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Study on lipid and phospholipid composition from the seed oil of *Dalbergia tonkinensis*

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Abstract. The content and composition of classes and molecular species of phospholipid extracted from *Dalbergia tonkinensis* Prain (Cây Sua) seed oil in Viet Nam have been investigated using HPLC-HRMS and LCMS-IT-TOF. The content of phospholipid subclasses and their molecular species of *D. tonkinensis* seed oil have been investigated for the first time. The results showed that *D. tonkinensis* seed oil contains all basic classes of lipid: polar lipid, sterol, triacylglycerol, free fatty acid, diacylglycerol, hydrocarbon, and wax. In which, the content of triacylglycerol accounts for the highest percentage of 76.38 %, while polar lipid and free fatty acid also have a relatively high content. This result indicated the presence of 12 specific phospholipid subclasses of the plant, including phosphatidylethanolamine, phosphatidylcholine, and phosphatidylinositol. The "molecular forms" of phospholipids of Sua seeds lipids have been identified. The results show that the phospholipids of Sua seeds have 3 classes of substances including phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylinositol (PI). In which PE has 5 molecular forms, PC has 5 molecular forms and has 2 molecular forms of PI. These are the first data was found and published in Sua seeds.

Key words: Lipid, fatty acid, Fabaceae, phospholipid, phospholipid subclasses, *Dalbergia tonkinensis*.

Classification numbers: 1.1.3, 1.4.1.

1. INTRODUCTION

There are over 300 species of *Dalbergia* genus (Fabaceae family). They are distributed in tropical and sub-tropical regions [1]. *Dalbergia odorifera's* wooden core has been used as spices

in food and medicinal herbs for the treatment of coronary artery disease and arrhythmia, cancer, and diabetes. In addition, bark decoction has been used to treat indigestion and seed oil has been used to reduce rheumatism [2, 3]. The biological effects of Dalbergia species are demonstrated through anti-inflammatory, antioxidant, and antibacterial activities. *Dalbergia tonkinensis* Prain is an angiosperm plant and known as a high-value timber tree. It is commonly distributed in provinces such as Thua Thien Hue, Vinh Phuc, Thai Nguyen provinces, and Ha Noi City (Viet Nam) [4]. This paper reports the analysis of the content and composition of lipid classes from the seeds of *D. tonkinensis*.

2. MATERIALS AND METHOD

2.1. Plant material

The seeds of *D. tonkinensis* were collected in November 2020 in Ha Noi city (Viet Nam) and identified by Dr. Nguyen Quoc Binh from the Viet Nam National Museum of Nature, Vietnam Academy of Science and Technology. The voucher specimen (VNMN-B2016.1) was deposited at the Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology.

2.2. Oil extraction

Soxhlet extraction was performed to obtain oil from *D. tonkinensis* species using a modified method of ISO 659:2009 [5]. In brief, 10 g of sample material were ground in a ball mill and then extracted in a Twisselmann apparatus for 6 hours with 200 mL of petroleum ether. The oil was obtained by removing the solvent.

2.3. Determination of content and composition of total lipid classes

The total lipids were covered on a thin plate (6 cm × 6 cm) by dotting 3 tracks corresponding to 3 volumes of 5, 10, and 15 µL. They were then subjected to chromatography with solvent systems of *n*-hexane/diethyl ether/acetic acid (85:15:1, v/v/v) and $CHCl_3$/MeOH/C_6H_6/amonia solution 28 % (65:30:10:6, v/v/v/v). The thin plates were dried in the atmosphere, then at 240 ^oC for 10 minutes and visualized using H_2SO_4 (10 %) in MeOH. Chromatograms were obtained by scanning on an Epson Perfection 2400 PHOTO scanner (Nagano, Japan) with a standard size and resolution. The compositions of the total lipid classes were identified by measuring area and color intensity using image analysis software (Sorbfil TLC Videodensitometer DV, Krasnodar, Russia) [6].

2.4. Identification of phospholipid

Phospholipids were analyzed with high-performance liquid chromatography (HPLC) - high-resolution mass spectrometry (HR-MS) to identify and quantify the chemical structure of phospholipids. The HPLC separation of PL was performed at a constant content of $(C_2H_5)_3N$/acid formic (0.08:1, v/v) in the solvent system that allowed to carry out efficient ionization under ESI conditions and obtain a stable ion signal by simultaneous registration of positive and negative ions. For polar lipids of *D. tonkinensis* seed, HPLC separation was performed using channel solvent systems of A: *n*-hexane/2-propanol/acid formic/$(C_2H_5)_3N$ (82:17:1:0.08, v/v/v/v) and B: 2-propanol/H_2O/acid formic/$(C_2H_5)_3N$ (85:14:1:0.08, v/v/v/v). The gradient was started at 5 % of B, increased to 80 % for 25 minutes at a flow rate of 0.2

mL/min. Polar lipids were detected by HR-MS and determined by comparison with authentic standards using a Shimadzu LC-MS Solution control and processing software (v.3.60.361, Shimadzu, Kyoto, Japan). The quantification of individual molecular species within each polar lipid class was carried out by calculating the peak area for the individually extracted ion chromatograms. Molecular forms of phospholipids from *D. tonkinensis* were analyzed by HR-MS using a Shimadzu LCMS-IT-TOF instrument (Kyoto, Japan) at the Institute of Organic Biochemistry, FEB, RAS [7, 8].

3. RESULTS AND DISCUSSION

3.1. Total lipid content of the sample

The total lipid content was determined by taking the lipid mass percent compared with the initial fresh sample. The fatty oil content *of D. tonkinensis* seeds (13.86 %) was lower than that of other samples belonging to Fabaceae like soybean oil (22.1 %). However, the results of Augustus [9] and Badami [10] showed that the oil content from two *Dalbergia* species ranged from 4.8 % (D. sissoo) to 7.4 % (*D. paniculatae*), indicating that the fatty oil content of D. tonkinensis seeds (13.86 %) was quite high [11].

3.2. The content and composition of lipid classes

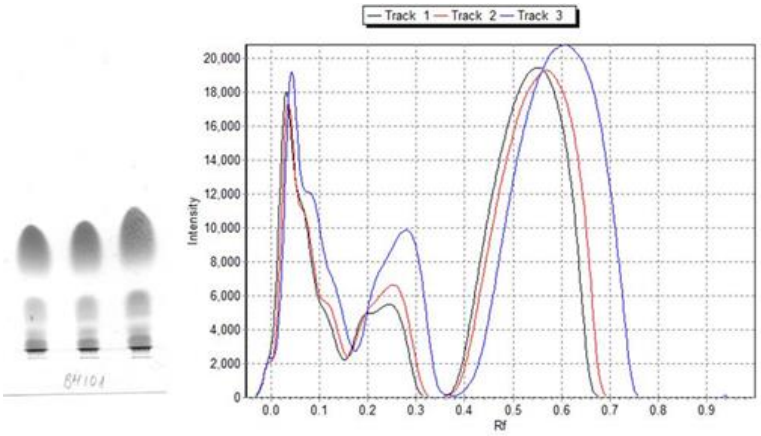

Figure 1. TLC images and chromatograms for calculating the lipid content of *D. tonkinensis* seed oil.

Table 1. The content and composition of lipid classes from the seed oil of *D. Tonkinensis.*

No.	Class name	Class symbol	**Content** (% of total lipid)
1	Polar lipid	PL	18.29
2	Sterol	ST	0.05
3	Diacylglycerol	DG	0.2
4	Free fatty acid	FFA	3.87
5	Triacylglycerol	TG	76.38
6	Other		1.21
7	**Total**		**100**

As shown in Table 1, the total lipid of *D. tonkinensis* seeds contains the following basic lipid classes: polar lipid (PL), sterol (ST), triacylglycerol (TG), and free fatty acid (FFA). In which, triacylglycerol has the highest content (76.38 %), similar to the triacylglycerol content in seed oil of *D. mammosa* (69.51 %), Afzelia xylocarpa (63.94 %), and Sapotaceae (63.2 %) [11, 12].

3.3. Identification result of phospholipid from the seed oil of *D. tonkinensis* Prain

To determine phospholipid subclasses in polar lipids of *D. tonkinensis* seed oil, we applied the HR-MS data of fractions of phospholipid reference substances [13]. The phospholipid compositions of *D.tonkinensis* seed oil were identified to be phosphatidylethanolamine (PE), phosphatidylcholine (PC), and phosphatidylinositol (PI) at a retention time of 20.099, 22.650, and 27.336 min, respectively (Figure 2). The phospholipids were detected by LCMS-IT-TOF.

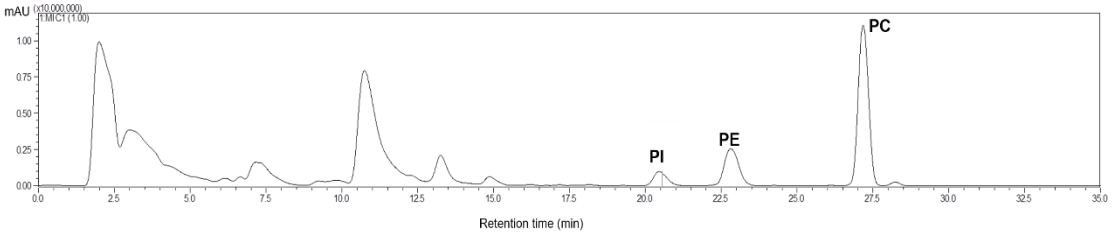

Figure 2. HPLC spectra of polar lipid of *Dalbergia tonkinensis* Prain seed oil.

3.3.1. Molecules of phosphatidylethalnolamine (PE)

PE in *D. tonkinensis* seed oil included diacyl species and contained no alkenyl-acyl or alkyl-acyl. Five species of PE molecules are shown in Table 2.

Table 2. Molecular species of phosphatidylethalnolamine.

PL	Symbol	m/z ($[M-H]^-$)	Molecular formula	Mass error (ppm)	Content (%)	Retention time (min)
PE 36:4	18:2/18:2	738.5053	$C_{41}H_{74}NO_8P$	4.10	29.06	22.655
PE 36:3	18:1/18:2	740.5298	$C_{41}H_{76}NO_8P$	8.40	12.65	22.433
PE 36:2	18:2/18:0	742.5452	$C_{41}H_{78}NO_8P$	0.18	9.60	22.948
PE 34:2	16:0/ 18:2	714.5006	$C_{39}H_{74}NO_8P$	3.96	40.08	22.927
PE 34:1	16:0/18:1	716.5344	$C_{39}H_{76}NO_8P$	15.10	8.62	22.735

The HR-MS of all compositions of the PE formulations exhibited the signal of positive ions $[M+H]^+$, ion clusters $[M+H+C_2H_8NO_4P]^+$, and negative ions $[M-H]^-$. With PE 34:2, a negative signal appeared at *m/z* 714.5006 (calcd. $[C_{39}H_{73}NO_8P]^+$ for 714.5152), and positive ions $[M+H]^+$ were detected at *m/z* 716.5240 ($[C_{39}H_{75}NO_8P]^+$). In the MS/MS mode of ions $[M-H]^-$ of PE 34:2, a strong signal was found at *m/z* 279.2309, corresponding to the negative ions of fatty acid 18:2, confirming the presence of fatty acid 18:2 in PE molecules. The fragmentation was very important for the identification of PE molecular species. Thus, the mass spectrum data indicated

that the molecular species was diacyl glycerophosphoethalnolamine PE 16:0/18:2. By the same method, the remaining four molecular species of PE were identified as: PE 34:1, PE 36:4, PE 36:3, and PE 36:2.

3.3.2. Molecular species of phosphatidyl choline (PC)

Molecular species of PC in *D. tonkinensis* seed are shown in Table 3.

Table 3. Molecular species of phosphatidylcholine.

PL	Symbol	m/z $(M+H)^+$	Molecular formula	Mass error (ppm)	Content (%)	Retention time (min)
PC 34:2	18:2/16:0	758.5691	$C_{42}H_{80}NO_8$ P	7.20	31.20	27.323
PC 36:2	18:2/18:0	786.5972	$C_{44}H_{84}NO_8P$	0.43	20.83	27.165
PC 36:4	18:2/18:2	782.5676	$C_{44}H_{80}NO_8P$	4.04	26.14	27.215
PC 36:3	18:2/18:1	784.5895	$C_{44}H_{82}NO_8P$	15.31	16.90	26.936
PC 34:2	18:1/18:0	788.6141	$C_{44}H_{86}NO_8P$	15.36	4.93	26.985

PC 34:2 (accounting for the highest content in PC with 31.2 %) formed acetylation molecular ions carrying a negative charge $[M+HCOO]^-$ at *m/z* 802.5536 corresponding to the formula of $[C_{43}H_{81}NO_{10}P]^-$, the ones carrying a positive charge $[M+H]^+$ at *m/z* 758.5691, calculated at 757.5622 (mass error 7.20 ppm) corresponding to the formula of $[C_{42}H_{81}NO_8P]^+$, and ions cluster $[M-CH_3]^-$ at 742.5305 corresponding to the composition of $[C_{41}H_{77}NO_8P]$. In the MS^{2-} spectrum, $C_3H_6O_2$ disappeared from ions $[M+HCOO]^-$ at *m/z* 802.5536 forming $[M-CH_3]^-$ at *m/z* 742.5305. This indicated that negative ions were formed by adding formiate ions to lipid molecules. Therefore, we could observe the appearance of two carboxylate negative ions 18:2 at *m/z* 279.2280 and 16:0 at *m/z* 255.2283 on the MS^{2-} spectrum of ions $[M-CH_3]$. Thus, PC 34:2 was characterized by diacyglycerophosphocholine 18:2/16:0. Using the same method, the remaining four molecular species of PC were also identified.

3.3.3. Molecular species of phosphatidylinositol (PI)

In this study, two molecular species of PI were identified (Table 4). The results indicated that there was no alkenyl acyl glycerophosphoinositol in PI. The two constituents of PI were diacyl glycerophosphoinositol with fatty acids 16:0, 18:0 and 18:2.

Table 4. Molecular species of phosphatidylinositol.

PL	Symbol	m/z $([M-H]^-)$	Molecular formula	Mass error (ppm)	Content (%)	Retention time (min)
PI 34:2	16:0/18:2	833.5123	$C_{43}H_{79}O_{13}P$	12.30	68.33	20.572
PI 36:2	18:0/18:2	861.5467	$C_{45}H_{83}O_{13}P$	3.77	31.67	20.468

For PI, only negative ions $[M-H]^-$ appeared. According to the fraction of MS^{2-} spectrum of ions $[M-H]^-$ of PI, several specific ions like PI 34:2 were also found in PI. PI 34:2 with *m/z* of 83.5123 corresponded to $[C_{43}H_{78}O_{13}P]^-$, calculated at 834.5258 with a mass error of 12.3 ppm. The peaks loosing inositol, acyl groups and carboxylate anions of fatty acid were detected in the MS^{2-} spectrum signal of negative ions PI 34:2. Moreover, specific ions at 255.2344 corresponding to anions of fatty acid 16:0 appeared when PI lost two acyl groups. This was an important fragmentation to identify the molecular species of PI. The compostion of PI 34:2 was diacyglycerophosphoinositol 16:0/18:2. Similarly, diacyglycerophosphoinositol 18:0/18:2 was found in PI 36:2.

4. CONCLUSION

The content of phospholipid subclasses and their molecular species of *D. tonkinensis* seed oil have been investigated for the first time. The results indicated that all basic classes of lipid were identified in *D. tonkinensis* seed oil to be polar lipid, sterol, triacylglycerol, free fatty acid, diacylglycerol, hydrocarbon, and wax, in which, the content of triacylglycerol was the highest (76.38 %). The results also indicated the presence of 12 molecular species in phospholipid subclasses including phosphatidylethanolamine, phosphatidylcholine, and phosphatidylinositol by LCMSIT-TOF, in which, PE 34:2, PC 34:2, and PI 36:2 accounted for the highest content.

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CRediT authorship contribution statement. Nguyen Thi Thuy: Methodology, Formal analysis, Investigation, Conceptualization. Pham Minh Quan: Software, Formal analysis. Pham Quoc Long: Supervision. Dao Thi Kim Dung: Formal analysis. Nguyen Thi Mai, Duong The Vi, Nguyen Thi Diep: Methodology, Data curation, Formal analysis. Doan Lan Phuong: Methodology, Writing-Review and Editing.

Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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