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Dinh Thi Huyen Trang

STUDY ON CHEMICAL COMPOSITION AND STRUCTURE ELUCIDATION OF ISOLATED COMPOUNDS FROM *Pandanus tonkinensis* MART.EX B.STONE USING MODERN

SUMMARY OF DISSERTATION ON ANALYTICAL CHEMISTRY

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LIST OF THE PUBLICATIONS RELATED TO THE DISSERTATION

1. Dinh Thi Huyen Trang, Pham Hung Viet, Duong Hong Anh, Bui Huu Tai, Ngo Quoc Anh, Nguyen Xuan Nhiem and Phan Van Kiem, 2022, Lignans and Other Compounds from the Roots of *Pandanus tonkinensis* with Their Lipid Peroxidation Inhibitory Activity, *Natural Product Communications*, 17(4), pp. 1-5.

2. Dinh Thi Huyen Trang, Duong Hong Anh, Quoc Anh Ngo, Pham Hung Viet, Bui Huu Tai, Nguyen Xuan Nhiem & Phan Van Kiem, 2022, Pandatonkinosides A and B: two new phenolic glycosides from the roots of *Pandanus tonkinensis* and their nitric oxide production inhibitory activities, *Natural Product Research*, 37(19), pp. 3253-3260.

3. Dinh Thi Huyen Trang, Pham Thu Trang, Do Minh Phuong, Duong Hong Anh, Ngo Quoc Anh, Phan Van Kiem, and Pham Hung Viet, 2023, The chemical composition from the fruits of *Pandanus tonkinensis* and their inhibitory NO production and lipid peroxidative inhibitory activities, *Vietnam Journal of Chemistry*, 61(special issue), pp. 1-7.

4. Dinh Thi Huyen Trang, Bui Van Trung, Ngo Quoc Anh, Duong Hong Anh and Pham Hung Viet, 2023, Determination of Pinoresinol 4-O-beta-D-glucopyranoside and Vladinol F as Markers in Pandanus tonkinensis Fruits by High Performance Liquid Chromatography, VNU Journal of Science: Medical and Pharmaceutical Sciences, Vol. 39, No. 2 (2023) 1-9.

INTRODUCTION

1. The urgency of the thesis

As one of the countries located in the tropical monsoon climate zone, Vietnam has a diverse and rich flora, with medicinal plants accounting for about 30%. Many research projects on medicinal plants of the Vietnamese flora have made great contributions to protecting human health. Plants that have medicinal effects on humans leave fewer side effects than drugs of synthetic origin. Therefore, with the increasing number of diseases such as cardiovascular disease, cancer, liver disease, etc., research on medicinal plants is of scientific and topical significance [1].

Pandanaceae is a family of flowering plants native to tropical and subtropical regions, distributed from West Africa to the Pacific. *Pandanus* is the largest and most important genus with about 600 species, which can be used as a food source and medicine. In Vietnam, the Pandanaceae family includes 23 species belonging to two genera: *Freycinetia* (3 species) and *Pandanus* (20 species). According to traditional medicine documents, there are 9 species of the *Pandanus* genus used as medicine in Vietnam, mainly effective in kidney diseases (diuretics, treatment of kidney stones, gallstones, urinary tract infections,...), liver diseases (hepatitis, cirrhosis and ascites), heat-clearing, fever reducing, skin diseases,...[2], [3].

Pandanus tonkinensis Mart. ex B. Stone, also known as Northern pineapple, is present from the Northern midland mountains to the Central region, Central Highlands, Binh Thuan, Long An. It is one of the 9 species mentioned above whose buds, leaves, roots and fruits can be used in medicine [2]. In the program of Science and Technology for Sustainable Development in the Northwest region, the results of investigation and research on the remedy for treating liver and gallbladder diseases were announced, in which the water extract of the remedy with two Stixis *suaveolens* and *Pandanus tonkinensis* have been shown to have good

hepatoprotective effects, higher than sylimarin [4]. Currently, there is no research on the chemical composition and pharmacological effects of *Pandanus tonkinensis* [5]. In order to obtain scientific evidence about ingredients, biological activities as well as quality control of medicinal herbs and preparation products in the direction of hepatoprotection, the thesis titled: "Study on chemical composition and structure elucidation of isolated compounds from *Pandanus tonkinensis* Mart.ex B.Stone using modern" were proposed and implemented.

2. The objectives of the thesis

- Analyze the composition, chemical structure and hepatoprotective activity of compounds from *Pandanus tonkinensis.*

- Identify markers for *Pandanus tonkinensis* in the direction of hepatoprotection and develop a process for quantitative analysis of markers in medicinal herbs to serve the quality control and development of products from this medicinal herb.

3. The main contents of the thesis

- Using modern extraction and isolation techniques and biophysical chemical methods to analyze the composition, chemical structure, and biological activity of compounds isolated from *Pandanus tonkinensis*.

- Selecting markers for *Pandanus tonkinensis* in the direction of hepatoprotection; Extract, purify, and analzse the purity of the marker for this medicinal herb.

- Developing and validating a quantitative ty process for markers in the medicinal herb *Pandanus tonkinensis*. Apply the process and analyze the content of marker in medicinal herbs collected in localities.

CHAPTER 1. OVERVIEW

Chapter 1 consists of 20 pages, presenting an overview of the literature on the genus *Pandanus*, research works on the chemical composition and biological activity of compounds isolated from the genus *Pandanus.*

Introduction to *Pandanus tonkinensis* Martelli ex BC Stone. Overview of methods to evaluate biological activity in the direction of hepatoprotection and markers .

CHAPTER 2: RESEARCH METHODS AND EXPERIENCE

Chapter 2 includes 12 pages, detailing methods of isolation, structure determination, and biological activity assessment methods for antioxidant and anti-inflammatory properties. Marker selection method, quantitative construction of markers.

CHAPTER 3 : RESULTS AND DISCUSSION

3.1. Isolation and identification of compounds from *Pandanus tonkinensis*

3.1.1. Procedure for isolating compounds from fruit Pandanus tonkinensis

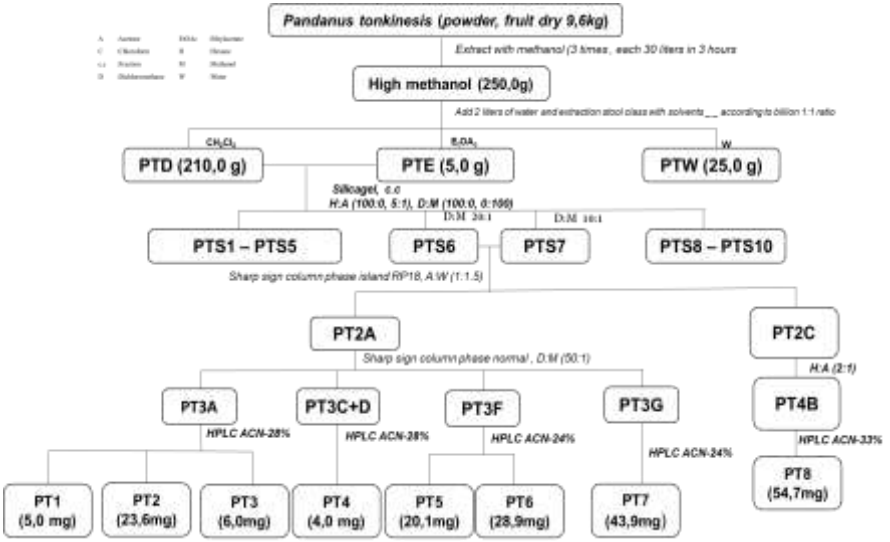


Figure 3.1. Diagram of isolation of compounds from *Pandanus tonkinensis fruit*

3.1.2. Isolation of compounds from Pandanus tonkinensis roots

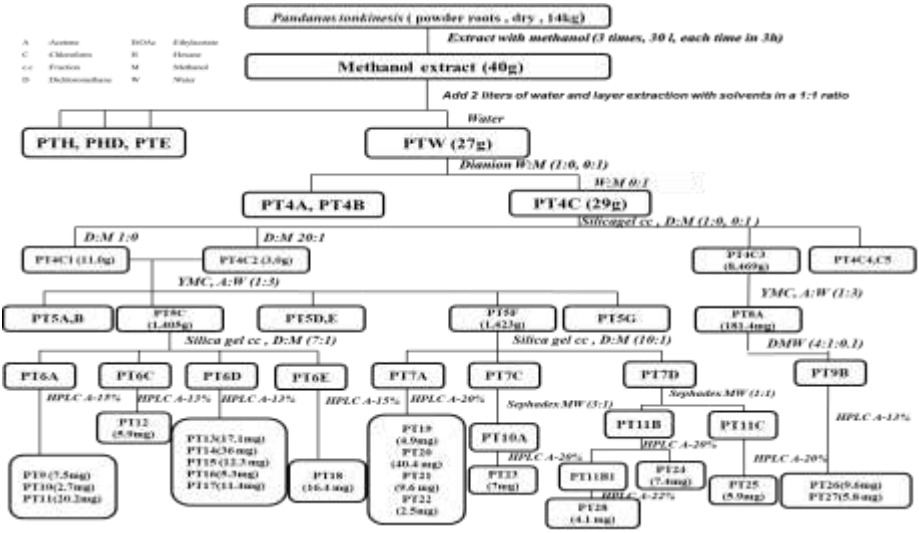
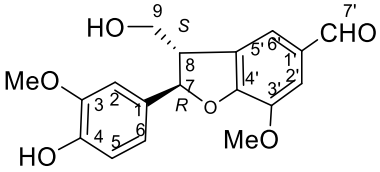


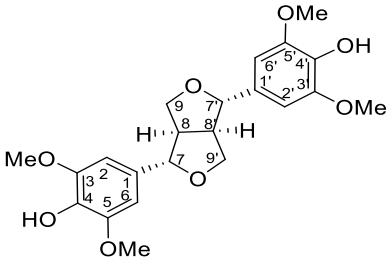
Figure 3.2. Diagram for isolating compounds from *Pandanus tonkinensis* roots

3.1.3. Identification of compounds isolated from Pandanus tonkinensis fruit

From Pandanus tonkinensis fruit, eight pure compounds were isolated, including: ficusal (PT1), syringaresinol (PT2), medioresinol (PT3), lariciresinol (PT4), secoisolariciresinol (PT5), vladinol F (PT6), luteoliflavan (PT7), isorhapontigenin (PT8).



PT1



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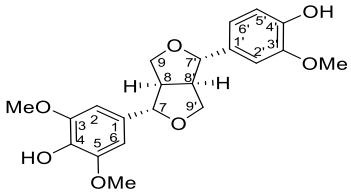

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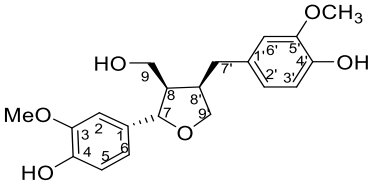

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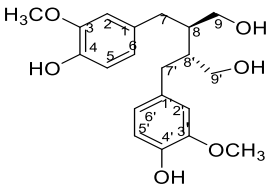

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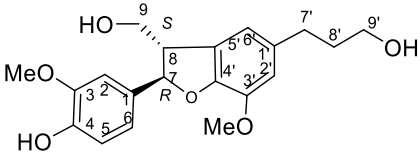

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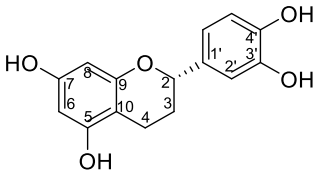

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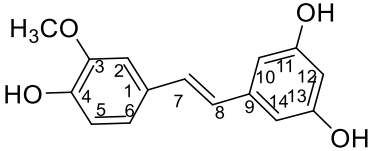


PT8

Figure 3.3. Chemical structures of compounds isolated from *Pandanus tonkinensis* fruit

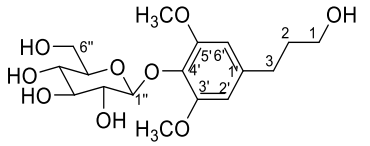
3.1.4. Identification of compounds isolated from Pandanus tonkinensis roots

From Pandanus tonkinensis roots , 20 compounds were isolated, including 3 new compounds and 17 known compounds.

3 new compounds include: (7S)-2,6-dimethoxyphenyl-7,9-propanediol-1-O- β -D-glucopyranoside **(PT10)**, trans-cinnamyl alcohol 9-O-(6′-O- α - L-arabinofuranosyl)- β -D-glucopyranoside **(PT25)**, 4-(3-hydroxypropyl)-2,6-dimethoxyphenol β -D-apiofuranosyl-(1→6)- β -D-glucopyranoside **(PT26)**.

17 known compounds include: dihydrosyringin **(PT9)**, (6S,9S)-roseoside **(PT11)**, 1-O- β -Dglucopyranosyl-2-{2,6-dimethoxy-4-[1-(E)-

propen-3-ol]phenoxyl}propan-3-ol **(PT12)**, 1-O-(β -D-glucopyranosyl)-2-[2-methoxy-4-(ω-hydroxypropyl)-phenoxyl]-propan-3-ol **(PT13)**, benzyl O-α -L-arabinopyranosyl-(1→6)- β -D-glucopyranoside **(PT14)**, 1-O-(β-D-glucopyranosyl)-2-[2,6-dimethoxy-4-(ω hydroxypropyl)-phenoxyl]-propan-3-ol **(PT15)**, (7R,8R) -threo-4,7,9,9′-tetrahydroxy-3,3′-dimethoxyl-8-O-4′-neolignan-4-O- β -D-glucopyranoside **(PT16)**, (7S,8S)-threo-4,7,9,9′-tetrahydroxy-3,3′-dimethoxyl-8-O-4′-neolignan-4-O-β-D-glucopyranoside **(PT17),** pinoresinol-4,4′-di-O-β-D-glucoside **(PT18)**, isoeucommin A **(PT19)**, pinoresinol 4'-O- β -D-glucopyranoside **(PT20)**, acanthoside B **(PT21)**, eucommin A **(PT22)**, rourinoside **(PT23)**, (7S,8R)-5-methoxydihydrodehydrodiconiferyl alcohol-4-O-β-D-glucopyranoside **(PT24)**, kelampayoside A **(PT27)**, urolignoside **(PT28)**.



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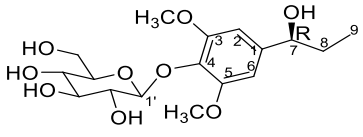

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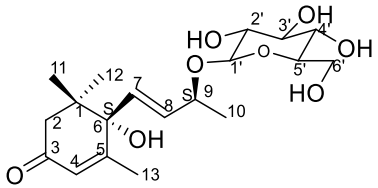

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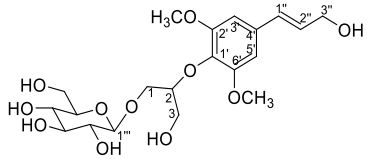

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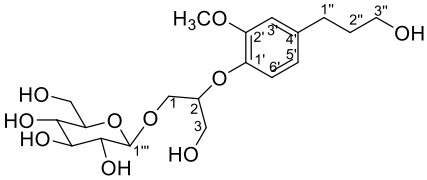

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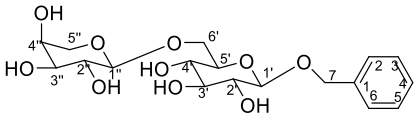

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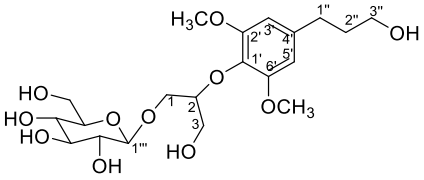

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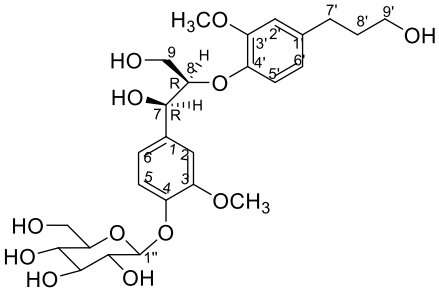

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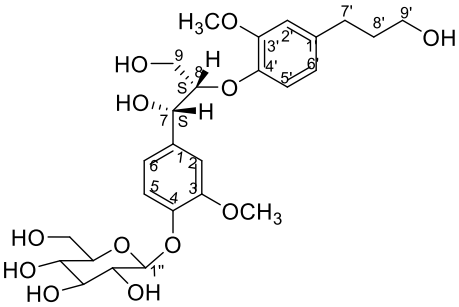

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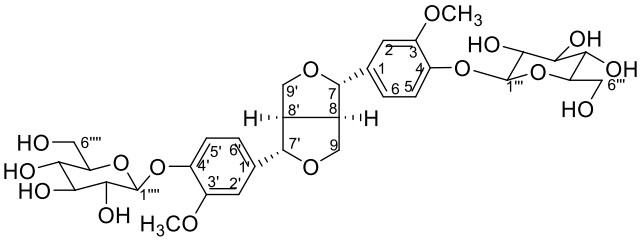

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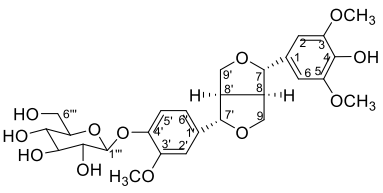

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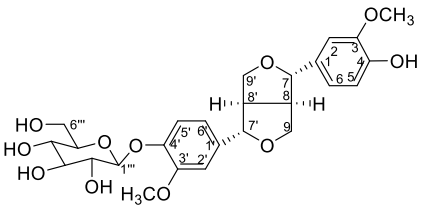

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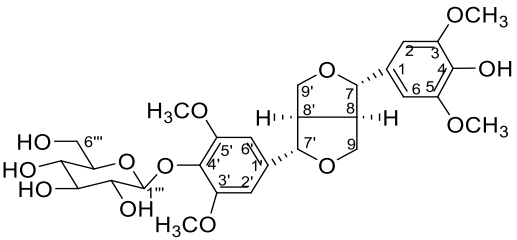

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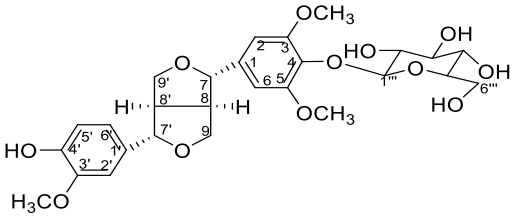

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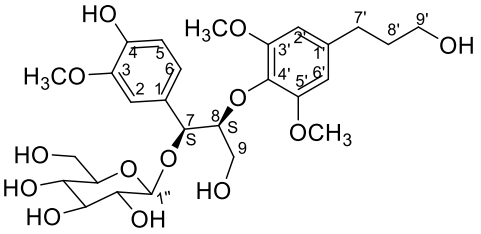

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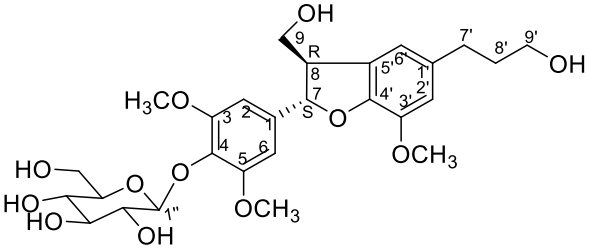

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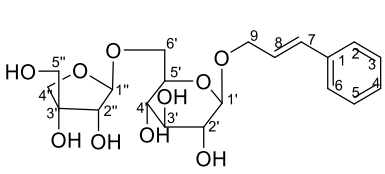

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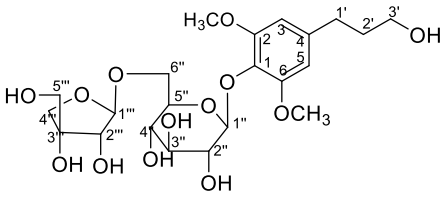

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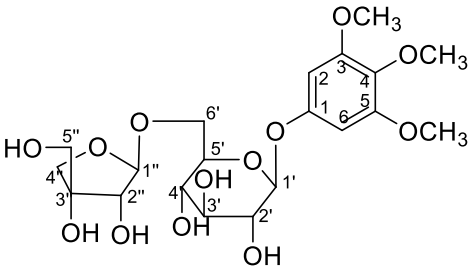

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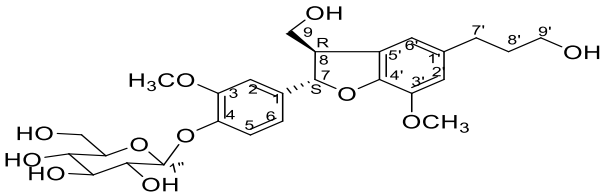


PT28

Figure 3.4. Chemical structures of compounds isolated from *Pandanus tonkinensis* roots

3.2. Evaluation of biological activity of isolated compounds

3.2.1. Anti-inflammatory activity of isolated substances through inhibition of NO production on RAW 264.7 cells stimulated by LPS

The anti-inflammatory activity of the isolated compounds is presented in table 3.1.

Table 3.1. NO production inhibitory activity of substances isolated from *Pandanus tonkinensis*

Matter	IC_{50} (μM)	Matter	IC_{50} (μM)	Matter	IC_{50} (μM)
PT1	17.4 ± 1.94	**PT11**	28.5 ± 1.76	**PT21**	>100
PT2	126 ± 7.65	**PT12**	>100	**PT22**	32.2 ± 3.47
PT3	39.3 ± 3.30	**PT13**	44.4 ± 6.24	**PT23**	1.93 ± 0.23
PT4	5.25 ± 0.39	**PT14**	>100	**PT24**	54.0 ± 2.03
PT5	23.0 ± 1.35	**PT15**	1.78 ± 0.13	**PT25**	83.3 ± 4.67
PT6	21.4 ± 2.08	**PT16**	34.4 ± 1.81	**PT26**	20.1 ± 2.07
PT7	7.08 ± 0.44	**PT17**	80.3 ± 7.26	**PT27**	5.84 ± 0.44
PT8	48.3 ± 5.11	**PT18**	>100	**PT28**	>100
PT9	37.0 ± 4.04	**PT19**	>100	**L-NMMA**	37.8 ± 3.2
PT10	94.0 ± 10.3	**PT20**	24.7 ± 1.08		

The above test results showed that 22 substances PT1-PT9, PT10, PT11, PT13, PT15-PT17, PT20, PT22-PT27 showed NO production inhibitory activity with IC 50 values ranging from 1.78 to 125.83 μM, of which 14 substances PT1, PT4-PT7, PT9, PT11, PT15, PT16, PT20, PT22, PT23, PT26, PT27 showed better anti-inflammatory ability through IC_{50} results than the positive control.

3.2.2. Antioxidant activity of isolated substances through inhibition of cell membrane lipid peroxidation

The results of determining the antioxidant activity of compounds isolated from *Pandanus tonkinensis* wild pineapple are presented in table 3.2.

Table 3.2. Membrane lipid peroxidation inhibitory activity of substances isolated from *Pandanus tonkinensis*

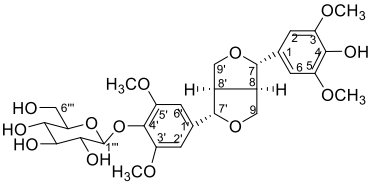
Matter	IC_{50} (μM)	Matter	IC_{50} (μM)	Matter	IC_{50} (μM)
PT1	>100	**PT11**	>100	**PT21**	27.5 ± 2.76
PT2	126 ± 5.57	**PT12**	>100	**PT22**	54.2 ± 3.54
PT3	>100	**PT13**	>100	**PT23**	>100
PT4	32.2 ± 1.42	**PT14**	>100	**PT24**	>100
PT5	20.2 ± 1.71	**PT15**	>100	**PT25**	>100
PT6	84.8 ± 6.69	**PT16**	>100	**PT26**	>100
PT7	26.3 ± 3.57	**PT17**	>100	**PT27**	>100
PT8	23.3 ± 1.67	**PT18**	>100	**PT28**	>100
PT9	>100	**PT19**	57.5 ± 5.53	**Trolox**	31.4 ± 2.20
PT10	>100	**PT20**	10.4 ± 0.71		

In the test, the positive control trolox performed stably giving an IC_{50} value of 31.4 ± 2.2 μM. 10 compounds including PT2, PT4-PT8, PT19 - PT22 showed inhibitory activity on cell membrane lipid peroxidation with IC_{50} values ranging from 10.36 to 126.39 μM. These 10 compounds show anti-oxidant ability, of which 5 substances PT5, PT7, PT8, PT20, PT21 have

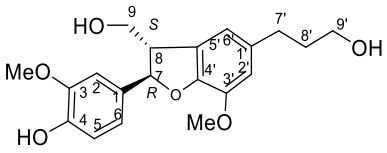
better anti-oxidant ability than the positive control.

3.3. Marker

In the step of determining medicinal ingredients, compound **PT20 (**pinoresinol 4-*O*-beta-D-glucopyranoside) and **PT6 (**vladinol F) were isolated from medicinal herbs with the mass of 40.4 mg and 28.9 mg, respectively, which are the highest in the root water extract and the third highest in the fruit solvent extract, respectively. Both of these compounds have anti-inflammatory and antioxidant activity according to screening tests. Based on the results of preliminary analysis of medicinal samples and single substances, two compounds PT20 and PT6 were selected as markers for the medicinal herb *Pandanus tonkinensis* **.**



PT20



PT6

Image 3.5. Chemical structures of pinoresinol 4'-O- β-D-glucopyranoside (PT20) and vladinol F (PT6) – two markers for the medicinal herb *Pandanus tonkinensis.*

3.4. Developing and validating a procedure for quantifying the compound pinorecinol 4'-O- β -D-glucopyranoside and vladinol F in the medicinal herb *Pandanus tonkinensis*

3.4.1. Survey the process of processing medicinal samples before analysis

Using 50% methanol or 50% ethanol solvents to extract the analytes (PT20 and PT6) from the medicinal herbs at a ratio of 10g of medicinal powder/100 ml of solvent, with each solvent extracted 3 times. Ultrasound for 30 minutes each time, then combine the extract, evaporate and redissolve

it in 5 mL of solvent to collect the first final extract. Repeat the above extraction process to collect the second final extract. Analyze the solutions. The 1st and 2nd final extraction of each solvent by high-performance liquid chormatography (HPLC), compare the results to select the solvent and number of extraction times to be able to extract the analytes most effectively. Survey results showed that the selected sample treatment conditions were 50% methanol and 1- time extraction.

3.4.2. Procedure for quantifying the compound p inorecinol 4'-O- β -D-glucopyranoside and vladinol F in the medicinal herb Pandanus tonkinensis

Through preliminary investigation, the following conditions were used for HPLC analysis using a diode array detector (DAD): C18 column (250 mm × 4.6 mm; 5μm); separation column temperature: 40^{o}C; mobile phase A (acetonitrile) and mobile phase B (0.1% acetic acid solution) with solvent gradient program: starting from mobile phase ratio A:B =10:90 (v:v), increasing to 30 :70 (v:v) for 30 minutes, increased to 90:10 (v:v) for the next 10 minutes, returned to the original 10:90 ratio for 10 minutes of column stabilization; mobile phase flow rate: 1.0 mL/min; detection wavelength at 228 nm.

Experimental solutions were prepared as follows: i) *Stock standard solutions PT20 and PT6:* prepared separately by accurately weighing about 5 mg of standard into a 5 mL volumetric flask, adding about 3 ml of 50% methanol, ultrasonic shaking to dissolve, then adjusting the volume to just the right amount and shaking well; ii) *Mixed standard solution* : accurately pipet 1.0 ml of PT20 stock standard solution and 1.0 mL of PT6 stock standard solution into a 20 ml volumetric flask, add enough 50% methanol to the mark, shake well ; iii) *Test solution*: Accurately weigh about 5.0 g of medicinal herbs into a 100 mL conical flask, add exactly 50.0 mL of 50% methanol, sonicate for 30 minutes (extract 3 times), combine the resulting

extract, then rotary evaporate until dry. Dissolve it in 5.0 mL of methanol to obtain the test solution.

Samples of the mixed standard solution and test solution were injected into the chromatography column with a volume of 10µl, the chromatogram was determined, and the position and area of the PT20 and PT6 signals were determined. The concentrations of PT20 and PT6 in the solution injected into the chromatograph were determined according to the standard curve method. The content of PT20, PT6 in medicinal herbs is calculated according to the concentration of PT20, PT6 in the injection solution into the machine and the parameters of the sample processing process such as the volume of injection solution (5ml) and the amount of medicinal herbs (calculated in dry medicinal herbs) according to the formula:

$$C_{\text{PT20, PT6 in pharmacy whether}}\ (\text{mg/g}) = \frac{C_{PT\,20,PT6\,\text{extraction}}\ \left(\frac{mg}{ml}\right) \times 5\ (ml)}{m_{medicine}\ (g) \times (1 - \text{moisture})}$$

3.4.3. Evaluation of the procedure for quantifying the compound pinorecinol 4'-O- β -D-glucopyranoside and the substance vladinol F in the medicinal herb Pandanus tonkinensis

The dosing procedure was validated according to the guidelines of the Association of Official Analytical Chemists (AOAC) and the International Conference on Harmonization of Procedures for Registration of Pharmaceuticals for Human Use (ICH) with criteria: specificity, system suitability, linearity, precision (repeatability and intermediate precision), precision, limit of detection and limit of quantification.

3.4.3.1. Specificity

Experiments show that on the chromatogram of the test solution, peaks with retention times of 20.230 minutes and 27.045 minutes appear, corresponding to peak PT20 (20.247 minutes) and peak PT6 (27.122 minutes) on the chromatogram of the mixed standard solution. fit. The UV spectrum of the peak with a retention time of 20.230 minutes obtained on the chromatogram of the test solution corresponds to the UV spectrum of the

PT20 peak obtained on the chromatogram of the mixed standard solution with λ max = 228.2 nm and 280.0 nm.

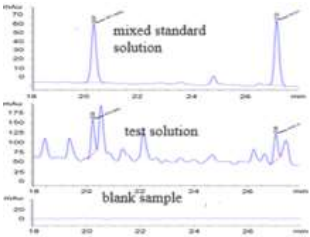


Figure 3.6 . Chromatogram of mixed standard solution, test solution and blank sample .

The UV spectrum of the peak with a retention time of 27.045 minutes obtained on the chromatogram of the test solution corresponds to the UV spectrum of the PT6 peak obtained on the chromatogram of the mixed standard solution with λ_{max} = 280.0 nm. The peaks PT20 and PT6 on the chromatogram of the test solution and the mixed standard solution are pure. The blank sample (methanol solvent) does not affect the analysis results. These results show that the test procedure meets the requirements for specificity.

3.4.3.2. System suitability

The results of evaluating the suitability of the system are presented in table 3.3. The relative standard deviation values of the retention time of peaks PT20 and PT6 when analyzing repeatedly (n=6) mixed standard solutions at a concentration level of 0.12% and 0.07%, respectively, met the requirements. requirement ≤ 1.0%, the relative standard deviation value of peak area of PT20 and PT6 peaks are 1.31% and 1.35% respectively, meeting the requirement ≤ 2.0%. The similarity coefficients of PT20 and PT6 between two concentration levels analyzed in duplicate are RF = 1.01 and RF = 1.00. The chromatographic conditions chosen for repeatable results in terms of retention time and peak area using the high-performance liquid

chromatography equipment system are suitable to ensure the stability of the analysis.

Table 3.3 . Results of evaluating the suitability of the system

	PT20 peak retention time (minute)	Area of peak PT20 (mAU.s)	PT6 peak retention time (minute)	PT6 peak area (mAU.s)
Average (n=6)	20,293	878.87132	27,115	899.61777
RSD (%)	0.12	1.31	0.07	1.35
Similarity coefficient RF = 1.01 RF = 1.00				

3.4.3.3. Standard curve range for quantification

The results of the linear range survey are presented in table 3.4 and figure 3.7. In the concentration range from 25.5 x 10^{-3} to 101.9 x 10^{-3} mg/mL for PT20, there is a linear dependence between peak area and PT20 concentration with a linear correlation coefficient R^2 = 0.9983. In the concentration range from 26.0 x 10^{-3} to 103.9 x 10^{-3} mg/mL for PT6, there is a linear dependence between peak area and PT6 concentration with linear correlation coefficient R^2 = 0.9974. The standard curves have good linearity for quantitative analysis of PT20 and PT6.

Table 3.4. Results of linear range survey to quantify PT20 and PT6

Marker	PT20		PT6	
	Concentration (mg/mL)	Peak area (mAU.s)	Concentration (mg/mL)	Peak area (mAU.s)
Standard range	25.5 x 10^{-3}	436.59933	26.0 x 10^{-3}	411.81412
	40.8 x 10^{-3}	708.55096	41.6 x 10^{-3}	749.87158
	51.0 x 10^{-3}	878.87132	51.9 x 10^{-3}	899.61777
	61.2 x 10^{-3}	1028.60651	62.3 x 10^{-3}	1071.64014
	76.4 x 10^{-3}	1345.65894	77.9 x 10^{-3}	1318,18884
	101.9 x 10^{-3}	1733.94653	103.9 x 10^{-3}	1799.81677
Regression equation	y = 17084x + 6.3385		y = 17376x - 11.091	
Correlation coefficients	R^2 = 0.9983 > 0.99		R^2 = 0.9974 > 0.99	
%Y	0.72% (< 2.0%)		1.23% (< 2.0%)	

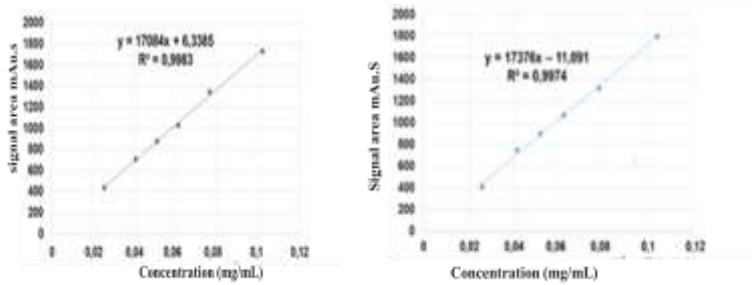


Figure 3.7 . Standard curves for quantification of pinoresinol 4-O-beta-D-glucopyranoside (PT20) and vladinol F (PT6).

3.4.3.4. Accuracy

The results obtained when analyzing 6 independent test samples on 2 different days, with 2 different testers, were used to evaluate the precision (repeatability and intermediate precision).

** Repeatability*

Table 3. 5 . Results of repeatability survey

Sample try	Quantity weigh (g)	Pic PT20 _ (mAU.s)	Pic PT6 _ (mAU.s)	Jaw quantity PT20 (mg/g)	Jaw amount of PT6 (mg/g)
first	5,10042	674.06952	616.18457	0.0404	0.0367
2	5,32086	704.51483	643.13428	0.0404	0.0368
3	5.52441	724.00456	665,42011	0.0400	0.0366
4	5.31051	706.41534	628.91473	0.0406	0.0360
5	May 20041	700.22448	632.86042	0.0411	0.0370
6	5,18079	699.01453	631.08778	0.0412	0.0371
			TB	0.0405	0.0366
			RSD (%)	1.13	1.02

Table 3.5. Results of evaluating the reproducibility of the method

	Day 1- tester 1 (n = 6)	Day 2- tester 2 (n = 6)	Two days (n = 12)
Average PT20 content (µ g/g)	40.5	41.2	40.9
RSD (%)	1.13	1.43	1.54
PT6 content average (µ g/g)	36.6	36.7	36.7
RSD (%)	1.02	1.67	1.32

Specific results in table 3.5 and table 3.6 show that the developed method has repeatability when the RSD (n = 6) for PT20 and PT6 are both less than 5.3% and the RSD (n = 12) is repeatable. For PT20 and PT6, both are less than 8%, meeting the requirements according to AOAC guidelines with test samples containing quantity from 0.001% to 0.01%.

3.4.3.5. Accuracy and working range

The results of evaluating the accuracy of the method are presented in table 3.7. The average value of PT20 recovery efficiency at all three addition levels ranges from 99% to 101% with relative standard deviation from 0.06% to 1.41%; With PT6, the average recovery efficiency at 3 concentration levels ranges from 101% to 102% and the relative standard deviation ranges from 0.55% to 1.31%. According to AOAC requirements, with samples with concentrations from 0.001% to 0.01%, the average sample recovery must reach from 90% to 107%, thus the analytical method ensures accuracy for quantifying PT20 and PT6.

Table 3.7 . Results of evaluating the accuracy of the method

Quantitative concentration level (% compared to test sample)	Average amount of standard added (µg)	Average amount of recovery standard added (µg)	Average recovery efficiency (%)	RSD (%) (n = 3)
PT20: 50%	6.29	6.38	101	0.06
100%	12.58	12.66	101	0.82
150%	25.17	25.03	99	1.41
PT6: 50%	6.41	6.44	101	0.96
100%	12.82	13.01	101	0.55
150%	25.64	25.76	100	1.31

3.4.3.6 . Limit of detection and limit of quantification:

Phase washy gradually solution standard and injection enter set bag sharp sign arrive hot PT20 level 0.76 µg/ml and PT6 0.78 µg/ml then collect, there are peaks PT20 and PT6 replied response urgent about 3 times degree interference road background . Phase washy urgent double solution on , injection enter set bag sharp sign , almost like Are not export presently replied corresponding to the above pic sharp sign stuff . Thus, the detection limit (IDL) with the standard solution is about 0.76 µg/ml PT20 and 0.78 µg/ml PT6. According to calculations, the limit of quantification (IQL) of the standard solution will be about 3.3 times the detection limit, corresponding to concentrations of 2.55 µg/ml PT20 and 2.60 µg/ml PT6 .

Mix mixed standard solutions ($IQL_{standard\ solution}$) and mixed standard solutions and add them to the medicinal extract base ($IQL_{spike\ solution}$) with PT20 and PT6 concentrations of 2.55 µg/ml and 2.60, respectively. µg/ml. Chromatographic analysis of each solution was repeated 6 times. On the chromatogram of the $IQL_{solution}$ and IQL_{spike} the PT20 and PT6 peaks appear clearly. The RSD (n = 6) of the area of the PT20 and PT6 peaks when analyzing the LOQ_{spike} is 2.87% and 2 .31% is less than the 7.3% required by AOAC. Thus, it can be confirmed that the limits of quantification for PT20 and PT6 are 2.55 µg/ml and 2.60 µg/ml, respectively, from the test solution.

Use the process sample conversion system: 5 g of medicinal material transferred to 5 ml of service test. The method detection limit (MDL) is calculated as 0.76 µg/g PT20 and 0.78 µg/ g PT6 dried medicinal herbs; The method quantification limit (MQL) is 2.55 µg PT20/g dry medicinal herb and 2.60 µg PT6/g dry medicinal herb.

Thus, the appraisal results in section 3.4.3 show that the process of simultaneously analyzing PT20 and PT6 in wild pineapple medicinal herbs using the HPLC method meets the requirements of specificity, system

suitability, and linearity, precision, accuracy (repeatability and intermediate precision), suitable for applying simultaneous qualitative and quantitative testing of PT20 and PT6 in medicinal materials.

3.5. Quantification of pinorecinol 4'-O- β -D-glucopyranoside (PT20) and vladinol F (PT6) in wild pineapple collected at some localities

Using the analytical procedure validated above, the average content of two markers in Northern wild pineapple species collected in Thanh Hoa, Hoa Binh, and Thai Nguyen was determined in the range of 25.0 ± 0.4 to 43,5 ±0.4 µg/g dry herb with pinoresinol 4-O-beta-D-glucopyranoside and 24.3 ±0.5 to 37.1 ±0.5 µ/g dry herb with vladinol F.

Table 3.8. Quantitative results of pinoresinol 4-O-beta-D-glucopyranoside (PT20) and vladinol F (PT6) in the wild pineapple species *Pandanus tonkinensis* collected in some localities

	Muong Lat - Thanh Hoa	Cam Thuy - Thanh Hoa	Luong Son - Hoa Binh	Dinh Hoa - Thai Nguyen
PT20: Jaw amount(µ g/g)	40.9	43.5	25.0	38.6
RSD %	1.54	0.9	1.62	0.37
PT6: Jaw amount(µ g/g)	36.7	37.1	24.3	30.7
RSD %	1.32	1.44	1.97	1.96

Figure 3.8 . Chromatogram analysis of pinoresinol 4-O-beta-D-glucopyranoside (PT20) and vladinol F (PT6) in *Pandanus tonkinensis.*

CONCLUSIONS

The research results achieved are the following:

1. Research on chemical composition

Using a combination of chromatographic methods and modern spectroscopic methods, 28 compounds were isolated and determined from the fruits and roots of *Pandanus tonkinensis*, including 3 new compounds.

- From the fruit of *P.tonkinensis* 8 known compounds were isolate including: ficusal (**PT1**), syringaresinol (**PT2**), medioresinol (**PT3**), lariciresinol (**PT4**), secoisolariciresinol (**PT5**), vladinol F (**PT6**), luteoliflavan (**PT7**), isorhapontigenin (**PT8**).

- From the roots of *P.tonkinensis*, 20 compounds were isolated. There are 3 new compounds: (7S) -2,6-dimethoxyphenyl-7,9-propanediol-1-O- β -D-glucopyranoside (**PT10**); Trans-cinnamyl alcohol 9-O-(6′-O- α -L-arabinofuranosyl)- β -D-glucopyranoside (**PT25**); 4-(3-hydroxypropyl)-2,6 dimethoxyphenol β -D-apiofuranosyl-(1→6)- β -D-glucopyranoside (**PT26**) and 17 known compounds dihydrosyringin (**PT9**); (6S,9S)-roseoside (**PT11**); 1-O- β -Dglucopyranosyl-2-{2,6-dimethoxy-4-[1-(E)-propen-3-ol]phenoxyl}propan-3-ol(**PT12**); 1-O-(β-D-glucopyranosyl)-2-[2-methoxy-4-(ω -hydroxypropyl)-phenoxyl]-propan-3-ol (**PT13**); benzyl O- α -L-arabinopyranosyl-(1→6)- β -D-glucopyranoside (**PT14**); 1-O-(β -D-glucopyranosyl)-2-[2,6-dimethoxy-4-(ω-hydroxypropyl)-phenoxyl]-propan-3-ol (**PT15**); (7R,8R)-threo-4,7,9,9′-tetrahydroxy-3,3′-dimethoxyl-8-O-4′-neolignan-4-O- β -D -glucopyranoside (**PT16**); (7S,8S)-threo-4,7,9,9′-tetrahydroxy-3,3′-dimethoxyl-8-O-4′-neolignan-4-O- β -D -glucopyranoside (**PT17**); pinoresinol-4,4′-di-O- β -D-glucoside (**PT18**); isoeucommin A (**PT19**); pinoresinol 4'-O- β -D-glucopyranoside (**PT20**); acanthoside B (**PT21**);eucommin A (**PT22**); rourinoside (**PT23**); (7S,8R)-5-methoxydihydrodehydrodiconiferyl alcohol-4-O- β -D-glucopyranoside (**PT24**); kelampayoside A (**PT27**); urolignoside (**PT28**).

2. Research on biological activity

Antioxidant activities of the isolated substances were evaluated by inhibition of NO production on RAW 264.7 cells, induction with LPS, and inhibition of membrane lipid peroxidation. Cells of 28 compounds isolated from the fruits and roots of *P.tonkinesis*. The results show that:

- 22 compounds PT1-PT11, PT13, PT15-PT17, PT20, PT22- PT27 have anti-inflammatory activities, of which 14 compounds PT1, PT4-PT6, PT7, PT9, PT11, PT15, PT16, PT20, PT22, PT23, PT26, PT27 have IC_{50} values from 1.78 - 37.03 indicating that they possess better anti-inflammatory abilities than the positive control L-NMMA (IC_{50} 37.8) according to IC_{50} results.
- 10 compounds including PT2, PT4-PT8, and PT19-PT22 showed antioxidant abilities, of which 5 substances PT5, PT7, PT8, PT20, and PT21 have IC_{50} values ranging from 10.36 - 27.45 indicating that they possess better antioxidant abilities than the positive control trolox (IC_{50} 31.4).

3. Research on markers

After selecting 02 compounds as markers for the medicinal herb *P. tonkinensis* which are pinorecinol 4'-*O*- β- D-glucopyranoside and vladinol F, the medicinal herb has been extracted and purified to a purity level of 98% with a weight of 100-200 mg to serve as a standard for testing medicinal herbs. A high-performance liquid chromatography method for quantitative analysis of two markers in medicinal herbs *P.tonkinensis* has been developed and validated. The results show that the analytical method is specific, with a linear standard curve range from 25.5 to 101.9 μg/ml (R^2 = 0,9983) and from 26.0 to 103.9 μg/ml (R^2 = 0,9974) for the two markers, with good precision (RSD < 2%), high precision (recovery efficiency between 99.4 and 101.5%), the limit of quantification with pinoresinol 4-O-beta-D-glucopyranoside and vladinol F were 2.55 μg and 2.60 μg per gram of dry medicinal material, respectively.

NEW FINDINGS OF THE THESIS

1. For the first time, the structure of 28 compounds from the fruit and root of *Pandanus tonkinensis* has been isolated and determined using modern physicochemical analysis methods.
2. Three new compounds from *Pandanus tonkinensis* were identified, including: (7*S*)-2,6-dimethoxyphenyl-7,9-propanediol-1-*O*- β -D-glucopyranoside (**PT10**); Trans-cinnamyl alcohol 9-*O*-(6′-*O*- α -L-arabinofuranosyl)- β -D-glucopyranoside (**PT25**) and 4-(3-hydroxypropyl)-2,6dimethoxyphenol β -D-apiofuranosyl-(1→6)- β -D-glucopyranoside (**PT26**).
3. For the first time, two compounds were selected as markers for medicinal herbs of the *Pandanus tonkinensis* species with an orientation of hepatoprotective effect, pinorecinol 4'-O- β- D-glucopyranoside and vladinol F, and a high-performance liquid chromatography analysis method was developed that is suitable for quantifying two markers in the medicinal herb *Pandanus tonkinensis*.

RECOMMENDATIONS

The two extracted and purified compounds pinorecinol 4'-O- β- D-glucopyranoside and vladinol F can be used as standards and validated quantitative analysis procedures to control the quality of medicinal materials and research. The production process of hepatoprotective products from *Pandanus tonkinensis* medicinal herb.