THE UNIVERSITY OF DANANG
UNIVERSITY OF SCIENCE AND EDUCATION

NGUYEN QUANG TRUNG

STUDY ON CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF POTENTIAL COMPOUNDS FROM GENUS *PAEDERIA* L. USING COMPUTATIONAL CHEMISTRY METHODS

Major: Organic chemistry
Major code: 9 44 01 14

SUMMARY OF DOCTORAL THESIS

Da Nang–2024

This thesis was completed at:

UNIVERSITY OF SCIENCE AND EDUCATION

ADVISOR:

1. Prof. Dr. Dao Hung Cuong

2. Dr. Vo Van Quan

1st REVIEWER: .....

2nd REVIEWER:.....

3rd REVIEWER:.....

The thesis will be defended at the Basic Thesis Evaluation Council (Organic Chemistry) at University of Science and Education on …

The thesis can be found in:

The library of Nation;

The library of University of Science and Education, The University of Danang.

INTRODUCTION

1. Preface

The *Paederia* L. genus (Rubiaceae) is a flowering plant genus that includes about 31 species, all of which are climbing plants distributed primarily in the subtropical and tropical regions of Asia and South America. In Vietnam, five species are present, among which *Paederia foetida*, *Paederia lanuginosa*, and *Paederia scandens* are the most common species. Traditionally, several species from this genus have been used in folk medicine to treat various ailments.

Numerous studies have been published regarding the chemical composition, biological activity, and pharmacology of extracts from *Paederia* L. plants and their medicinal values, mainly focusing on *Paederia foetida* and *Paederia scandens*. Additionally, some studies have also evaluated the biological activities of certain compounds isolated from the genus *Paederia* L., indicating that their activities are mediated through antioxidant mechanisms, showcasing potential anti-cancer, anti-inflammatory, antibacterial, cardiovascular protective, neuroprotective, antidiabetic, and anti-arthritis properties. However, there have been no studies assessing the antioxidant activity of compounds derived from the genus *Paederia* L., the mechanisms of antioxidant reactions, or the relationship between the structures and activities of these compounds.

2. Aims of the thesis

– Extract and analyze the chemical composition of selected species from the genus *Paederia* L., followed by an exploration and assessment of antioxidant activity of extracts;

–Isolate specific chemical compounds from various parts of selected *Paederia* L. species and elucidate their structures;

–Investigate and assess appropriate methodologies for quantifying the antioxidant activity of potential compounds derived from the genus *Paederia* L.*;*

– Examine and evaluate the antioxidant activity of potential compounds from *Paederia* L. genus utilizing the chosen computational chemistry techniques.

3. Contents of the thesis

– Extract and identify various chemical components from the leaf extract of *Paederia lanuginosa* and *Paederia foetida*, followed by assessment of the antioxidant activity of specific extracts;

– Isolate and elucidate the structures of isolated compounds obtained from the fractions of *Paederia lanuginosa* and *Paederia foetida* leaf extract;

– Investigate and determine an appropriate methodology for calculation the antioxidant activity of potential compounds identified in *Paederia lanuginosa*, *Paederia foetida* and *Paederia scandens*;

– Calculate the radical scavenging and antioxidant activities of potential compounds isolated from *Paederia lanuginosa*, *Paederia foetida* and *Paederia scandens* using the appropriate computational chemistry methods.

4. New contributions of the thesis

– From the leaves of *Paederia* lanuginosa, 10 compounds were isolated, including:

+ Three compounds first isolated from the genus *Paederia*, including 1-hexacosanol, phytol, and quercitrin;

+ Five compounds first isolated from the leaves of *Paederia* lanuginosa, including *β*-sitosterol, stigmasterol, arachidic acid, rutin, and linarin;

– For the first time, the accuracy of 17 density functional theory (DFT) methods was assessed. As a results, the M06-2X method was chosen as the most suitable method for calculating the antioxidant activity of aromatic compounds containing X-H bonds (X = C, N, O, S) with a reasonable computational resource expenditure and appropriate accuracy;

– For the first time, the radical scavenging activity and the antioxidant properties of some typical compounds (cleomiscosin B, 1,3–dihydroxy–2,4–dimethoxy–9,10–anthraquinone, 2–hydroxy–1,4–dimethoxy–9,10–anthraquinone, 1–methoxy–2–methoxymethyl–3–hydroxy–9,10–anthraquinone, 1–hydroxy–2–hydroxymethyl–9,10–anthraquinone, 1–methyl–2,4–dimethoxy–3–hydroxyanthraquinone, 1–methoxy–3–hydroxy–2–ethoxymethylanthraquinone), 6'–O–E–feruloylmonotropein, 10'–O–E–feruloylmonotropein, kaempferol and quercitrin) from the genus *Paederia* were successfully evaluated.

5. The layout of the thesis

The thesis contains 142 pages, including 42 tables and 53 figures. There are four main chapters and other parts (introduction, conclusion and recommendations, the list of published scientific papers, and references). The four main chapters, including:

Chapter 1. Literature overview, 42 pages.

Chapter 2. Materials and methods, 08 pages.

Chapter 3. Experimentation, 06 pages.

Chapter 4. Results and discussion, 78 pages.

PART 1. OVERVIEW

The literature review highlights the research that has been conducted on the following topics, both domestically and internationally:

1.1. Overview of some species in the genus *Paederia* and their uses in folk medicine.

1.2. Current research on the chemical composition and biological activity of certain species in the genus *Paederia*.

1.3. Research on antioxidant activity using computational chemistry methods.

PART 2. MATERIALS AND METHODS

2.1. Materials

– *Paederia lanuginosa* and *Paederia foetida* species were collected from households in Cau Nhi Dong neighborhood, Dien An ward, Dien Ban town, Quang Nam province, Viet Nam were used for chemical composition investigation

– Potential compounds from *Paederia lanuginosa*, *Paederia foetida* and *Paederia scandens* species were screened for antioxidant activity using computational chemistry method

2.2. Chemicals, tools, equipments and computational softwares

– Chemicals used in atomic absorption spectroscopy for determining metal composition (Pb, Cd, As, Hg, Mg, Fe and Zn standard solutions (1000 mg/L, Merck)), DPPH free radical scavenging activity assay (L-ascorbic acid 99.7%, Fisher; 2,2-diphenyl-1-picrylhydrazyl 95%, Alfa Aesar), solvents (methanol, hexane, EtOAc, Xilong Scientific Co., Ltd), thin layers (silica gel 60 F_{254}, Merck; RP-18 F_{254S}, Merck), packing materials (Sephadex LH–20 (Sigma–Aldrich), silicagel (60N, neutral, 40-50 μm, Kanto

Chemical., Inc.), silicagel YMC RP-18 (30 - 50 μm, Fujisilisa, Chemical Ltd.), and chromatography columns for extraction and purification.

– Equipment includes atomic absorption spectrophotometer, UV-VIS-NIR spectrophotometer, high-resolution mass spectrometer, and NMR spectrometer.

– Computational software: Gaussian 16 and other supportive softwares.

2.3. Research methods

2.3.1. Extraction methods

Raw material samples were extracted using a combination of solid-liquid extraction and liquid-liquid extraction method.

2.3.2. Identification of chemical components in the extracts

Extracts were identified for their chemical components using GC–MS technique.

2.3.3. Antioxidant activity assay of the extracts

DPPH free radical scavenging activity was tested following the method of Kamkar et al. with some adjustments.

2.3.4. Compound isolation methods

A combination of thin-layer chromatography and column chromatography was used.

2.3.5. Structural determination of compounds

A combination of spectroscopic methods including high-resolution mass spectrometry, one-dimensional (1H–NMR, 13C–NMR, DEPT) and two-dimensional NMR (HSQC, HMBC, COSY, NOESY), comparing with references.

2.3.6. Method for calculating antioxidant activity of compounds

– The performance of 17 different DFT functionals was compared

for the calculation of the bond dissociation energy (BDE) values of X–H (X = C, N, O, S) bonds of aromatic compounds. The effect of the size of the basis set (expansions of 6-31(G)) was also assessed for the initial geometry and zero point energy calculations, that was followed by the single point BDE calculations with different model chemistries: M08–HX, M06–2X, M05–2X, M06, M05, BMK, MPW1B95, B1B95, B98, B97–2, LC–ωPBE, B3LYP, cam–B3LYP, B2PLYP, MPWB1K, BB1K and BB95 functionals with the 6-311+(3df,2p) basis set.

– Calculation free radical scavenging activity of potential compounds:

+ The thermodynamic parameters (BDE, PA, and IE) were initial screened to identify which mechanism (FHT, SPLET, and SETPT) is more favorable.

+ The reaction rate constant (*k*) was calculated following transition state theory (TST) at a standard state of 1M, 298.15 K. For reactions following the SET mechanism, Marcus theory was employed to compute the energy barrier via the free energy of the reaction and the nuclear reorganization energy. Additionally, a correction used for reactions rate closed to diffusion-limite, according to Collins–Kimball theory, calculated in solvent at 298.15 K.

PART 3. EXPERIMENTATION

3.1. Sample processing

The *Paederia* plant samples with all parts collected were identified at the Department of Biology, University of Science–Hue University. The leaves, after collection, were processed to remove impurities, washing thoroughly, dried, and prepared as raw material samples.

3.2. Determination of metal composition in fresh raw material samples

Fresh leaves of *Paederia* plants were ashed, dissolved in concentrated acid solution, brought to an appropriate volume, and the metal content was then determined using atomic absorption spectroscopy.

3.3. Extraction of samples and investigating antioxidant activity of the extracts

The extraction process of dried *Paederia lanuginosa* leaves using methanol solvent yielded 45 g of crude extract. This crude extract was then fractionated, and the solvent was removed to yield hexane extract (16 g), EtOAc extract (12 g), and water extract (17 g), respectively. Similarly, the extraction of dried *Paederia foetida* leaves using methanol solvent yielded 51 g of crude extract. This crude extract was also fractionated, yielding hexane extract (14 g), EtOAc extract (18 g), and water extract (19 g), respectively. The fractionated extracts were partially used to identify the chemical components and DPPH free radical scavenging activity assay. The diagram of the extraction process for *Paederia* plant leaves is shown in **Figure 3.1**.

3.4. Isolation of compounds from *Paderia lanuginosa* leaves

Diagram of hexane fraction and EtOAC fraction isolation are presented in **Figure 3.2** and **Figure 3.3**.

3.5. Evaluation of antioxidant activity of potential compounds in the genus *Paederia* L. using computational chemistry methods

- Seventeen DFT methods including M06–2X, M05–2X , M06 , M05, BMK , MPW1B95, B1B95, B98, B97–2, LC–wPBE, B3LYP, cam–B3LYP, B2PLYP, MPWB1K, BB1K, BB95, M08–HX, and several widely used basis sets ((6–31G(d), 6–31+G(d), 6–31+G(d,p), 6–311G(d,p), 6–311++G(d,p)) were surveyed to evaluate the computational capabilities of BDE for X–H bonds (where X = C, N,

O, S) in compounds containing aromatic rings (Ar–X–H). The scheme of evaluation is illustrated in **Figure 3.4**.

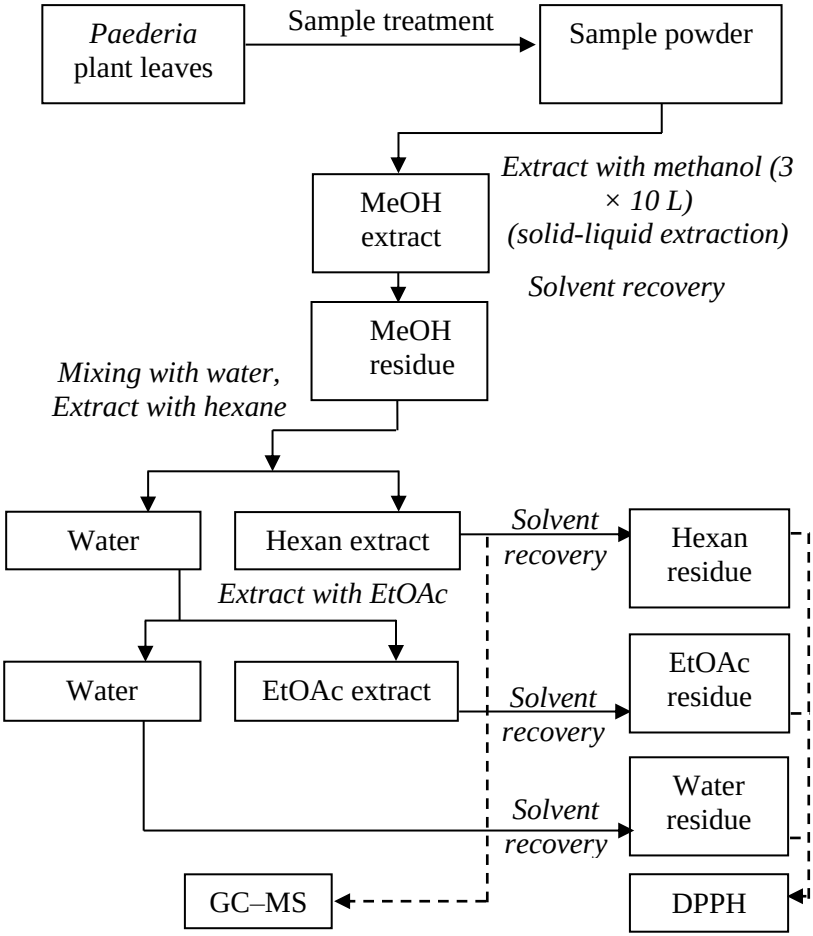

Figure 3.1. Diagram of the extraction process for *Paederia* plant leaves

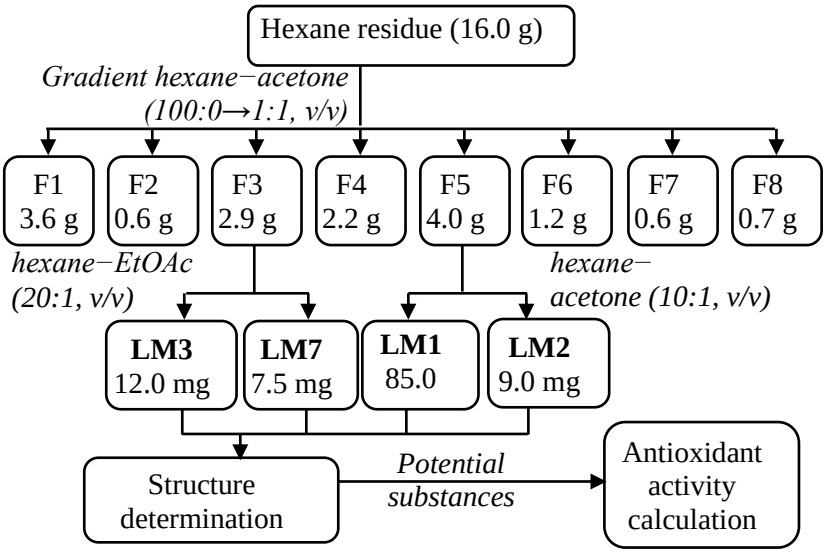

Figure 3.2. Diagram of hexan fraction solation of *Paederia lanuginosa* leaves

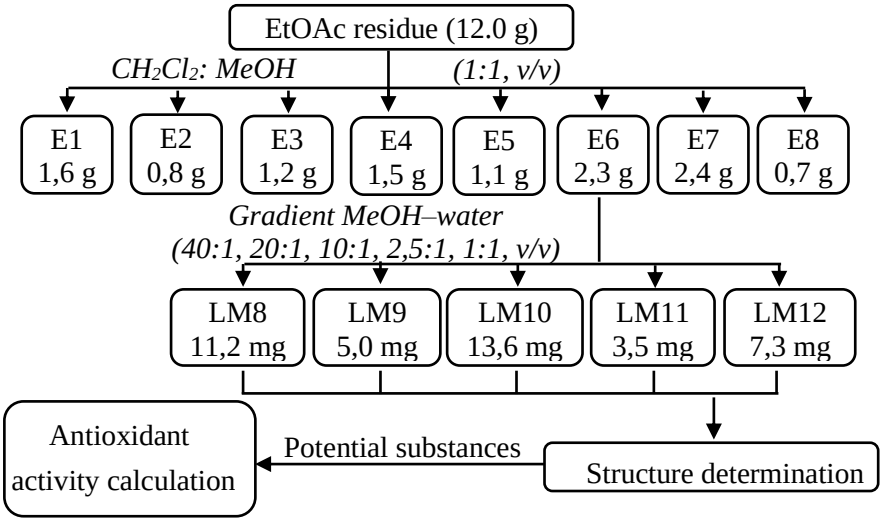

Figure 3.3. Diagram of EtOAc fraction isolation of *Paederia lanuginosa* leaves

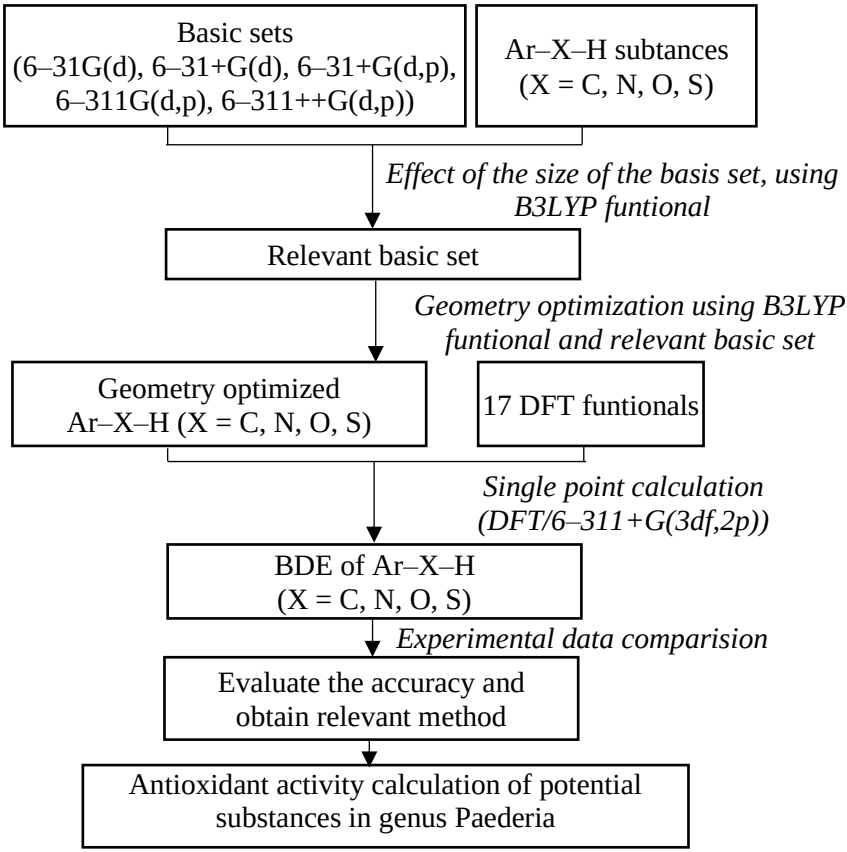

Figure 3.4. Diagram of method evaluation for BDE calculation of Ar–X–H (X = C, N, O, S)

– The potential compounds selected for antioxidant activity evaluation, derived from the genus *Paederia*, include three flavonoid compounds (kaempferol, quercetin, quercitrin) isolated from *Paederia lanuginosa*, three derivatives of phenolic acid (6'–O–E–feruloylmonotropein, 10'–O–E–feruloylmonotropein, cleomiscosin B), and six anthraquinone compounds (1,3–dihydroxy–2,4–dimethoxy–9,10–anthraquinone, 2–hydroxy–1,4–dimethoxy–9,10–

anthraquinone, 1–methoxy–2–methoxymethyl–3–hydroxy–9,10–anthraquinone, 1–hydroxy–2–hydroxymethyl–9,10–anthraquinone, 1–methyl–2,4–dimethoxy–3–hydroxyanthraquinone, 1–methoxy–3–hydroxy–2–ethoxymethylanthraquinone) isolated from *Paederia scandens*. Additionally, some their isomers such as cleomiscosin A and cleomiscosin C were also selected for assessing their structure–activity relationship.

PART 4. RESULTS AND DISCUSSIONS

4.1. Results of plant identification

Samples of *Paederia lanuginosa* Wall. and *Paederia foetida* L. species were identified at the Department of Biology, University of Science – Hue University.

4.2. Metal content in fresh raw material leaves

The heavy metal content in fresh leaves of *Paederia lanuginosa* and *Paederia foetida* are within acceptable limits for food according to QCVN 8–2:2011/BYT (*). The analysis results are presented in **Table 4.1**.

Table 4.1. Result of metal content of *Paederia* fresh leaves

Elements	*P. lanuginosa* leaves (mg/kg)	*P. foetida* leaves (mg/kg)	Limitation (mg/kg) (*)
Pb	<0.02	<0.02	0.3
Cd	<0.02	<0.02	0.2
As	<0.02	<0,02	1.0
Hg	<0.02	<0.02	/
Fe	8.67	13.5	/
Zn	8.06	5.04	/
Mg	370	530	/

4.3. Chemical constituents of extracts

4.3.1. Chemical constituents of Paederia lanuginosa leaves extract

The GC–MS results showed that 55 compounds were found in the hexane, ethyl acetate (EtOAc), and aqueous extracts. Notably, several constituents were detected in multiple extracts, including palmitic acid, stearic acid, phytol, methyl palmitate, methyl stearate, methyl linoleate, and 2–methoxy–4–vinylphenol. Among these, certain compounds exhibited high concentrations, such as methyl oleate (41.1 % [b]), phytol (22.1 % [a]), methyl linoleate (14.3 % [b], 5.1 % [a]), vitamin E (13.9 % [a]), γ-sitosterol (11.9 % [a]), linolenic acid (7.3 % [a]), oleic acid (7.0 % [b]), methyl palmitate (6.7 % [b]), 5-hydroxymethylfurfural (6.4 % [c]), stigmasterol (5.7 % [a]), squalene (5.5 % [a]), and campesterol (4.6 % [a]). Among the identified constituents, some compounds have been documented in prior studies concerning the essential oil composition of *Paederia foetida* and *Paederia scandens* utilizing the GC–MS technique, such as palmitic acid, stearic acid, phytol, sitosterol, linolenic acid, oleic acid, stigmasterol, squalene, linalool, linoleic acid, terpineol, and 9, 12, 15–octadecatrienoic acid, as well as 2, 3–dihydroxypropyl ester (Z, Z, Z). In contrast to previous studies, no chemical constituents of *Paederia lanuginosa* has been reported using this technique until now.

4.3.2. Chemical constituents of Paederia foetida leaves extract

The GC–MS results shown that 37 compounds were identified in the hexane, ethyl acetate, and water extracts. Among these, several constituents were present in multiple extracts and exhibited significant concentrations such as 9,12,15–Octadecatrienoic acid, (Z,Z,Z)–(17.718 %[a]), vitamin E (6.547 %[b]), 4H–Pyran–4–one, 2,3–dihydro–3,5–dihydroxy–6–methyl–(8,938 %[c]), campesterol (2,992 %[a]),

neophytadiene (8,355 %[b]), palmitic acid (8.325 %[a]), oleic acid (0.345 %[b]), phytol (6.496 %[a]), squalene (3.942 %[b]), stigmasterol (4.364 %[a]), γ–sitosterol (7.612 %[a]). Serveral compounds such as n–hexadecanoic acid, oleic acid, phytol, squalene, stigmasterol, γ–sitosterol, 5–methyl–2–furancarboxaldehyde have been documented in prior studies concerning the essential oil composition of *Paederia foetida*.

4.4. Antioxidant activity of *Paederia lanuginosa* and *Paederia foetia* residues

Results of DPPH free radical scavenging activity assay of residues and ascorbic acid are presented in **Table 4.13**

Table 4.13. DPPH free radical scavenging activity assay

Residues	IC_{50} (µg/mL)			
	Hexane	**EtOAc**	**Water**	**Ascorbic acid**
P. lanuginosa	7.36 ± 0.15	14.09 ± 0.17	37.93 ± 0.46	2.67 ± 0.026
P. foetia	19.83 ± 0.27	2.95 ±0.07	104.1 ± 6.7	

As shown in **Table 4.13**, all residues exhibited the higher IC_{50} values than that of ascorbic acid. Notably, the EtOAc residue from the leaves of *Paederia foetida* demonstrated the best antioxidant activity, yielding an IC_{50} value of 2.95 µg/mL, which is comparable to ascorbic acid (2.67 µg/mL). The hexane residue exhibited a lower IC_{50} value compared to both EtOAc and aqueous residue of *Paederia lanuginosa leaves*. Conversely, the EtOAc of *Paederia foetida leaves* exhibited a lower IC_{50} value than that of hexane and aqueous residue. Those observation aligns with the results of the chemical constituents using the GC–MS technique on the extracts of these two plant species, highlighting a greater abundance and concentration of compounds with promising antioxidant potential.

4.5. Structure of isolated compounds

4.5.1. Isolated compounds from hexane residue of Paederia lanuginosa leaves

*a. Mixture of β–sitosterol: stigmasterol (3:2) (LM1) (**Figure 4.10**)*

NMR data:

– **δ_H (ppm)** (^{1}H–NMR $CDCl_3$, 500 MHz): 5,35 (m, H–6); 3,52 (m, H–3); 5,16 (dd, *J* = 15,0; 8,4 Hz, H–22); 5,02 (dd, *J* = 15,0; 9,0 Hz, H–23); 1,03 (s, H–21) và δ_H 1,02 (s, H–26) (stigmasterol); 0,92 (s, H–21) và 0,83 (s, H–26) (β–sitosterol); 1,01 (s, H–19; 0,85 (s, H–18); 0,84 (s, H–27);

– **δ_C (ppm)** (**^{13}C–NMR** $CDCl_3$, 125 MHz): 140,8 (C–5); 121,7 (C–6); 71,8 (C–3; 138,3 (C–22); 129,3 (C–23); 56,9–11,9 (C–1; C–2; C–4; C–7 ÷ C–21; C–24 ÷ C–29).

*b. Arachidic acid (LM2) (**Figure 4.11**)*

NMR data:

– **δ_H (ppm)** (^{1}H–NMR $CDCl_3$, 500 MHz): 2,35 (2H, t, *J* = 7,2 Hz, H–2); 1,62 (2H, m, H–3–4); 0,88 (3H, t, *J* = 7,2 Hz, H–20); 1,25 (H–5 ÷ H–19);

– **δ_C (ppm)** (**^{13}C–NMR** $CDCl_3$, 125 MHz): 178,0 (C–1); 14,1–33,7 (C2–C20).

*c. 1–hexacosanol (LM3) (**Figure 4.12**)*

NMR data:

– **δ_H (ppm)** (^{1}H–NMR $CDCl_3$, 500 MHz): 3,64 (2H, t, *J* = 6,6, H–1); 0,88 (3H, t, *J* = 7,2 Hz, H–26; 1,25 và 1,56 (H–2 ÷ H–25);

– **δ_C (ppm)** (**^{13}C–NMR** $CDCl_3$, 125 MHz): 63,1 (C–1); 32,9 (C–2); 14,1 (C–26); 22,7–31,9 (C–3 ÷ C–25).

*d. (E)–Phytol (LM7) (**Figure 4.13**)*

– **δ_H (ppm)** (^{1}H–NMR $CDCl_3$, 500 MHz): 5,41 (qt, *J* = 7,2; 1,2 Hz,

H–2; 4,15 (d, *J* = 7,2 Hz, H–1; 2,00 (H–4); 1,67 (s, H–20); 1,52 (H–15; 0,86–0,88 (H_3–16 ÷ H_3–19);

– **δ_C (ppm)** (**^{13}C–NMR** $CDCl_3$, 125 MHz): 123,1 (C–2); 140,3 (C–3); 59,4 (C–1); 16,2–39,9 (C–4 ÷ C–20).

4.5.2. Isolated compounds from EtOAc residue of Paederia lanuginosa leaves

*a. Kaempferol (LM8) (**Figure 4.14**)*

NMR data:

– **δ_H (ppm)** (^{1}H–NMR $CDCl_3$, 500 MHz): 6,93 (dd, $^3J_{H-H}$ = 9,0, $^4J_{H-H}$ = 1,8, H–2′/H–6′); 8,04 (dd, $^3J_{H-H}$ = 9,0, $^4J_{H-H}$ = 1,8 Hz, H–3′/H–5′); 6,19 (d, $^4J_{H-H}$ = 2,4 Hz, H–6) và 6,44 (d, $^4J_{H-H}$ = 1,8 Hz, H–8);

– **δ_C (ppm)** (**^{13}C–NMR** $CDCl_3$, 125 MHz): 175,9 (C–4); 146,8 (C–2); 160,7 (C–9); 135,6 (C–3); 156,1 (C–5);163,9 (C–7); 159,2 (C–4 ′).

*b. Quercitrin (LM9) (**Figure 4.15**)*

NMR data:

– **δ_H (ppm)** (^{1}H–NMR $CDCl_3$, 500 MHz): 6,87 (1H, d, $^3J_{H-H}$ = 8,4 Hz, H–5′); 7,25 (1H, dd, $^3J_{H-H}$ = 8,4, $^4J_{H-H}$ = 2,4 Hz, H–6′); 7,30 (1H, d, $^3J_{H-H}$ = 2,4 Hz, H–2′); 6,20 (1H, d, $^4J_{H-H}$ = 2,4 Hz, H–6); 6,39 (1H, d, $^4J_{H-H}$ = 2,4 Hz, H–8); 12,65 (1H, s, H–5O); 0,82 (3H, d, $^3J_{H-H}$ = 6,0 Hz, H–6″);

– **δ_C (ppm)** (**^{13}C–NMR** $CDCl_3$, 125 MHz): 177,7 (C–4); 104,0 (C–10); 120,7 (C–1′), 145,2 (C–3′); 148,4 (C–4′), 93,6 (C–8); 98,6 (C–6); 115,4 (C–2′); 115,6 (C–5′); 121,1 (C–6′); 17,4 (C–6″); 70,0 (C–5″); 70,3 (C–3″); 70,5 (C–2″); 71,1 (C–4″); 101,8 (C–1″).

*c. Quercetin (LM10) (**Figure 4.16**)*

NMR data:

– **δ_H (ppm)** (^{1}H–NMR $CDCl_3$, 500 MHz): 6,19 (d, $^4J_{H-H}$ = 1,8 Hz); δ_H 6,41 (d, $^4J_{H-H}$ = 1,8 Hz); 6,89 (d, $^3J_{H-H}$ = 9,0 Hz); 7,54 (dd, $^3J_{H-H}$ =

8,4, $^4J_{H-H}$ = 1,8 Hz); 7,68 (d, $^4J_{H-H}$ = 2,4 Hz); 10,75 (s_{br}, 7–OH); 12,45 (s, 5–OH); 9,27 (s, 3′–OH); 9,32 (s, 4′–OH);

– δ_C **(ppm)** (**^{13}C–NMR** $CDCl_3$, 125 MHz): 175,8 (C–4); 135,7 (C–3); 156,1 (C–5); 163,8 (C–7); 146,8 (C–3′); 147,7 (C–4′).

*d. Rutin (LM11) (**Figure 4.18**)*

NMR data:

– δ_H **(ppm)** (^{1}H–NMR $CDCl_3$, 500 MHz): 6,84 (1H, d, *J* = 7,8 Hz, H–5′); 7,54 (1H, dd, *J* = 7,8; 1,8 Hz, H–6′); 6,19 (1H, d, *J* = 1,8 Hz, H–6); 6,38 (1H, d, *J* = 2,4 Hz, H–8); 7,53 (1H, d, *J* = 2,4 Hz, H–2′); 12,59 (1H, s, 5–OH); 4,39 (1H, d, *J* = 1,2 Hz, H–1‴); 5,34 (1H, d, *J* = 7,2 Hz, H–1″); 1,0 (3H, d, *J* = 6,0 Hz, H–6‴);

– δ_C **(ppm)** (**^{13}C–NMR** $CDCl_3$, 125 MHz): 177,3 (C–4); 156,6 (C–2); 133,3 (C–3); 161,2 (C–5); 164,0 (C–7); 156,4 (C–9); 144,7 (C–3′); 148,4 (C–4′); 103,9 (C–10); 121,6 (C–1′); 93,5 (C–8); 98,6 (C–6); 115,2 (C–2′); 116,2 (C–5′); 121,1 (C–6′).

*e. Linarin (LM12) (**Figure 4.20**)*

NMR data:

– δ_H **(ppm)** (^{1}H–NMR $CDCl_3$, 500 MHz): 6,46 (1H, d, *J* = 2,4 Hz, H–6); 6,79 (1H, d, *J* = 1,8 Hz, H–8); 6,94 (1H, s, H–3); 8,05 (2H, d, *J* = 9,0 Hz, H–2′/H–6′); 7,15 (2H, d, *J* = 9,0 Hz, H–3′/H–5′); 3,87 (3H, s, 4′–OCH_3); 5,06 (1H, d, *J* = 7,2 Hz, H–1″); 4,57 (1H, s, H–1‴); 1,09 (3H, d, *J* = 6,6 Hz, H–6‴);

– δ_C **(ppm)** (**^{13}C–NMR** $CDCl_3$, 125 MHz): 182,0 (C–4); 163,9 (C–2); 161,1 (C–5), 162,4 (C–7); 157,0 (C–9); 162,9 (C–4′); 105,4 (C–10); 122,7 (C–1′); 94,8 (C–8); 99,9 (C–6); 103,8 (C–3); 128,4 (C–2′/C–6′); 114,7 (C–3′/5′); 99,6 (C–1″); 73,1 (C–2″); 76,2 (C–3″), 69,6 (C–4″), 75,7 (C–5″), 66,1 (C–6″); 100,5 (C–1‴); 70,3 (C–2‴); 70,7 (C–3‴); 72,0 (C–4‴); 68,3 (C–5‴); 17,8 (C–6‴).

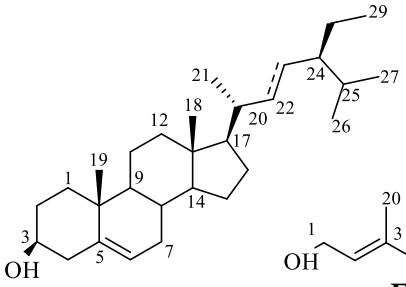

Figure 4.10. Structure of LM1

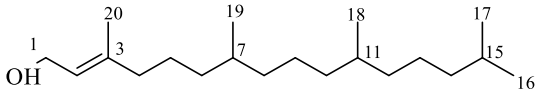

Figure 4.10. Structure of LM7

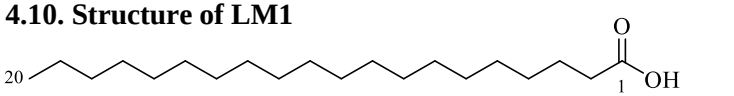

Figure 4.11. Structure of LM2

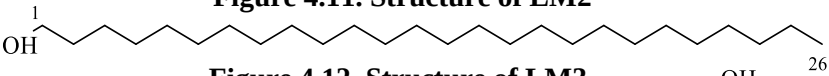

Figure 4.12. Structure of LM3

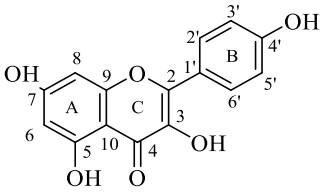

Figure 4.13. Structure of LM8

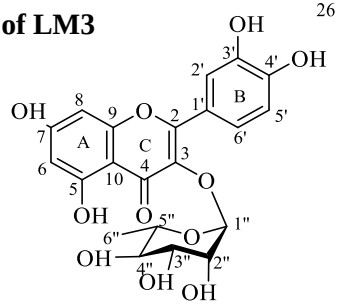

Figure 4.14. Structure of LM9

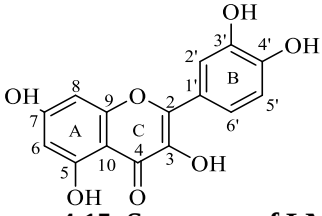

Figure 4.15. Structure of LM10

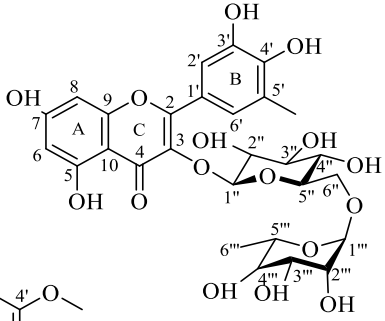

Figure 4.17. Structure of LM11

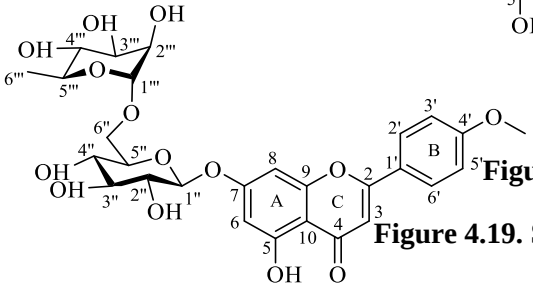

Figure 4.19. Structure of LM12

Evaluation of antioxidant activity of potential compounds using computational chemistry methods

4.5.3. Assessment of DFT methods for antioxidant activity calculation

a. Effect of the size of basis set

In the first section we assess the variation affected by the size of the basis sets by cross-comparison of the data. Arguably any improvement in the numerical values should manifest as a difference between the values calculated with different basis sets, and therefore the analysis does not require referencing to experimental data to reveal the ideal size of basis set. The results showed that, the geometry optimization with larger basis sets did not yield significant differences compared to smaller basis sets; hence even without comparison to experimental data, it would be clear that they could not deliver much improvement of accuracy (range from 0,1% to 0,3%).. Consequently, subsequent calculations were performed using the smallest basis set model (DFT/6–311+G(3df,2p)//B3LYP/6–31G(d)) to assess the accuracy of the methods in calculating the BDE values for C−H, N−H, O−H, and S−H bonds in compounds containing aromatic rings, based on comparisons with experimental data.

b. Calculation of BDE values for C−H bonds

c. Calculation of BDE values for N−H bonds

d. Calculation of BDE values for O−H bonds

e. Calculation of BDE values for S−H bonds

f. Average deviation

To evaluate the accuracy and effectiveness of the computational methods, the average of Mean Unsigned Error (MUE) and Maximum Absolute Error (MaxAE) for each funtional were compared for an

overall assessment of their performance in calculating the BDE values. The results are presented in **Table 4.18**.

Table 4.18. The average absolute (in kcal/mol) and relative (in %) deviation of BDE values from the experimental reference values

Funtional	*Mean unsigned error*		*Max absolute error*	
	MUE [a]	MaxAE [b]	MUE [a]	MaxAE [b]
M06–2X	**1.2**	**3.7**	**1.4**	**4.5**
M05–2X	**1.4**	**3.7**	**1.6**	**4.6**
M06	3.2	5.5	3.7	6.2
M05	4.6	7.0	5.4	8.1
BMK	2.3	4.4	2.7	5.0
MPW1B95	2.7	5.8	3.2	6.6
B1B95	3.2	6.2	3.9	7.0
B98	4.3	6.8	5.1	7.6
B97–2	4.7	7.2	5.6	8.1
LC–ωPBE	3.3	5.9	3.9	6.7
B3LYP	4.3	6.8	5.0	7.6
cam–B3LYP	2.5	4.9	2.9	5.6
B2PLYP	8.2	10.6	9.8	12.2
MPWB1K	2.2	5.3	2.6	6.0
BB1K	2.5	5.6	3.0	6.3
BB95	5.5	8.2	6.5	9.1
M08–HX	**1.5**	**4.1**	**1.7**	**5.3**

[a] *MUE (mean unsigned error)*

[b] *MaxAE (maximum absolute error)*

The results indicated that, M06−2X, M05−2X, and M08−HX yielded higher accuracy than any other tested functionals. M06−2X

was the best performer, with the lowest absolute and relative value deviation. M05−2X and M08−HX had good accuracy; however, the MUE and MaxAE values were slightly higher than those of M06−2X. All other functionals had higher errors in BDEs compared to the Minnesota functionals. Therefore, based on computed data, the M06−2X, M05−2X, and M08-HX functionals offered the most effective and accurate methods to compute BDE values of Ar−X−H (X = C, N, O, S) bonds. Based on these comparision results, M06–2X funtional is selected for computing the thermodynamic and kinetic parameters of the free radical scavenging reactions of several potential antioxidant compounds found in species of the genus *Paederia*. The potential compounds include three flavonoids five derivatives of phenolic acid, and six anthraquinone compounds.

4.5.4. Antioxidant activity of cleomiscosins

4.5.5. Antioxidant activity of anthraquinones

4.5.6. Antioxidant activity of feruloylmonotropeins

4.5.7. Antioxidant activity of flavonoids

The potential compounds evaluated for antioxidant activity using the selected computational method have their results summarized in **Table 4.38**.

Table 4.38. $HOO^{\bullet}$ free radical scavenging activity of studied compounds

Group	Compound	Phase	$k_{overall}$ (M^{-1} s^{-1})	Main mechanism	Contribution
Glycoside	Feruloyl monotropein	Gas	2.59×10^2 – 5.30×10^3	FHT	H_2A
		Pentyl ethanoate	24.0–25.5	FHT	H_2A

		Water	9.45×10^6 -3.66×10^7	SET	A^{2-}
Phenolic	Cleomiscosin	Gas	7.52×10^2 -6.28×10^4	FHT	H_2A
		Pentyl ethanoate	3.47×10^2 -6.44×10^4	FHT	H_2A
		Water	4.03×10^7 -8.66×10^7	SET	HA^-
	Flavonoid	Water	1.4×10^6 -8.0×10^6	SET	A^{2-}
Anthraquinone	Anthraquinone	Gas	3.1–66.3	FHT	H_2A
		Water	5.56×10^{-3} -8.95×10^1 $(3.42 \times 10^6$ $-3.70 \times 10^8)^*$	SET	HA^- $(H_2A)^*$

H_2A: neutral molecule, HA^-: anion, A^{2-} : dianion
(*) Rate constant of $O_2^{\bullet -}$ free radical scavenging reaction

From the calculation data, it is evident that the compounds found in the genus *Paederia* exhibited weak antioxidant activity in non-polar environments (gas phase and pentyl ethanoate solvent), where the predominant free radical scavenging mechanism was defined by the FHT. Conversely, these compounds demonstrated significant potential antioxidant activity in polar environments (aqueous medium, pH 7.4). The findings indicated that the HOO• free radical scavenging activity of the feruloyl monotropeins, cleomiscosins, and flavonoids range from 1.4×10^6 M^{-1} s^{-1} to 8.66×10^7 M^{-1} s^{-1}, which was approximately 10^2 to 10^3 times higher than those of common antioxidants such as Trolox ($k = 1.30 \times 10^5$ M^{-1} s^{-1}), and was comparable to ascorbic acid ($k = 9{,}97 \times 10^7$ M^{-1} s^{-1}) and resveratrol ($k = 5.62 \times 10^7$ M^{-1} s^{-1}).

In contrast, the O2•$^-$ free radical scavenging activity of the

anthraquinones ranges from 3.42×10^6 M^{-1} s^{-1} to 3.70×10^8 M^{-1} s^{-1}, which was significantly higher than that of common antioxidants such as ascorbic acid and quercetin (*k* values ranging from 0.1×10^3 M^{-1} s^{-1} to 3.0×10^3 M^{-1} s^{-1}). Additionally, the SET was primarily pathway of HOO• free radical scavenging reaction in polar environments, where the dissociated states (anion, dianion) played a significant role in their antioxidant activity.

CONCLUSIONS

In conducting thesis research, we have achieved the objectives and research content outlined in the proposal, which includes the analysis of the chemical composition of several species belonging *Paederia* L. and the evaluation of their antioxidant activity using the computational chemistry methods.

1. Constituents and antioxidant activity of extracts:

1.1. The extracts from the leaves of the two species demonstrated the good DPPH free radical scavenging activity. Notably, the EtOAC extract of *Paederia foetida leaves* exhibited highest antioxidant activity with the lowestIC_{50} value of 2.95 μg/mL, closely comparable to ascorbic acid (2.67 μg/mL).

1.2. Several biologically active and pharmacological compounds were identified using GC–MS technique, including 55 compounds from the extracts of *Paederia lanuginosa* leaves and 37 compounds from the extracts of *Paederia foetida* leaves.

1.3. 10 pure compounds were isolated from the hexane and EtOAC extracts of *Paederia lanuginosa* leaves, among which three compounds were reported for the first time from the genus *Paederia*: 1-hexacosanol, phytol, and quercitrin. Furthermore, five compounds isolated for the first time from *Paederia lanuginosa* leaves include β-

sitosterol, stigmasterol, arachidic acid, rutin, and linarin. Two other compounds, kaempferol and quercetin, had been isolated from *Paederia lanugin* leaves in previous studies.

2. Antioxidant activity of potential compounds of genus *Paederia*:

2.1. The M06-2X method was selected among the 17 surveyed DFT funtionals for computing the antioxidant activity of potential compounds in the genus *Paederia*. The compounds studied include cleomiscosin (cleomiscosin A, cleomiscosin B, cleomiscosin C), anthraquinones (1,3–dihydroxy–2,4–dimethoxy–9,10–anthraquinone, 2–hydroxy–1,4–dimethoxy–9,10–anthraquinone, 1–methoxy–2–methoxymethyl–3–hydroxy–9,10–anthraquinone, 1–hydroxy–2–hydroxymethyl–9,10–anthraquinone, 1–methyl–2,4–dimethoxy–3–hydroxyanthraquinone, 1–methoxy–3–hydroxy–2–ethoxymethylanthraquinone), feruloyl monotropein (6'–O–E–feruloylmonotropein, 10'–O–E–feruloylmonotropein), and flavonoids (kaempferol, quercetin, quercitrin).

2.2. The results indicated that the studied compounds exhibited excellent antioxidant activity in polar environments, compared to representative antioxidants such as Trolox, ascorbic acid, and resveratrol. Specifically, the rate constants for the HOO• free radical scavenging reaction for the feruloyl monotropein, cleomiscosin, and flavonoid compounds ranged from 1.4×10^6 M^{-1} s^{-1} to 8.66×10^7 M^{-1} s^{-1}, while the rate constants for the $O2^{\bullet -}$ free radical scavenging reaction for anthraquinone compounds ranged from 3.42×10^6 M^{-1} s^{-1} to 3.70×10^8 M^{-1} s

2.3. The results also demonstrated that the antioxidant activity of the studied compounds was primarily attributed to the dissociated state (anion, dianion) with the main reaction mechanism being the SET

mechanism, where the –OH functional groups directly linked to the aromatic rings of these compounds played a crucial role.

In addition to the results achieved, the thesis still has some limitations, such as not having isolated all the extractive fractions and evaluated the antioxidant activity of these fractions.

RECOMMENDATIONS

Based on the results of the isolation and antioxidant activity calculation of various compounds from species of the genus *Paederia* in Quang Nam, Vietnam, we recommend the following:

– Continue the investigation and isolation of extract fractions of *Paederia lanuginosa* and *Paederia foetida* leaves for promising antioxidant acitivity compounds;

– Further evaluate the antioxidant activity of other potential compounds from genus *Paederia*, specifically focusing on aromatic ring compounds, such as flavonoid glycosides, phenolic derivatives, and terpenoids.

PUBLICATIONS WITHIN THE SCOPE OF THESIS

[1] **Nguyen Quang Trung**, Nguyen Minh Thong, Dao Hung Cuong, Tran Duc Manh, Loc Phuoc Hoang, Nguyen Khoa Hien, Pham Cam Nam, Duong Tuan Quang (2021), "Radical scavenging activity of natural anthraquinones: A theoretical insight." *ACS omega*, 6.20, 13391-13397. (https://pubs.acs.org/doi/full/10.1021/ acsomega . 1c01448)

[2] **Nguyen Quang Trung**, Adam Mechler, Nguyen Thi Hoa, Quan V. Vo (2022), "Calculating bond dissociation energies of X− H (X= C, N, O, S) bonds of aromatic systems via density functional theory: a detailed comparison of methods." *Royal Society Open Science* 9.6, 220177. (https://royalsocietypublishing.org/doi/full/ 10.1098/ rsos.220177)

[3] **Nguyen Quang Trung**, Nguyen Thi Thu Thanh, Nguyen Thi Hoa, Adam Mechler, Quan V. Vo (2023), "Feruloylmonotropeins: promising natural antioxidants in Paederia scandens." *RSC advances* 13.9, 6153-6159. (https://pubs.rsc.org/en /content/articlehtml/2023/ra/d3ra00458a)

[4] **Nguyen Quang Trung**, Adam Mechler, Quan V. Vo (2024), " Computational assessment of the radical scavenging activity of cleomiscosins." *RSC advances* , 2024,14, 23629-23637 (https://pubs.rsc.org/en/content/articlelanding /2024/ra /d4ra03260h)

[5] **Nguyễn Quang Trung**, Đào Hùng Cường, Võ Văn Quân (2022), "Nghiên cứu thành phần hoá học của lá cây Mơ lông (*Paederia lanuginosa*) thu hái tại Quảng Nam, Việt Nam." *Tạp chí Hoá học và ứng dụng*, 268-276

[6] **Nguyen Quang Trung**, Dao Hung Cuong, Vo Van Quan (2023) "Structure and antioxidant ability of flavonoids from leaves of

Paederia lanuginosa from Quangnam, Vietnam." *The University of Danang - Journal of science and technology*, 124-135. (https://jst-ud.vn/jst-ud/article/view/8495)