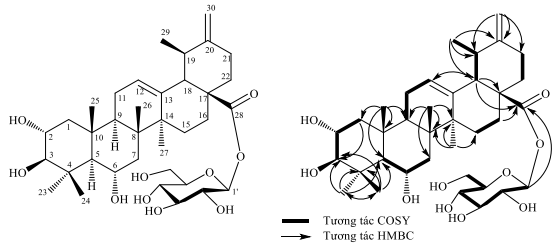



*Figure 0.34. Chemical structure and important HMBC and COSY correlations of **SP1***

*Table 0.18. The NMR data of **SP1***

| C | $\delta_C$[a,b] | $\delta_H$[a, c] (mult *J* = Hz) | C | $\delta_C$[a,b] | $\delta_H$[a, c] (mult *J* = Hz) |
|---|---|---|---|---|---|
| 1 | 47.9 | 0.94 (dd, 14.4, 9.5) *ax*<br>1.93 (dd, 14.4, 4.5) *eq* | 19 | 38.4 | 2.46 (m) |
| 2 | 69.1 | 3.63 (ddd, 10.0, 9.5, 4.5) *ax* | 20 | 154.2 | - |
| 3 | 84.6 | 2.89 (d, 10.0) *ax* | 21 | 33.2 | 2.25 (m), 2.37 (m) |
| 4 | 41.0 | | 22 | 39.7 | 1.67 (m), 1.92 (m) |
| 5 | 61.3 | 1.01 (d, 10.5) *ax* | 23 | 32.2 | 1.35 (s) |
| 6 | 68.8 | 3.99 (ddd, 10.5, 10.5. 4.0) *ax* | 24 | 17.5 | 1.04 (s) |
| 7 | 45.5 | 1.60 (m), 1.64 (m) | 25 | 18.0 | 1.09 (s) |
| 8 | 42.0 | - | 26 | 19.1 | 0.93 (s) |
| 9 | 48.4 | 1.61 (dd, 9.5, 5.5) | 27 | 23.9 | 1.24 (s) |
| 10 | 41.3 | - | 28 | 177.2 | - |
| 11 | 25.4 | 1.98 (m), 2.00 (m) | 29 | 16.7 | 1.05 (d, 7.0) |
| 12 | 127.4 | 5.31 (br t, 3.5) | 30 | 105.5 | 4.71 (brs), 4.66 (brs) |
| 13 | 138.8 | - | 1′ | 95.8 | 5.37 (d, 7.5) |
| 14 | 43.6 | - | 2′ | 73.9 | 3.32 (dd, 9.0, 7.5) |
| 15 | 29.2 | 1.18 (m), 2.03 (m) | 3′ | 78.3 | 3.42 (t, 9.0) |
| 16 | 25.2 | 1.90 (m), 2.31 (m) | 4′ | 71.2 | 3.37 (t, 9.0) |
| 17 | 49.5 | - | 5′ | 78.6 | 3.34 (m) |
| 18 | 56.3 | 2.33 (d, 11.0) | 6′ | 62.4 | 3.67 (dd, 12.0, 5.0)<br>3.81 (dd, 12.0, 2.0) |

*[a]measure in $CD_3OD$, [b]125MHz, [c]500M*

Compound **SP1** is a colorless powder. The IR spectrum of compound **SP1** showed the presence of functional groups hydroxy (3401 $cm^{-1}$), carboxyl (1734 $cm^{-1}$), olefinic 1646 $cm^{-1}$) and ether 1070 $cm^{-1}$), On the HR-ESI-MS high-resolution mass spectrum of **SP1**, a

pseudo molecular ion peak *m/z* 683.3563 $[M+Cl]^-$ ($\Delta$ = - 0.6 ppm) appeared combined with $^{13}C$ NMR spectral data. and HSQC allows determining the molecular formula of **SP1** as $C_{36}H_{56}O_{10}$. The $^1H$, $^{13}C$ NMR and HSQC spectra of **SP1** showed characteristic signals of a 5-ring, 6-membered triterpene and 6 carbon of a hexose sugar unit (Table 4.18), The NMR spectrum data of **SP1** are quite similar to the corresponding data of the compound Syzygiumursanolide C (**SP4**) except for the appearance of a signal of a methyl group replacing the hydroxymethylene group at C-23 [16]. The glucose lin moiety linked to the aglycone at the position of the ketone carbon (C-28) was determined based on the interaction on the HMBC spectrum between the anomer proton H-1′ ($\delta_H$ 5.37) with C-28 ($\delta_C$ 177.2 ), Three hydroxy groups were identified to be attached to carbon at C-2, C-3 and C-6 based on the HMBC interaction between H-23 ($\delta_H$ 1.35)/H-24 ($\delta_H$ 1.04) with C- 3 ($\delta_C$ 84.6)/C-4 ($\delta_C$ 41.0)/ C-5 ($\delta_C$ 61.3) and COSY interactions between H-3 ($\delta$H 2.89)/H-2 ($\delta_H$ 3) protons .63) and H-5 ($\delta_H$ 1.01)/H-6 ($\delta_H$ 3.99), The bond orientation of the hydroxy groups is determined through the interaction constant and spatial interaction between protons on the ROESY spectrum. The large interaction constants $^3J_{HH}$ = 9.5 Hz for H-2/H-3 and $^3J_{HH}$ = 10.5 Hz for H-5/H-6 indicate H-2/H-3 and H-5/ protons. H-6ax has a *trans*/*axial* orientation. Spatial interactions of protons on the ROESY spectrum between $H_{ax}$-2 ($\delta_H$ 3.63)/H3-24 ($\delta_H$ 1.04), H-3 ($\delta_H$ 2.89 )/H3-23 ($\delta_H$ 1.35), H-3/H-5 ($\delta_H$ 1.01), H-6 ($\delta_H$ 3.99)/H3-25 ($\delta_H$ 1.35) and H-6/ H3-26 ($\delta_H$ 0.93) determined the $\alpha$ configuration of H-2 and H-6 and the $\alpha$ configuration of H-3. The interactions on the ROESY spectrum mentioned above show *trans* bonds between rings A/B, B/C of the 5-ring triterpene framework of the ursane framework.

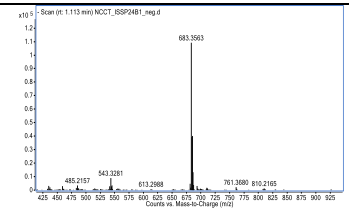



*Figure 0.35. The (-)-HR-ESI-MS spectrum of* ***SP1***

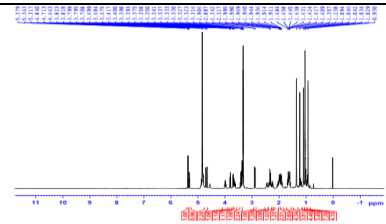


*Figure 0.36. The $^{1}H$ NMR spectrum of* ***SP1***

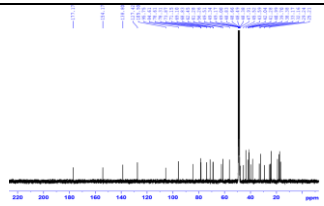


*Figure 0.37. The $^{13}C$ NMR spectrum of* ***SP1***

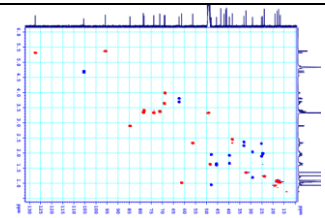


*Figure 0.38. HSQC spectrum of* ***SP1***

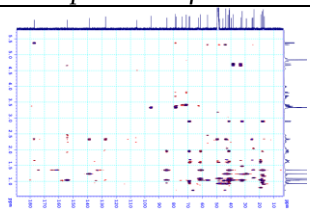


*Figure 0.39. HMBC spectrum of* ***SP1***

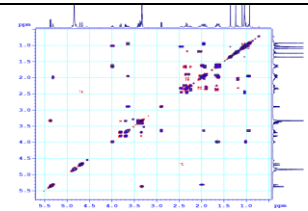


*Figure 0.40. $^{1}H$-$^{1}H$ COSY spectrum of* ***SP1***

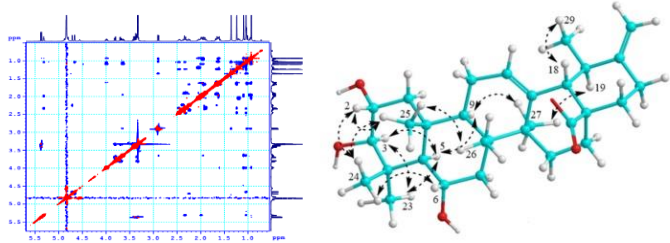



*Figure 0.41. The NOESY spectrum and the important correlations of* ***SP1***

The interaction between $H_3$-27 ($\delta_H$ 1.24) and H-19 ($\delta_H$ 2.46) has determined the two D/E rings coupled together in *cis* and the binding

orientation of the two protons H-27 and H-19 is *α*/*axial*. Based on the binding orientation of H-19 (*α*/*axial*), the H3-29 proton was determined to be *α*-equatorial. Then the ROESY interaction between H3-29 and H-18 determined the *α*-orientation of H-18.The glycoside bond was also determined to be *α*-form through the large interaction constant $J$ = 7.5 Hz of the anomer proton at $\delta_H$ 5.37 and the interaction constant between carbinol protons in the sugar portion including $J_{H\text{-}1'/H\text{-}2'}$ = 7.5 Hz, $J_{H\text{-}2'/H\text{-}3'}$ = 9.0 Hz, $J_{H\text{-}3'/H\text{-}4'}$ = 9.0 Hz and $J_{H\text{-}4'/H\text{-}5'}$ = 9.0 Hz determined that protons H-1′, H-2′, H-3′, H-4′, H-5′ all occupy the *axial* position, characteristic of a *α*-glucopyranosyl branch. The sugar part was determined to be D-glucose by acid hydrolysis of compound **SP1** to obtain glucose and determine the specific polar rotation of this sugar. The positive rotation angle signal of the sugar molecule (+27.6) was obtained, confirming the D-glucose sugar structure [17]. From NMR spectrum data, compound **SP1** was identified as 2*α*,3*α*,6*α*-trihydroxyurs-12,20(30)-dien-28-oic acid 28-*O*-*α*-D-glucopyranosyl ester. This is a new compound and was named syzybulloside A.

The structure determination of 17 compounds (**SP1-SP17**) isolated from *S. bullockii* was similarly performed by analyzing spectral data and comparing with reference materials. As a result, 3 new compounds **(SP1-SP3**) named syzybulloside (A-C) and 14 known compounds (**SP4-SP17**) were identified. The three new compounds all have a 5-ring triterpene framework in which the methyl group at position C-23 has replaced the hydroxymethylene group, which is new and different from the known 5-ring triterpene compound **SP4-SP6**. The relative configurations of the new compounds were determined using the ROESY spectroscopy method. The bonding

orientation of hydroxy groups at positions C-2, C-3, C-6 are 2*α*, 3*β*, 6*α*, respectively. The remaining compounds have structures of flavanols (**SP15, SP16**), megastigmanes and derivatives (SP10-SP13), and other structures (**SP7, SP8**), The chemical structures of the compounds are shown in Figure 4.63.

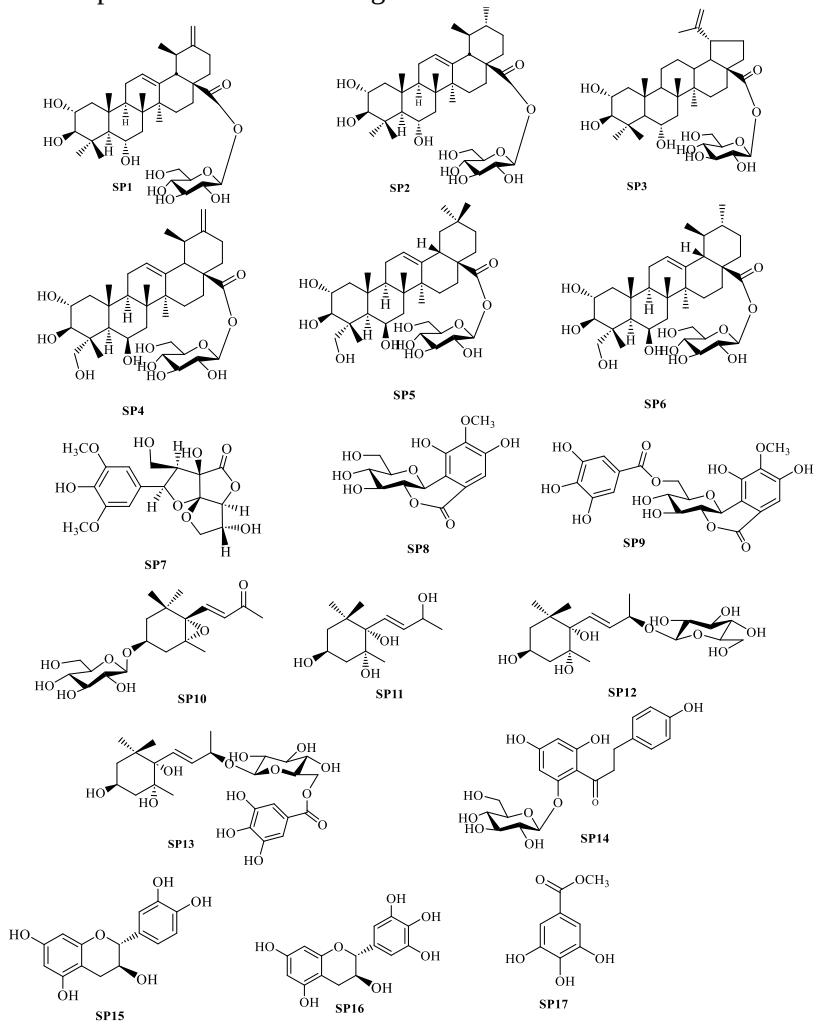



*Figure 4.63. Chemical structures of compounds **SP1-SP17***

### *4.2.2. The NO production inhibitory activity of compounds isolated from S. bullockii*

In the MTT assay, at the concentration of 100 µM, the compounds **SP1-SP17** did not cause cytotoxicity. From the results in Table 3.2, it shows that the isolated compounds (except the weakly active compound **SP11**) all show the ability to effectively inhibit NO production on the RAW 264.7 cell line with activated Lipopolysaccharide at high concentrations. The average inhibition IC50 ranges from 1.42 to 13.70 µM, much lower than the positive control L-NMMA ($IC_{50}$ = 33.8 µM),

## 4.3. Chemical composition and inhibitory activity on NO production of *S. attopeuense*

### *4.3.1. Determination of chemical structures of compounds isolated from S. attopeuense*

From *S. attopeuense*, 17 compounds (**SA1-SA17**) were isolated. Through analysis of mass spectrometry data, 1- and 2-dimensional nuclear magnetic resonance spectroscopy, it was identified that among the 17 compounds, there are 4 new substances (**SA1-SA4**) named syzyceroside A-D (**SA1-SA4**) and 13 known compounds (**SA5-SA17**). The chemical structures of the compounds are shown in Figure 4.85.

### *4.3.2. The NO production inhibitory activity of compounds isolated from S. attopeuense*

Seventeen compounds **SA1-SA17** did not show toxicity on the tested cell lines. The anti-inflammatory activity test results in Table 3.3 show that compounds **SA1-SA3**, **SA5** and **SA6** are stilbene derivatives capable of inhibiting NO production on the RAW 264.7 cell line that has been activated by LPS with $IC_{50}$ values: 18.37 ± 1.38, 31.23 ± 2.18, 35.12 ± 2.53, 28.24 ± 1.79 and 34.89 ± 2.13µM, respectivity. These data compared to the control Dexamethasone has an $IC_{50}$ of 15.37 ± 1.42 µM.

The new compound **SA1** with configuration of *Z* (*cis*) at the C7/C8 double bond shows much better activity than compounds **SA2, SA3, SA5** and **SA6** with configuration of *E* (*trans)* structure. Compounds **SA7, SA8, SA10, SA15, SA16** very weakly inhibit NO production with $IC_{50}$ values ranging from 76.39 to 95.14. The remaining compounds are considered to have no anti-inflammatory activity because the $IC_{50}$ is greater than 100 µM. This result shows that stilbene plays an important role in inhibiting NO production on LPS-stimulated RAW264.7 cell line.

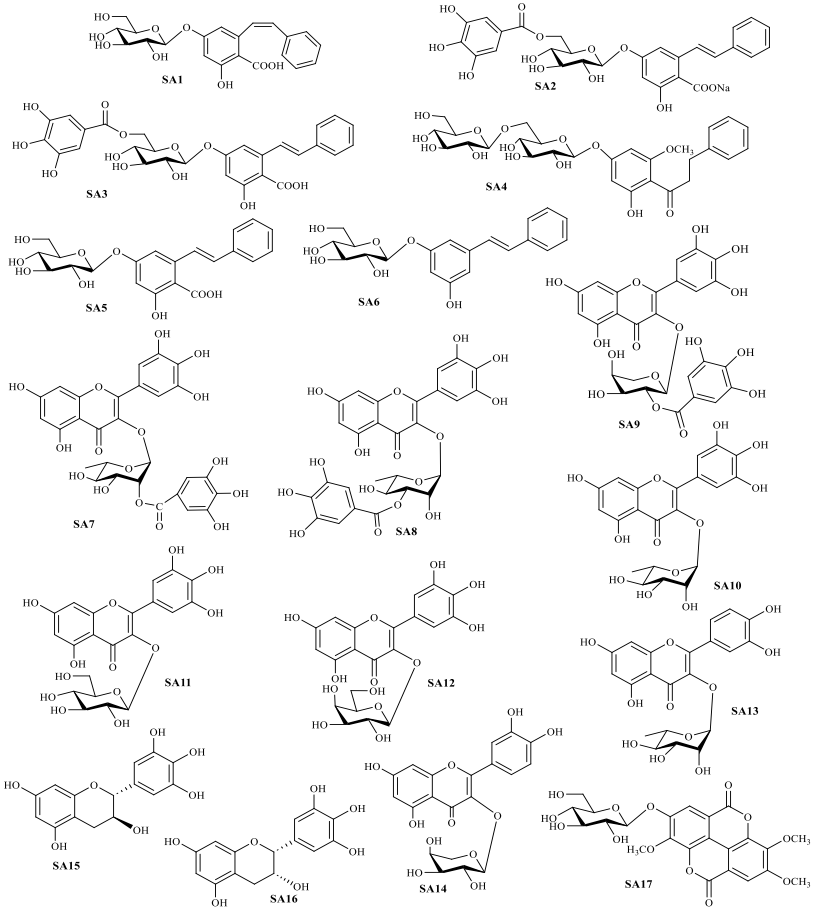



*Figure 4.85. Chemical structures of compounds **SA1-SA17***

## CONCLUSIONS

This is the first study on the chemical composition and anti-inflammatory activity through inhibiting NO production on the RAW264.7 cell line of three species *S. cersiforme* collected in Vinh Phuc province, *S. bullockii* and *S. attopeuense* collected in Quang Tri province. Using a combination of chromatographic and modern spectroscopic methods, 54 compounds were isolated and determined, including 9 new compounds from 3 species: *S. cerasiforme*, *S. bullockii* and *S. attopeuense*.

**1. Research on chemical composition**

- From the leaves of *S. cerasiforme*, 20 compounds (**SL1-SL20**) were isolated and determined, including 2 new compounds **SL1** (5,7-dihydroxy-2-isopropyl-6,8-dimethyl-4H chromen-4-one), **SL2** (5,7-dihydroxyflavanone 7-*O*-*α*-D-(6″-*O*-galloyl glucopyranoside) and 18 known compounds (**SL3-SL20**): pinocembrin-7-*O*-*α*-D-glucopyranoside, strobinin, demethoxymatteucinol, (2S)-hydroxynaringenin-7-*O*-*α*-D-glucopyranoside, afzelin, quercetin, kaplanin, endoperoxide G3, vomifoliol, litseagermacrane, 3-epibetulinic acid, betulonic acid, schleicheol 2, (7S,8R)-dihydrodehydrodiconiferyl alcohol, benzyl-6′-*O*-galloyl-*α*-D-glucopyranoside, 3-methoxy-4-hydroxyphenol 1-*O*-*α*-D- (6′-*O*-galloyl)-glucopyranoside, secoisolariciresinol.

- From the leaves and branches of *S. bullockii*, 17 compounds (**SP1**-**SP17**) were isolated and structurally determined, including 3 new compounds (**SP1**-**SP3**) named syzybulloside (A-C) and 14 known compounds. known (**SP4-SP17**): chebuloside II, 2*α*,3*α*,6*α*,23-tetrahydroxyurs-12-en-28-oic acid 28-*O*-*α*-D-glucopyranosyl ester, amarusine A, bergenine, 11-*O*-galloylbergenin, icariside B2, (3*S*,5*R*,6*S*,7*E*,9*S*)-megastigman-7-ene-3,5,6,9-tetraol,

actinidioionoside, actinidioionoside 6′-*O*-gallate, phloridzoside, (+)-catechin, ( +)-gallocatechin, methyl gallate.

- From the leaves and branches of *S. attopeuense*, 17 compounds (**SA1-SA17**) were isolated and determined, including 4 new substances (**SA1-SA4**) named syzyceroside A-D (**SA1-SA4**) and 13 new compounds (**SA1-SA4**) known substances: quadranoside IV, pinosilvin 3-*O*-*α*-D-glucopyranoside, gaylussacin, myricetin-3-*O*-(2′′-*O*-galloyl)-*α*-L-rhamnopyranoside, myricetin-3-*O*-(3′′-*O*-galloyl)-*α*-L-rhamnopyranoside, myricetin 3-*O*-*α*-D-(2′′-galloyl)-*α*-arabinopyranoside, myricetin-3-*O*-*α*-L-rhamnopyranoside, myricetin-3-*O*-*α*-D-glucopyranoside, myricetin-3-*O*-*α*-D-galactopyranoside, quercitrin, guaijaverin, (+)-gallocatechin, ellagic acid 3,3',4'-tri-*O*-methyl ether 4-*O*-*α*-D -glucopyranoside.

**2. Research on the NO production inhibitory activity**

The inhibitory activity of NO production of 54 compounds isolated from three species of *S. cerasiforme, S. bullockii, S. attopeuense*, have been evaluate for anti-inflammatory ability on the RAW264.7 cell line that stimulated by LPS. The results showed that flavanone compounds have more significant inhibitory activity on NO production than flavone compounds, terpenoid compounds show good activity and glycosidlse derivatives of stilbene plays an important role in inhibiting NO production in LPS-stimulated RAW264.7 cell line.

- Among the 20 compounds from *S. cerasiforme*, compounds **SL1**, **SL2**, **SL5**, **SL6**, **SL10, SL17** showed significantly NO inhibition with $IC_{50}$ value ranging from 6.69 to 12.28 µM. Compound **SL19** showed moderate inhibition ($IC_{50}$ 25.51 µM) compared to the positive control, L-NMMA ($IC_{50}$ 32.50 µM). The remaining compounds showed weaker inhibitory activity with $IC_{50}$ values ranging from 33.17 to 86.51 µM.

- Among the 17 compounds isolated from *S. Bullockii*, except for **SP11**, the remaining compounds showed significantly activity with $IC_{50}$ values ranging from 1.42 to 13.70 µM, compared to the positive control compound, L-NMMA, $IC_{50}$ = 33.8 µM.

- Among the 17 compounds isolated from *S. attopeuense*, compounds **SA1-SA3, SA5** and **SA6** exhibited moderate inhibition activity with $IC_{50}$ values ranging from 18.37 to 35.12 µM compared to the positive control compound, dexamethasone, $IC_{50}$ 15.37 µM). Compounds **SA7, SA8, SA10, SA15, SA16** showed weakly activity with $IC_{50}$ values ranging from 76.39 to 95.14 µM. The remaining compounds were inactive with $IC_{50}$ > 100 µM.

## NEW CONTRIBUTIONS OF THE THESIS

- This is the first study on the chemical constituents and thier NO production inhibition activity of *S. cerasiforme, S. bullockii, S. attopeuense*.

- Isolation and identification of 2 new compounds from *S.cerasiforme*: **SL1** (5,7-dihydroxy-2-isopropyl-6,8-dimethyl-4H chromen-4-one) and **SL2** (5,7-dihydroxyflavanone 7-*O*-*α*-D-(6″-*O*-galloyl glucopyranoside). These new compounds showed significantly inhibitory activity with $IC_{50}$ values of 12.28 and 8.52 µM, respectively.

- Isolation and identification of 3 new compounds from *S. bullockii* (syzybulloside A-C). These new compounds presented strongly NO inhibitory activity with $IC_{50}$ values of 11.58, 13.61 and 6.93, respectively. In addition, **SP4** also showed significantly inhibition activity with $IC_{50}$ = 7.09 µM.

- Isolation and identification of 4 new compounds from *S. attopeuense* (syzyceroside A-D). Compounds **SA1** (syzyceroside A), **SA2** (syzyceroside B), **SA3** (syzyceroside C) showed moderate inhibitory activity, whereas, **SA4** (syzyceroside D) was inactivity.

# LIST OF THE PUBLICATIONS RELATED TO THE DISSERTATION

1. **Bui Hai Ninh**, Duong Thi Dung, Bui Huu Tai, Pham Hai Yen, Nguyen Xuan Nhiem, Truong Thi Thu Hien, Do Thi Trang, Nguyen Van Tuyen, Le Tuan Anh, Nguyen Thi Hoai, Phan Van Kiem. *New isopropyl chromone and flavanone glucoside compounds from the leaves of Syzygium cerasiforme (Blume) Merr. & L.M.Perry and their inhibition of nitric oxide production*. Chemistry & Biodiversity, 2023, doi.org/10.1002/cbdv.202201048.
2. Bui Huu Tai, **Bui Hai Ninh**, Pham Hai Yen, Duong Thi Dung, Nguyen Huy Hoang, Nguyen Xuan Nhiem, Nguyen Van Tuyen, Le Tuan Anh, Phan Van Kiem. *New nitric oxide production inhibitors from Syzygium bullockii*. Journal of Natural Medicines, 2023, doi: 10.1007/s11418-023-01725-7.
3. Phan Van Kiem, **Bui Hai Ninh**, Bui Huu Tai, Nguyen Xuan Nhiem, Pham Thi Hai Yen, Nguyen Huy Hoang, Do Thi Trang, Duong Thi Dung, Nguyen Van Tuyen, Le Tuan Anh. *Undescribed phenolic glycoside from Syzygium attopeuense and their inhibition of nitric oxide production*. Chemistry & Biodiversity, 2023, doi: 10.1002/cbdv.202301037.
4. **Bui Hai Ninh**, Duong Thi Dung, Nguyen Van Tuyen, Bui Huu Tai, Phan Van Kiem. *Chemical constituents of Syzygium cerasiforme leaves and their nitric oxide inhibitory activity in LPS-activated RAW264.7 cells*. Vietnam Journal of Chemistry, 2023, doi: 10.1002/vj*ch*.202300107.