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RESEARCH ON CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF TWO SPECIES *IMPATIENS CHAPAENSIS* AND *IMPATIENS PARVISEPALA*

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PREFACE

1. The urgency of the thesis

Traditional medicine is a significant and frequently appreciated component of healthcare. It has a long history of use in each nation for maintaining health and treating and preventing disease. Many herbal remedies with good clinical effects but have not been studied in depth for their chemical compositions, pharmacological effects, and toxicity. Research to exploit, inherit, apply, and develop medicinal plant resources has been and will be a matter of great scientific, economic, and social significance in our country.

The development of modern science offers a great chance to further analyze medicinal herbs, chemical components, and pharmacological effects through experimental studies. Many reports were annually published in domestic and foreign scientific journals across isolating biological compounds from different medicinal plants, which serve as the scientific basis for the use of folk remedies in treatment effectively, as well as to promote the development of the medicinal and pharmaceutical fields in healthy improving and serving people's life.

Impatiens species (Balsaminaceae family) were known in folk medicine for the treatment of various diseases related to inflammation and diabetes in many parts of the world. The results of chemical and biological studies of these plants showed a large number of new secondary metabolites with interesting structures exhibiting valuable biological activities such as anti-inflammatory, hypoglycemic, anti-cancer, antioxidant,…However, there are no reports on *Impatiens* species growing in Vietnam in spite of the fact that these herbs have been widely used for the treatment of various diseases in Vietnamese folk medicine.

Based on above-mentioned scientific basis, we chose the topic "Research on chemical constituents and biological activities of two species *Impatiens chapaensis* and *Impatiens parvisepala*".

2. Research aims of the thesis

- To study the chemical constituents of two *Impatiens* species, including: Moc tai Sapa (*Impatiens chapaensis*) and Bong nuoc dai hoa nho (*Impatiens parvisepala*).
- To search for the anti-inflammatory and hypoglycemic compounds from these two species.

3. Research content of the thesis

- To study the isolation of compounds from two *Impatiens* species: *I. chapaensis* and *I. parvisepala*;
- To study the structure determination of isolated compounds;
- To evaluate the anti-inflammatory activity (via NO inhibition effect) of isolated compounds;
- To evaluate the hypoglycemic activity (via α-glucosidase inhibition effect) of isolated compounds.

CHAPTER 1. OVERVIEW

The overview summarized the domestic and foreign studies on the following problems:

1.1. Overview of the *Impatiens* genus

1.1.1. Botanical features of the Impatiens plants

1.1.2. Traditional medicinal use of the Impatiens plants

1.1.3. Overview of the chemical compositons of the Impatiens genus

1.1.4. Overview of the biological activities of the Impatiens genus

1.2. Overview of two species: Moc tai Sapa (*Impatiens chapaensis*) and Bong nuoc dai hoa nho (*Impatiens parvisepala*)

1.2.1. Moc tai Sapa (Impatiens chapaensis)

1.2.2. Bong nuoc dai hoa nho (*Impatiens parvisepala*)

CHAPTER 2. RESEARCH MATERIALS AND METHODS

2.1. Plant samples

In the thesis, the materials used for study were two plant samples:

❖ Sample 1: The whole plant of *Impatiens chapaensis* Tard. was collected in Hoanglien national park, Sapa district, Laocai province,

Vietnam in October 2019. A voucher specimen VHH.SP 10.2019.1 was deposited in the Institute of Chemistry, VAST, Hanoi, Vietnam.

❖ Sample 2: The whole plant of *Impatiens parvisepala* S. X. Yu & Y. T. Hou was collected in Phia Oac-Phia Den national park, Nguyenbinh district, Caobang province, Vietnam in May 2020. A voucher specimen VHH.CB 05.2020.1 was deposited in the Institute of Chemistry, VAST, Hanoi, Vietnam.

The scientific name of all these two plants were identified by Assoc. Prof. Dr. Vu Tien Chinh, Vietnam National Museum of Nature, VAST, Hanoi, Vietnam.

2.2. Research methods

2.2.1. Extraction and isolation methods

- Extraction method: using the total extraction method, illustrated in Figure 2.3 and Figure 2.7.

- Isolation method: using column chromatographic method with different adsorbents like silica gel, sephadex LH-20, and RP-18 with suitable solvent systems (Figure 2.4, 2.5, 2.6, 2.8, 2.9).

2.2.2. Methods of structure determination

Chemical structures of isolated compounds were elucidated by combining modern spectroscopic methods such as one-dimensional (^{1}H- và ^{13}C-NMR), two-dimensional (HSQC, HMBC, COSY và NOESY) nuclear magnetic resonance spectroscopy, mass spectra (ESI-MS, HR-ESI-MS) and CD spectra.

2.2.3. Methods of biological evaluation

2.2.3.1. Anti-inflammatory activity

The anti-inflammatory activity was evaluated through the inhibitory effect of NO production of RAW 264.7 cells with the use of N^{G}-methyl- L-arginine acetate (L-NMMA) as positive compound. The results were determined based on the IC_{50} value.

2.2.3.2. Hypoglycemic activity

The hypoglycemic activity was evaluated through the α-glucosidase inhibitory effect using acarbose as a positive control. The results were determined based on the IC_{50} value.

2.3. Extraction and isolation of compounds from two investigated plants

2.3.1. I. chapaensis

2.3.1.1. Plant sample preparation and extraction

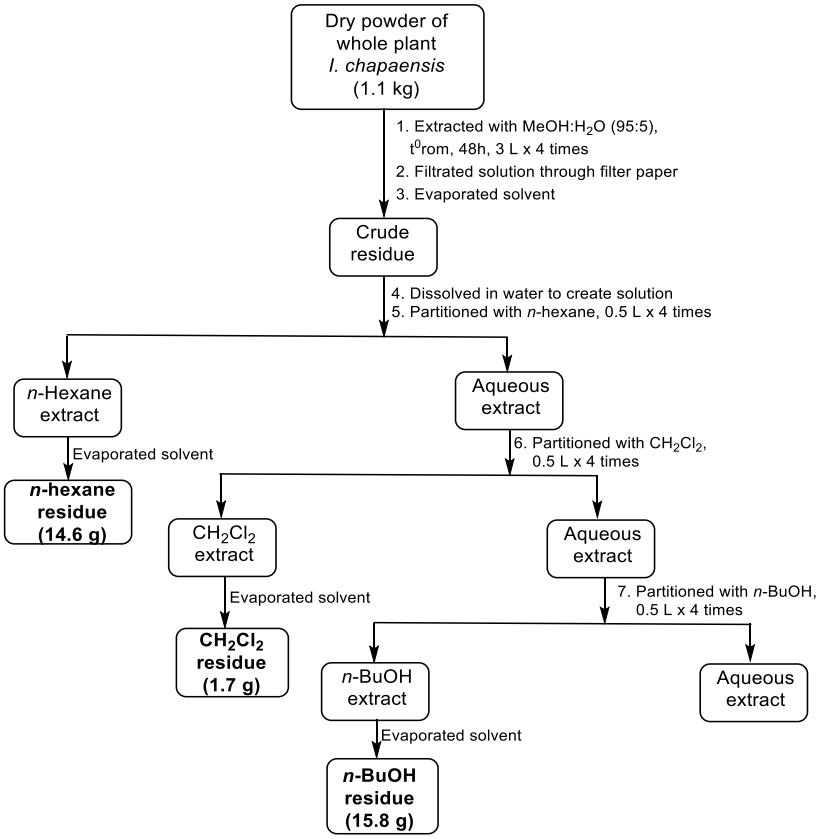
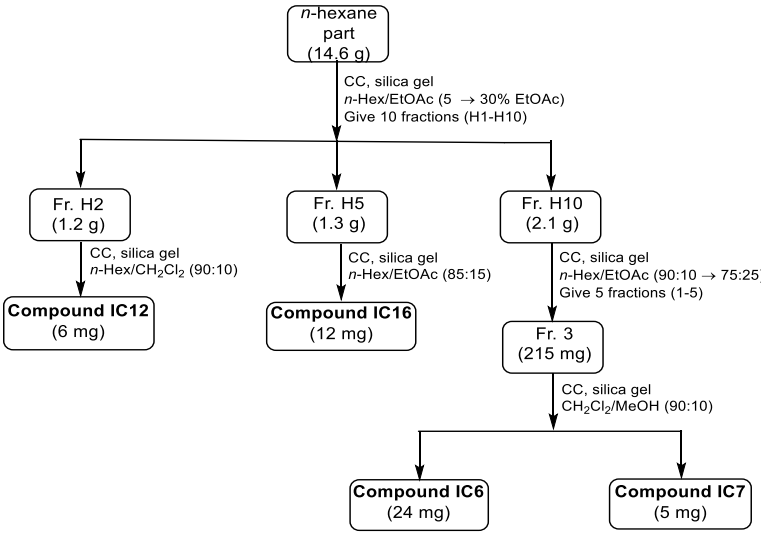


Figure 2.1. Plant sample preparation and extraction of *I. chapaensis*

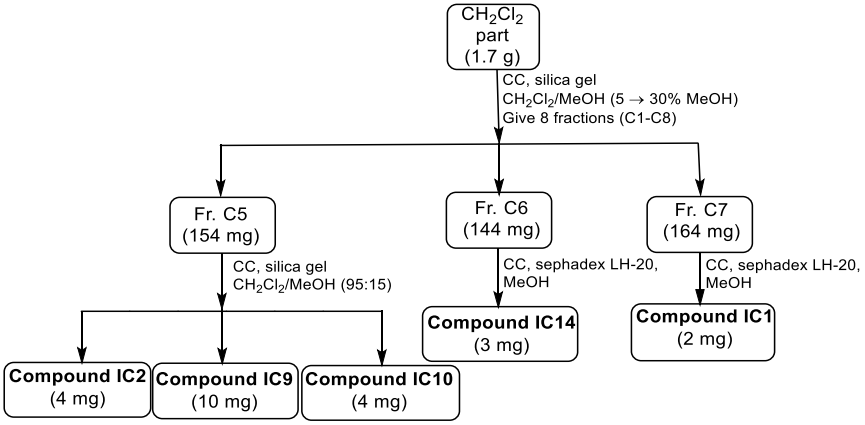
2.3.1.2. Procedures of compound isolation

❖ Isolation of compounds from *n*-hexane extract:



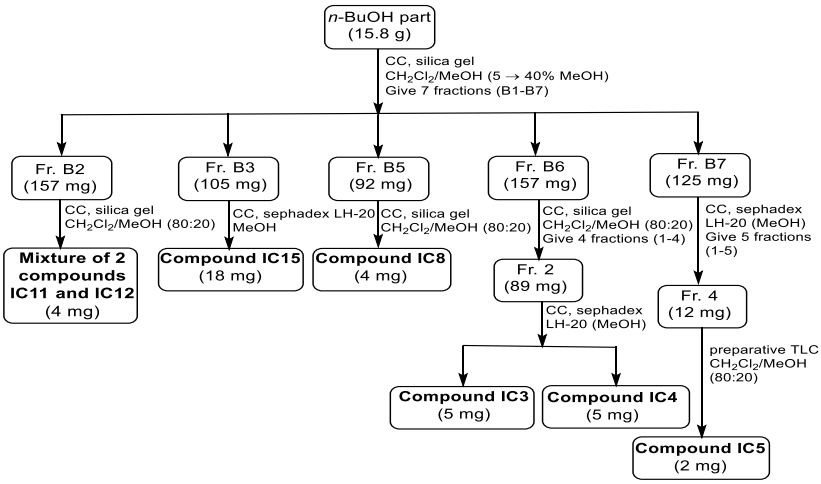
Hình 2.2. Isolation of compounds from *n*-hexane extract of *I. chapaensis*

❖ Isolation of compounds from dichloromethane extract:



Hình 2.3. Isolation of compounds from CH_2Cl_2 extract of *I. chapaensis*

❖ Isolation of compounds from *n*-BuOH extract:



Hình 2.4. Isolation of compounds from *n*-BuOH extract of *I. chapaensis*

2.3.1.3. Spectroscopic data of compounds isolated from I. chapaensis

❖ Compound **(*S*)-naringenin** (**IC1**): colorless gum, 2 mg (0.00018% of the dry sample weight), R_f = 0.5 (*n*-hexane/EtOAc = 1/1), (–)-ESI-MS: *m/z* 271.8 $[M-H]^-$), molecular formula $C_{15}H_{12}O_5$ (M = 272.0);

^{1}H-, ^{13}C-NMR (500/125 MHz, CD_3OD) (Table 3.1).

❖ Compound **(*S*)-pinocembrin** (**IC2**): yellow crystal, 4 mg (0.00036% of the dry sample weight), R_f = 0.5 (*n*-hexane/EtOAc = 3/1), molecular formula $C_{15}H_{12}O_4$ (M= 256.0);

^{1}H-, ^{13}C-NMR (500/125 MHz, CD_3OD) (Table 3.2).

❖ Compound **kaempferol** (**IC3**): yellow powder, 5 mg (0.00045% of the dry sample weight), R_f = 0.45 (CH_2Cl_2/MeOH = 95/5), molecular formula $C_{15}H_{10}O_6$ (M= 286.0);

^{1}H-, ^{13}C-NMR (500/125 MHz, CD_3OD) (Table 3.3).

❖ Compound **quercetin** (**IC4**): yellow powder, 5 mg (0.00045% of the dry sample weight), R_f = 0.35 (CH_2Cl_2/MeOH = 95/5), molecular formula $C_{15}H_{10}O_7$ (M = 302.0);

^{1}H-, ^{13}C-NMR (500/125 MHz, CD_3OD) (Table 3.4).

❖ Compound **(±)-3',5',5,7-tetrahydroxyflavanone** (**IC5**): yellow powder, 2 mg (0.00018% of the dry sample weight), R_f = 0.40 (CH_2Cl_2/MeOH = 95/5), molecular formula $C_{15}H_{10}O_6$ (M = 288.0);

^{1}H-, ^{13}C-NMR (500/125 MHz, CD_3OD) (Table 3.5).

❖ Compound **phlorizin** (**IC6**): yellow oil, 24 mg (0.00218% of the dry sample weight), R_f = 0.60 (EtOAc/MeOH = 4/1), molecular formula $C_{21}H_{24}O_{10}$ (M = 436.1);

^{1}H-, ^{13}C-NMR (500/125 MHz, CD_3OD) (Table 3.6).

❖ Compound **2,4-dihydroxydihydrochalcone-6-O-*β*-D-glucopyranoside** (**IC7**): yellow oil, 5 mg (0.00045% of the dry sample weight), R_f = 0.65 (CH_2Cl_2/MeOH = 6/1), (–)-ESI-MS: *m/z* 418.9 $[M-H]^-$, molecular formula $C_{21}H_{24}O_9$ (M = 420.1);

^{1}H-, ^{13}C-NMR (500/125 MHz, CD_3OD) (Table 3.7).

❖ Compound **isoquercitrin** (**IC8**): yellow solid, 4 mg (0.00036% of the dry sample weight), R_f = 0.50 (EtOAc/MeOH = 4/1), molecular formula $C_{21}H_{20}O_{12}$ (M = 464.1);

^{1}H-, ^{13}C-NMR (500/125 MHz, CD_3OD) (Table 3.8).

❖ Compound **methyl 4-hydroxybenzoate** (**IC9**): white solid, 10 mg (0.00091% of the dry sample weight), R_f = 0.35 (*n*-hexane/EtOAc = 3/1), molecular formula $C_8H_8O_3$ (M = 152.2);

^{1}H-, ^{13}C-NMR (500/125 MHz, $CDCl_3$) (Table 3.9).

❖ Compound **methyl 2,4,6-trihydroxybenzoate** (**IC10**): yellow solid, 4 mg (0.00036% of the dry sample weight), R_f = 0.25 (*n*-hexane/EtOAc = 3/1), (+)-ESI-MS: *m/z*: 222.7 $[M+K]^+$, molecular formula $C_8H_8O_5$ (M = 184.0);

^{1}H-, ^{13}C-NMR (500/125 MHz, $CDCl_3$) (Table 3.10).

❖ Compound **isotachioside** (**IC11**): white powder, 1.8 mg (0.00016% of the dry sample weight), R_f = 0.55 (EtOAc/MeOH = 4/1), molecular formula $C_{13}H_{18}O_8$ (M = 302.0);

^{1}H-, ^{13}C-NMR (500/125 MHz, CD_3OD) (Table 3.11).

❖ Compound **uridine** (**IC12**): white powder, 2.2 mg (0.00020% of the dry sample weight), R_f = 0.55 (EtOAc/MeOH = 4/1), molecular formula $C_9H_{12}N_2O_6$ (M = 244.1);

^{1}H-, ^{13}C-NMR (500/125 MHz, CD_3OD) (Table 3.12).

❖ Compound **spinasterol** (**IC13**): white powder, 6 mg (0.00055% of the dry sample weight), R_f = 0.50 (*n*-hexane/EtOAc = 75/25), molecular formula $C_{29}H_{48}O$ (M = 412.3);

^{1}H-, ^{13}C-NMR (500/125 MHz, $CDCl_3$) (Table 3.13).

❖ Compound **isofraxidin** (**IC14**): white solid, 3 mg (0.00027% of the dry sample weight), R_f = 0.50 (*n*-hexane/acetone = 2/1), (+)-ESI-MS: *m/z* 222.8 $[M+H]^+$, molecular formula $C_{11}H_{10}O_5$ (M = 222.0);

^{1}H-, ^{13}C-NMR (500/125 MHz, CD_3OD) (Table 3.14).

❖ Compound **(7*R*,8*S*)-yemuoside YM1** (**IC15**): white powder, 18 mg (0.00164% of the dry sample weight), R_f = 0.60 (EtOAc/MeOH = 4/1), (–)-ESI-MS: *m/z* 505 $[M-H]^-$, molecular formula $C_{25}H_{30}O_{11}$ (M = 506.1);

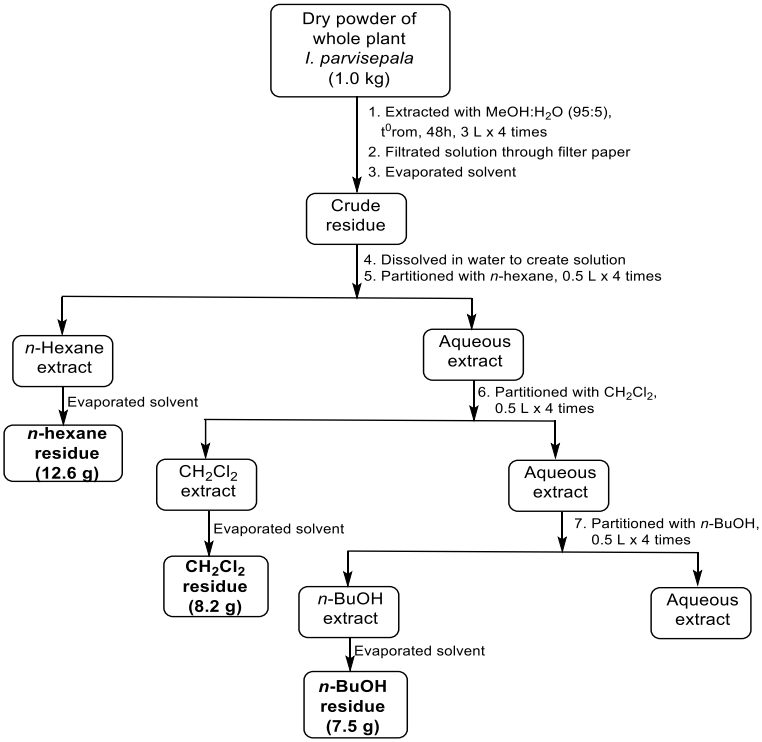
^{1}H-, ^{13}C-NMR (500/125 MHz, CD_3OD) (Table 3.15).

❖ Compound **(*S*)-dehydrovomifoliol** (**IC16**): colorless oil, 12 mg (0.00109% of the dry sample weight), R_f = 0.25 (*n*-hexane/EtOAc = 4/1), $[\alpha]_D^{25}$ = + 135.8 (*c* = 0.1, CH_3OH), (–)-ESI-MS: *m/z*: 220.7 $[M-H]^-$, molecular formula $C_{13}H_{18}O_3$ (M = 222.0);

^{1}H-, ^{13}C-NMR (500/125 MHz, $CDCl_3$) (Table 3.16).

2.3.2. I. parvisepala

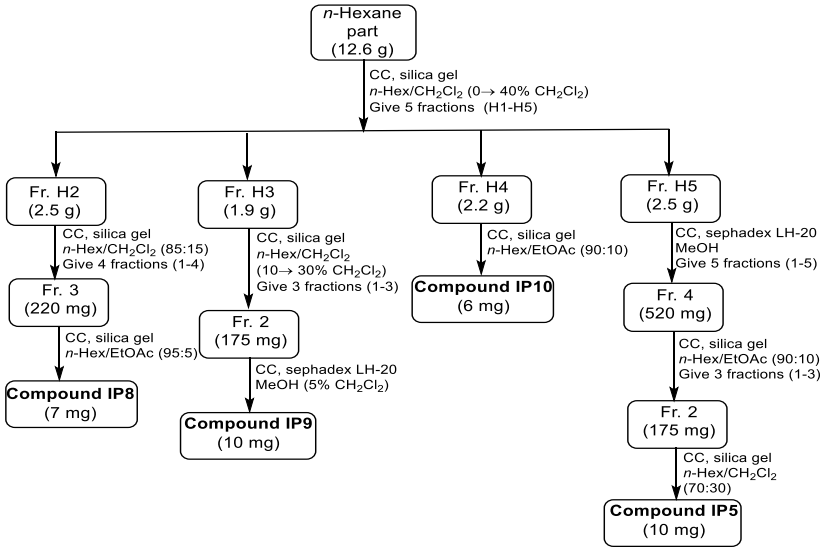
2.3.2.1. Plant sample preparation and extraction



Hình 2.7. Plant sample preparation and extraction of *I. parvisepala*

2.3.2.2. Procedures of compound isolation

- Isolation of compounds from *n*-hexane extract:



Hình 2.8. Isolation of compounds from *n*-hexane extract of *I. parvisepala*

❖ <u>Isolation compounds from *n*-BuOH extract:</u>

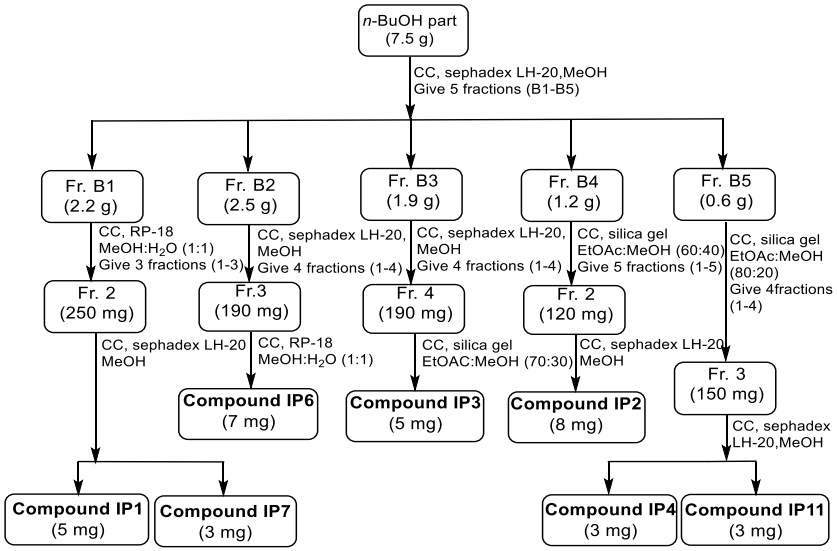


Figure 2.9. Isolation of compounds from *n*-BuOH extract of *I. parvisepala*

2.3.2.3. Spectroscopic data of compounds isolated from I. parvisepala

❖ Compound **kaempferol-3-*O*-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside** (**IP1**): yellow solid, 5 mg (0.0005% of the dry sample weight), R_f = 0.35 (MeOH/H_2O = 1/1, reverse phase thin layer chromatography RP-18), (–)-ESI-MS: *m/z* 593.0 $[M-H]^-$; (+)-ESI-MS: *m/z* 617.1 $[M+Na]^+$, molecular formula $C_{27}H_{30}O_{15}$ (M = 594.0);

^{1}H-, ^{13}C-NMR (600/150 MHz, CD_3OD) (Table 3.18).

❖ Compound **apigenin 7-*O*-*β*-D-glucopyranoside** (**IP2**): yellow solid, 8 mg (0.0008% of the dry sample weight), R_f = 0.5 (EtOAc/MeOH = 4/1), (–)-ESI-MS: *m/z* 430.9 $[M-H]^-$, molecular formula $C_{21}H_{20}O_{10}$ (M = 432.1);

^{1}H-, ^{13}C-NMR (600/150 MHz, CD_3OD) (Table 3.19).

❖ Compound **isoquercitrin** (**IP3**): yellow powder, 4 mg (0.0004% of the dry sample weight), R_f = 0.5 (EtOAc/MeOH = 6/4), (–)-ESI-MS: *m/z* 462.9 $[M-H]^-$; (+)-ESI-MS: *m/z* 487.0 $[M+Na]^+$, molecular formula $C_{21}H_{20}O_{10}$ (M = 464.1);

^{1}H-, ^{13}C-NMR (600/150 MHz, CD_3OD) (Table 3.20).

❖ Compound **phlorizin** (**IP4**): yellow oil, 3 mg (0.0003% of the dry sample weight), R_f = 0.6 (EtOAc/MeOH = 4/1), (–)-ESI-MS: *m/z* 435.0 $[M-H]^-$, molecular formula $C_{21}H_{24}O_{10}$ (M = 436.1);

^{1}H-, ^{13}C-NMR (600/150 MHz, CD_3OD) (Table 3.21).

❖ Compound **lupeol** (**IP5**): white solid, 10 mg (0.0010% of the dry sample weight), R_f = 0.40 (*n*-hexane/EtOAc = 85/15, (+)-ESI-MS: *m/z* 427.2 $[M+H]^+$, molecular formula $C_{30}H_{50}O$ (M = 426.3);

^{1}H-, ^{13}C-NMR (600 MHz, CD_3OD) (Table 3.22).

❖ Compound **ginsenoside Rg1** (**IP6**): colorless solid, 7 mg (0.0007% of the dry sample weight), R_f = 0.55 (EtOAc/MeOH = 7/3, HR-ESI-MS: *m/z* 835.4609 $[M-Cl]^-$ (calcd. for $[C_{42}H_{72}O_{14}Cl]^-$, 835.4616), molecular formula $C_{42}H_{72}O_{14}$ (M = 800.5);

^{1}H-, ^{13}C-NMR (600/150 MHz, pyridin-d_5) (Table 3.23)

❖ **New compound: 3-*O*-{[*α*-L-arabinopyranosyl-(1→3)-]-*β*-D-glucopyranosyl-(1→2)]}-*β*-D-glucuronopyranoside 16*α*-*O*-acetyl-3*β*,22*α*,28*β*-trihydroxy-olean-12-ene** (named **iparvisepala-1**) (**IP7**): white solid, 3 mg (0.0003% of the dry sample weight), R_f = 0.50

(EtOAc/MeOH = 6/4, HR-ESI-MS: *m/z* 1021.4789 $[M+Cl]^-$ (calcd. for $[C_{49}H_{78}O_{20}Cl]^-$, 1021.4780), molecular formula $C_{49}H_{78}O_{20}$ (M = 986.5);

^{1}H-, ^{13}C-NMR (600/150 MHz, CD_3OD) (Table 3.25).

❖ Compound ***α*-tocopherylquinone** (**IP8**): yellow oil, 7 mg (0.0007% of the dry sample weight), R_f = 0.35 (*n*-hexane/EtOAc = 85/15, molecular formula $C_{29}H_{50}O_3$ (M = 446.3);

^{1}H-, ^{13}C-NMR (600/150 MHz, $CDCl_3$) (Table 3.26).

❖ Compound **phytol** (**IP9**): yellow oil, 10 mg (0.0010% of the dry sample weight), R_f = 0.45 (*n*-hexane/EtOAc = 85/15), (–)-ESI-MS: *m/z* 295.1 $[M-H]^-$, molecular formula $C_{20}H_{40}O$ (M = 296.3);

^{1}H-, ^{13}C-NMR (600/150 MHz, $CDCl_3$) (Table 3.27).

❖ Compound **1-[nonadeca-(9*Z*,12*Z*)-dienoyl]-*sn*-glycerol** (**IP10**): white solid, 6 mg (0.0006% of the dry sample weight), R_f = 0.35 (*n*-hexane/acetone = 2/1), (–)-ESI-MS: *m/z* 367.1 $[M-H]^-$, molecular formula $C_{22}H_{40}O_4$ (M = 368.3);

^{1}H-, ^{13}C-NMR (600/150 MHz, CD_3OD) (Table 3.28).

❖ Compound **uracil** (**IP11**): colorless solid, 3 mg (0.0003% of the dry sample weight), R_f = 0.30 (CH_2Cl_2/MeOH = 9/1), (+)-ESI-MS: *m/z* 112.6 $[M+H]^+$, molecular formula $C_4H_4N_2O_2$ (M = 112.0);

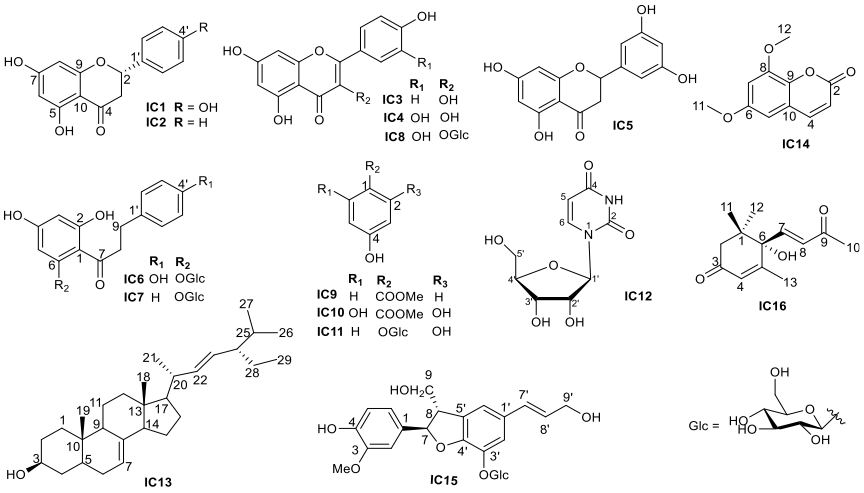
^{1}H NMR (600 MHz, CD_3OD, δ_H, ppm, *J*/Hz): 5.63 (d, *J* = 7.5 Hz, H-5), 7.41 (d, *J* = 7.5 Hz, H-6).

CHAPTER 3. RESULTS AND DISCUSSION

3.1. Analyzing, determining the structure of compounds isolated from *I. chapaensis*

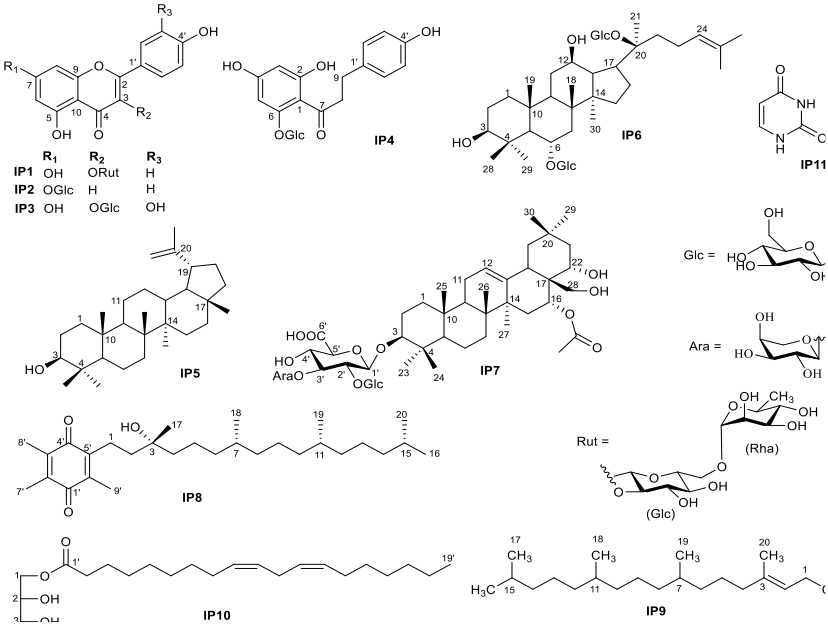
The results on phytochemical study of this plant showed that, 16 compounds (**IC1 – IC16**) were isolated, which cover 8 flavonoids: (*S*)-naringenin (**IC1**), (*S*)-pinocembrin (**IC2**), kaempferol (**IC3**), quercetin (**IC4**), (±)-3',5',5,7-tetrahydroxyflavanone (**IC5**), phlorizin (**IC6**), 2,4-dihydroxydihydrochalcone-6-*O*-*β*-D-glucopyranoside (**IC7**), and isoquercitrin (**IC8**); 3 monophenols: methyl 4-hydroxybenzoate (**IC9**), methyl 2,4,6-trihydroxybenzoate (**IC10**), and isotachioside (**IC11**); and 5 miscellaneous: uridine (**IC12**), spinasterol (**IC1**3), isofraxidin (**IC14**),

(7*R*,8*S*)-yemuoside YM1 (**IC15**), and (*S*)-dehydrovomifoliol (**IC16**). Among these, 9 compounds (**IC1**, **IC5, IC6, IC7**, **IC10, IC11, IC12, IC15,** and **IC16**) were isolated for the first time from *Impatiens* genus.

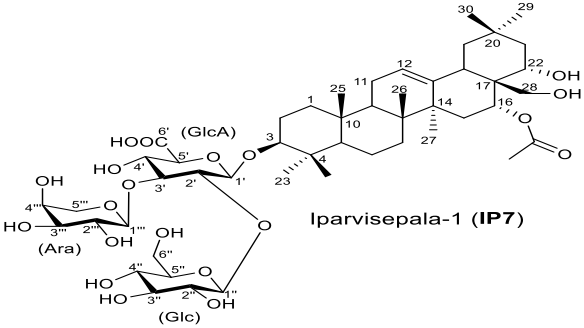


3.2. Analyzing, determining the structure of compounds isolated from *I. parvisepala*

The phytochemical analysis of this plant led to the isolation of 11 compounds (**IP1** – **IP11**), including 4 flavonoids: kaempferol-3-*O*-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside (**IP1**), apigenin 7-*O*-*β*-D-glucopyranoside (**IP2**), isoquercitrin (**IP3**), and phlorizin (**IP4**); 3 triterpenoids: lupeol (**IP5**), ginsenoside Rg1 (**IP6**), and iparvisepala-1 (**IP7**); and 4 miscellaneous: *α*-tocopherylquinone (**IP8**), phytol (**IP9**), 1-[nonadeca-(9*Z*,12*Z*)-dienoyl]-*sn*-glycerol (**IP10**), and uracil (**IP11**). Among these, saponin **IP7** was found to be a new compound, while 7 compounds (**IP1**, **IP5**, **IP6** và **IP8** – **IP11**) were firstly isolated from *Impatiens* genus.

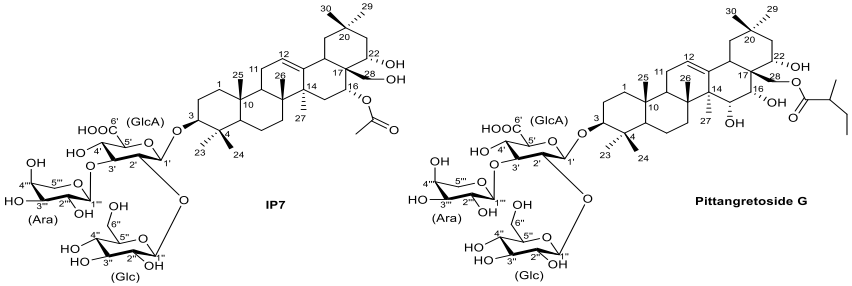


❖ **New compound: IP7**



Compound **IP7** was obtained as white powder. It indicated a $[M+Cl]^-$ quasimolecular ion peak at *m/z* 1021.4701 (calcd. for $C_{49}H_{78}O_{20}Cl$ 1021.4775) in the negative HR-ESI-MS. The negative ion mode showed *m/z* 985.4992 $[M-H]^-$ ($C_{49}H_{77}O_{20}$), which was fragmentated in MS/MS to yield peaks corresponding to the loss of one hexose unit at *m/z* 805.4418 $[(M-H)-180]^-$, to the subsequent loss of one pentose unit at *m/z* 655.3882 $[(M-H)-180-150]^-$, to the continuous loss of glucuronic acid at 515.3740

[(M-H)-180-150-140]$^-$. From that, the molecular formula of **IP7** was determined as $C_{49}H_{78}O_{20}$. NMR spectra (Table 3.25) of this compound suggested it is a triterpenoid saponin, revealing typical signals of olean-12-ene at δ_H 5.37 t, 3.0 Hz (H-12) of double bond at δ_C 125.3 (C-12), 142.6 (C-13) and seven singlet methyls, which resonanced in the range of δ_H from 0.9 to 1.1; three oxygen-methin and one oxymethylene groups at δ_H 3.23 (d, *J* = 4.2, 12.0 Hz, H-3), 5.48 (m, H-16), 4.07 (dd, *J* = 5.5, 12.0 Hz, H-22), 3.30 (m, H-28a) and 3.60 (m, H-28b) correspond to 91.8 (C-3), 72.7 (C-16), 73.9 (C-22) và 69.8 (C-28), respectively. These suggestions were confirmed by the cross peaks between δ_H 5.48 (H-16)/1.95 (H_2-15); δ_H 4.07 (H-22)/1.48, 1.66 (H-21); δ_H 5.37 (H-12)/1.91 (H-11); δ_H 4.48 (H-1')/3.82 (H-2'); and δ_H 3.82 (H-2')/3.77 (H-3') in the COSY spectrum, as well as HMBC correlations between δ_H 4.07 (H-22)/δ_C 72.7 (C-16); δ_H 3.30 and 3.60 (H-28)/δ_C 72.7 (C-16), 44.4 (C-17), 42.5 (C-18) and 73.9 (C-22); and δ_H 3.62 (H-5')/δ_C 176.1 (COOH) (Figure 3.56). The signals at δ_H 2.03, δ_C 22.2 and 171.8 as well as the downfield shift of H-16 (5.48 ppm) suggested the connection position of acetyl group at C-16. The configurations were determined as 3*β*, 16*α*, 22*α*, 28*β* due to observed cross peaks between δ_H 3.23 (H-3)/ 0.8 (H-5), 1.09 (H-23); δ_H 5.48 (H-16)/ 3.6 (H-28); δ_H 4.07 (H-22)/ 1.01 (H-30) in NOESY spectrum. The comparison of above spectral analysis with the published data led to conclude that aglycone part of **6** was 16*α*-*O*-acetyl-3*β*,22*α*,28*β*-trihydroxy-olean-12-ene (Grabowska et al. 2017). Corresponding to MS data, three anomeric groups at δ_H 4.48 (d, 7.8), δ_C 105.7; δ_H 4.98 (d, 7.8), δ_C 103.2 và δ_H 4.64 (d, 7.2), δ_C 104.7 confirmed the presence of three sugar units. The connection positions between glycon with aglycon moieties, as well as between glycon units were determined basing on HMBC correlations (Figure 3.56): between H-1 of glucuronopyranose (4.49 ppm) with C-3 (91.8); H-1' of glucopyranose (4.98 ppm) with C-2' of glucuronopyranose (78.8 ppm); H-1'' of arabinopyranose (4.64 ppm) with C-3' of glucuronopyranose (86.3 ppm). The sugar moiety of **IP7** was well accordant with those of saponin Pittangretoside G published in the literature.



Hình 3.1. Structure of compounds **IP7** and pittangretoside G

Bảng 3.1. ^{1}H- and ^{13}C-NMR data of sugar moiety of compound **IP7** as compared to that of compound pittangretoside G published in literature

C	Compound **IP7** (CD_3OD)		pittangretoside G (CD_3OD)	
	δ_H	δ_C	δ_H	δ_C
3-OGlcA			**3-OGlcA**	
1'	4.48 (d, *J* = 7.8 Hz)	105.7, CH	4.55 (d, *J* = 7.5 Hz)	104.8, CH
2'	3.82 (m)	78.8, CH	3.84	77.3, CH
3'	3.77 (t, *J* = 8.4)	86.3, CH	3.77	85.5, CH
4'	3.64 (m)	72.9, CH	3.65	71.5, CH
5'	3.62 (m)	78.3, CH	3.77	76.2, CH
6'	-	176.1, C	-	172.5
2'-OGlc			**2'-OGlc**	
1"	4.98 (d, *J* = 7.8 Hz)	103.2, CH	4.99 (d, *J* = 7.5 Hz)	102.0, CH
2"	3.19 (m)	76.2, CH	3.16 (t, *J* = 8.0 Hz)	75.4, CH
3"	3.37 (m)	78.2, CH	3.38 (t, *J* = 8.0 Hz)	77.2, CH
4"	3.10 (m)	72.6, CH	3.10 (t, *J* = 9.0 Hz)	71.7, CH
5"	3.37 (m)	78.2, CH	3.33	77.4, CH
6"	3.57 (dd, *J* = 7.2, 12.0 Hz) 3.84 (dd, *J* = 1.8, 10.2 Hz)	63.6, CH_2	3.59 3.85	62.6, CH_2
3'-OAra			**3'- OAra**	
1"'	4.64 (d, *J* = 7.2 Hz)	104.7, CH	4.61 (d, *J* = 7.0 Hz)	104.1, CH
2"'	3.62 (m)	72.1, CH	3.64	71.5, CH
3"'	3.57 (m)	74.6, CH	3.52	73.6, CH
4"'	3.80 (m)	70.1, CH	3.84	69.1, CH
5"'	3.94 (dd, *J* = 2.4, 12.6 Hz) 3.61 (m)	67.7, CH_2	3.94 3.63	66.6, CH

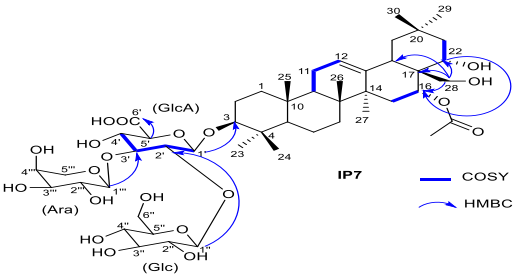


Figure 3.56. COSY and HMBC correlations of compound Iparvisepala-1 (**IP7**)

Basing on above-mentioned spectrum data analysis, structure of compound **IP7** was identified as 3-*O*-{[*α*-L-arabinopyranosyl-(1-3)-]-*β*-D-glucopyranosyl-(1-2)]}-*β*-D-glucuronopyranoside 16*α*-*O*-acetyl-3*β*,22*α*,28*β*-trihydroxy-olean-12-ene (named Iparvisepala-1). The structure of **IP7** was almost identical to those of saponin 3-*O*-{[*β*-D-glucopyranosyl-(1-3)-]-[*β*-D-glucopyranosyl-(1-2)]}-*β*-D-glucuronopyranoside 16-*O*-acetyl-3*β*,22*α*,28*β*-trihydroxy-olean-12-ene (named IPS-1) with only one difference of the sugar unit connected to C-3′ of glucuronopyranose (Figure 3.57, Table 3.25).

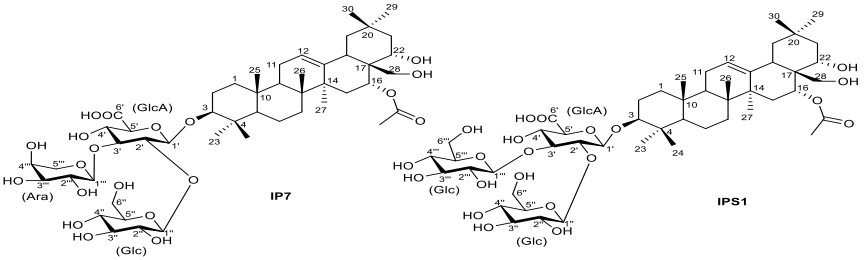


Figure 3.57. Structure of compounds **IP7** and **IPS1**

Table 3.25. ^{1}H- and ^{13}C-NMR data of compound **IP7** as compared to that of **IPS1** published in literature

C	Compound **IP7** (CD_3OD)		**IPS1** (CD_3OD: D_2O (95:5))	
	δ_H	δ_C	δ_H	δ_C
1	1.02 (m) 1.63 (m)	39.9, CH_2	1.00/1.64	41.3, CH_2

2	2.01 (m) 1.73 (m)	27.4, CH_2	1.74/1.96	28.2, CH_2
3	3.23 (dd, *J* = 4.2, 12.0 Hz)	91.8, CH	3.22 (dd, *J* = 5.4, 13.3 Hz)	93.4, CH
4	-	41.1, C	-	41.8, C
5	0.80 (d, *J* = 12.0 Hz)	56.9, CH	0.78 (d, *J* = 11.9 Hz)	58.3, CH
6	1.57 (m) 1.40 (m)	19.2, CH_2	1.56/1.42	20.5, CH_2
7	1.57 (m) 1.35 (m)	33.9, CH_2	1.57/1.35	35.2, CH_2
8	-	40.5, C	-	42.4, C
9	1.68 (m)	48.0, CH	1.65	49.3, CH
10	-	37.8 C	-	39.1, C
11	1.91 (m)	24.5, CH_2	1.92	25.8, CH_2
12	5.37 (t, *J* = 3.6 Hz)	125.3, CH	5.35 (t, *J* = 3.6 Hz)	126.6, CH
13	-	142.6, C	-	143.6, C
14	-	42.8, C	-	43.8, C
15	1.51 (m) 1.95 (m)	31.5, CH_2	1.48/1.95	32.9, CH_2
16	5.48 (m)	72.7, CH	5.47 (m)	74.3, CH
17	-	44.4, C	-	45.7, C
18	2.20 (dd, *J* = 3.6, 13.8 Hz)	42.5, CH	2.20 (dd, *J* = 3.53, 14.0 Hz)	44.0, CH
19	2.25 (m) 1.13 (m)	48.0, CH_2	2.26/1.11	49.4, CH_2
20	-	32.2, C	-	33.4, C
21	1.48 (m) 1.7 (m)	45.0, CH_2	1.48/1.66	46.3, CH_2
22	4.07 (dd, *J* = 5.5, 12.0 Hz)	73.9, CH	4.06 (dd, *J* = 5.6, 12.13 Hz)	75.2, CH
23	1.09 (s)	28.2, CH_3	1.07 (s)	29.6, CH_3
24	0.89 (s)	16.9, CH_3	0.89 (s)	18.3, CH_3
25	1.00 (s)	16.2, CH_3	0.98 (s)	17.4, CH_3
26	1.01 (s)	17.3, CH_3	0.96 (s)	18.6, CH_3
27	1.34 (s)	27.4, CH_3	1.31 (s)	28.7, CH_3
28	3.30 (m) 3.60 (m)	69.8, CH2	3.30/3.60	71.3, CH_2
29	0.98 (s)	33.9, CH_3	0.96 (s)	35.2, CH_3
30	1.01 (s)	25.4, CH_3	0.99 (s)	26.7, CH_3

$CH_3\underline{C}OO$	-	171.8, C	-	173.9, C
$\underline{C}H_3COO$	2.03 (s)	22.2, CH_3	-	23.6, CH_3
3-OGlcA			**3-OGlcA**	
1'	4.48 (d, *J* = 7.8 Hz)	105.7, CH	4.50 (d, *J* = 7.2 Hz)	106. 7, CH
2'	3.82 (m)	78.8, CH	3.85 (m)	80.0, CH
3'	3.77 (t, *J* = 8.4)	86.3, CH	3.84 (m)	87.7, CH
4'	3.64 (m)	72.9, CH	3.65 (m)	73.7, CH
5'	3.62 (m)	78.3, CH	3.65 (m)	79.3, CH
6'	-	176.1, C	-	-
2'-OGlc			**2'-OGlc**	
1''	4.98 (d, *J* = 7.8 Hz)	103.2, CH	4.98 (d, *J* = 7.8 Hz)	104.4, CH
2''	3.19 (m)	76.2, CH	3.21	77.4, CH
3''	3.37 (m)	78.2, CH	3.42	79.3, CH
4''	3.10 (m)	72.6, CH	3.15	73.7, CH
5''	3.37 (m)	78.2, CH	3.34	79.5, CH
6''	3.57 (dd, *J* = 7.2, 12.0 Hz) 3.84 (dd, *J* = 1.8, 10.2 Hz)	63.6, CH_2	3.59 3.86	64.7, CH_2
3'-OAra			**3'-OGlc**	
1'''	4.64 (d, *J* = 7.2 Hz)	104.7, CH	4.77 (d, *J* = 7.8 Hz)	105.1, CH
2'''	3.62 (m)	72.1, CH	3.32	76.7, CH
3'''	3.57 (m)	74.6, CH	3.42	79.3, CH
4'''	3.80 (m)	70.1, CH	3.38	72.8, CH
5'''	3.94 (dd, *J* = 2.4, 12.6 Hz) 3.61 (m)	67.7, CH_2	3.34	79.5, CH
6'''	-	-	3.69/3.89	63.7, CH_2

3.2. Results on biological evaluation

3.2.1. Anti-inflammatory activity

The results of anti-inflammatory assay illustrated that compound **IC9** weakly inhibited NO production with the IC_{50} value of 704.23 ± 42.92 µM. The positive control, L-NMMA, had an IC_{50} value of 27.75 ± 1.61 µM. Whereas, the other four compounds (**IC5, IC7, IC10,** and **IC15**) showed no inhibitory effect on NO production at the tested concentrations 200, 100, 20, 4, and 0.8 µg/mL) (IC_{50} > 200 (µg/mL) (Table 3.30).

Table 3.30. NO inhibitory activities of compounds isolated from *I. chapaensis*

Tested compounds	IC_{50}
IC5	>200 (µg/mL)
IC7	>200 (µg/mL)
IC9	107.15 ± 6.53 (µg/mL) (704.23 ± 42.92 µM)
IC10	>200 (µg/mL)
IC15	>200 (µg/mL)
L-NMMA	6.89 ± 0.40 (µg/mL) (27.75 ± 1.61 µM)

3.2.1. Hypoglycemic activity

- ***I. chapaensis***

The results showed that flavanone **IC5** was indicated as the most potent *α*-glucosidase inhibitor with the IC_{50} value of 101.38 ± 8.96 µM, as compared to positive control acarbose (IC_{50} 227.14 ± 13.71 µM). Besides, compound **IC14** weakly suppressed on tested enzyme (IC_{50} = 1656.5 ± 39.68 µM). None of the remaining five tested compounds (**IC7**, **IC9**, **IC10**, **IC15,** and **IC16**) showed any inhibitory activity, regardless of investigated concentrations 4, 20, 100, and 500 µg/mL (IC_{50} > 500 (µg/mL) (Table 3.31).

Table 3.31. *α*-Glucosidase inhibitiory activities of compounds isolated from *I. chapaensis* species

Tested compounds	IC_{50}
IC5	28.91 ± 2.58 (µg/mL) (101.38 ± 8.96 µM)
IC7	>500 (µg/mL)
IC9	>500 (µg/mL)
IC10	>500 (µg/mL)
IC14	367.74 ± 8.81 (µg/mL) (1656.5 ± 39.68 µM)
IC15	>500 (µg/mL)
IC16	>500 (µg/mL)
Acarbose	146.64 ± 8.85 (µg/mL) (227.14 ± 13.71 µM)

❖ ***I. parvisepala***

In vitro evaluation of the *α*-glucosidase inhibitory potential was carried out on three isolated metabolites (**IP2**, **IP8,** and **IP10**) to result the most suppressor effect of apigenin-7-*O*-glucoside (**IP2**) on the tested enzyme (IC_{50} = 12.53 ± 0.39 µM), which was significantly better than that observed for the positive control, acarbose (IC_{50} = 197.53 ± 2.68 µM). Meanwhile, 2 compounds (**IP8** and **IP10**) failed to inhibit the tested enzyme at the studied concentrations 4, 20, 100, and 500 µg/mL (IC_{50} > 500 (µg/mL) (Table 3.32).

Table 3.22. *α*-Glucosidase inhibition effect of compounds isolated from *I. parvisepala*

Tested compounds	IC_{50}
IP2	5.42 ± 0.17 (µg/mL) (12.53 ± 0.39 µM)
IP8	> 500 (µg/mL)
IP10	> 500 (µg/mL)
Acarbose	127.53 ± 1.73 (µg/mL) (197.53 ± 2.68 µM)

❖ **New compound: Iparvisepala-1 (IP7)**

New triterpene saponin iparvisepala-1 (**IP7**) was tested for hypoglycemic activity via *α*-glucosidase inhibition effect using acarbose as a positive control. However, **IP7** was found to be insensitive to the tested enzyme at the investigated concentrations 0.39, 1.56, 6.25, 25, and 100 µg/mL (IC_{50} > 100 (µg/mL) (Table 3.33).

Table 3.33. *α*-Glucosidase inhibitory effect of new compound Iparvisepala-1 (**IP7**)

Compounds	IC_{50}
IP7	> 100 (µg/mL)
Acarbose	134.56 ± 3.02 (µg/mL) (208.74 ± 4.67 µM)

CONCLUSION AND RECOMMENDATION

1. Study on chemical constituents of two *Impatiens* species (*I.chapaensis* and *I. parvisepala*)

27 compounds were isolated and structurely elucidated from two investigated species. Among these, compound Iparvisepala-1 (**IP7**) was new to science, while 16 compounds were found for the first time from *Impatiens* genus, as follows:

- 16 compounds (**IC1 – IC16**) were purified and structure elucidated from *I. chapaensis*, including 8 flavonoids : (*S*)-naringenin (**IC1**), (*S*)-pinocembrin (**IC2**), kaempferol (**IC3**), quercetin (**IC4**), (±)-3',5´,5,7-tetrahydroxyflavanone (**IC5**)**,** phlorizin (**IC6**)**,** 2,4-dihydroxydihydrochalcone-6-*O*-*β*-D-glucopyranoside (**IC7**), and isoquercitrin (**IC8**); 3 monophenols: methyl 4-hydroxybenzoate (**IC9**), methyl 2,4,6-trihydroxybenzoate (**IC10**), and isotachioside (**IC11**); and 5 miscellaneous: uridine (**IC12**)**,** spinasterol (**IC13**), isofraxidin (**IC14**), (7*R*,8*S*)-yemuoside YM1 (**IC15**), and (*S*)-dehydrovomifoliol **(IC16**). Among these, 9 compounds (**IC1**, **IC5, IC6, IC7**, **IC10, IC11, IC12, IC15,** and **IC16**) were isolated for the first time from *Impatiens* genus.
- 11 compounds (**IP1 – IP11**) were isolated and structure determined from *I. parvisepala*, including 4 flavonoids: kaempferol-3-*O*-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside (**IP1**), apigenin 7-*O*-*β*-D-glucopyranoside (**IP2**), isoquercitrin (**IP3**), and phlorizin (**IP4**); 3 triterpenoids: lupeol (**IP5**), ginsenoside Rg1 (**IP6**), and iparvisepala-1 (**IP7**); and 4 miscellaneous: *α*-tocopherylquinone (**IP8**), phytol (**IP9**), 1-[nonadeca-(9*Z*,12*Z*)-dienoyl]-*sn*-glycerol (**IP10**), and uracil (**IP11**). Among these, compound **IP7** was found as a new triterpene saponin, while 7 compounds kaempferol-3-*O*-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside (**IP1**, **IP5**, **IP6**, **IP8, IP9**, **IP10,** and **IP11**) were the first representatives of the genus *Impatiens*.

2. Evaluation on anti-inflammatory and hypoglycemic activities of two *Impatiens* species

5 isolates (**IC5**, **IC7**, **IC9**, **IC10,** and **IC15**) were investigated for anti-inflammatory activity through the inhibition effect on NO production. The results showed that methyl 4-hydroxybenzoate (**IC9**) weakly inhibited NO production with the IC_{50} value of 704.23 ± 42.92 µM, as compared to standard compound L-NMMA (IC_{50} = 27.75 ± 1.61 µM).

Out of 11 compounds (**IC5**, **IC7**, **IC9**, **IC10**, **IC14**, **IC15**, **IC16**, **IP2**, **IP7**, **IP8,** and **IP10**) isolated from two investigated species, 2 flavonoids (±)-3',5',5,7-tetrahydroxyflavanone (**IC5**) and apigenin 7-*O*-*β*-D-glucopyranoside (**IP2**) showed strong effect on *α*-glucosidase inhibition with the respective IC_{50} of 101.38 ± 8.96 µM and IC_{50} = 12.53 ± 0.39 µM, much better than that observed for positive control acarbose (IC_{50} = 197.53 ± 2.68 µM).

- **Recommendation**

1. Continue to study on phytochemistry of some *Impatiens* species growing in Vietnam and search for anti-inflammatory and hypoglycemic compounds from these sources.
2. The results on biological investigations showed the potential inhibitory effect of 2 flavonoids **IC5** and **IP2** on *α*-glucosidase, therefore, further studies need to be done to use these metabolites in the field of health protection.

NEW CONTRIBUTIONS OF THE THESIS

- For the first time two species *I. chapaensis* and *I. parvisepala* were studied on chemical constituents: 27 compounds were purified and structurally determined. Among these, one compound Iparvisepala-1 (**IP7**) was new and 16 compounds were firstly isolated from *Impatiens* genus.
- For the first time 5 compounds (**IC5**, **IC7**, **IC9**, **IC10,** and **IC15**) isolated from *I. chapaensis* was evaluated on anti-inflammatory activity: compound methyl 4-hydroxybenzoate (**IC9**) indicated weak effect on inhibition of NO production.
- For the first time 7 compounds (**IC5**, **IC7**, **IC9**, **IC10,** and **IC14**-**IC16**) isolated from *I. chapaensis* and 4 compounds (**IP2**, **IP7**, **IP8**, and **IP10**) isolated from *I. parvisepala* were evaluated on hypoglycemic activity: 2 flavonoids, including (±)-3',5',5,7-tetrahydroxyflavanone (**IC5**) and apigenin 7-*O*-*β*-D-glucopyranoside (**IP2**) showed promising activity against *α*-glucosidase, which were much better than that obtained for positive control acarbose.

LIST OF THE PUBLICATIONS RELATED TO THE DISSERTATION

1. **Nguyen Thi Thuy Linh**, Trinh Thi Thuy, Nguyen Thanh Tam, Ba Thi Cham, Khieu Thi Tam, Nguyen Hoang Sa, Do Thi Thao, Vu Tien Chinh, Nguyen Thi Hoang Anh. *Chemical constituents of Impatiens chapaensis Tard. and their α-glucosidase inhibition activities*, Natural product research, 2021, 36 (12), 3229-3233. Doi: 10.1080/14786419.2021.1956923.
2. **Nguyen Thi Thuy Linh**, Trinh Thi Thuy, Nguyen Thanh Tam, Ba Thi Cham, Bui Huu Tai, Do Thi Thao, Dinh Gia Thien,Vu Tien Chinh, Nguyen Thi Hoang Anh. *Chemical constituents of Impatiens parvisepala and their α-glucosidase inhibition activity*, Natural product research, 2023, 37 (16), 2647-2652. Doi: 10.1080/14786419.2022.2127705.